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Poster Presentations

2 | Evaluate the sensitivity of Rome IV criteria among patients with suspected irritable bowel syndrome: A multi-center cross-sectional study in Asian outpatients

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Background & Aims: The indication of Rome IV criteria in Asians remains unclear considering the different bowel symptom clusters from patients in Western countries. Moreover, reports on agreement between symptom-based criteria yield very different results, while validation of Rome IV is limited. This study aims to explore the sensitivity of Rome IV criteria among patients with suspected irritable bowel syndrome (IBS), and to describe the agreement between the various symptom-based criteria.

Methods: A multi-center cross-sectional study was designed. Patients suspected by their physicians were referred for enrollment. The Manning and Rome II-IV criteria were evaluated through face-to-face interviews and patients completed the questionnaires including Gastrointestinal Symptom Rating Scale, IBS severity scoring system, Irritable Bowel Syndrome Quality of Life measurement, and the Hospital Anxiety and Depression Scale.

Results: A total of 576 were included. The Rome IV criteria were fulfilled by 344 patients (sensitivity = 0.60, 95% CI: 0.56-0.64). Rome IV-positive patients more frequently reported urgency to defecate, loose stools, more than 3 bowel movements per day, and abdominal pain. Rome IV-positive patients had higher anxiety and depression scores, more severe IBS symptoms and lower quality of life compared with Rome IV-negative patients. The various symptom-based criteria identify slightly different subpopulations. Rome IV has only moderate agreement with other criteria. Most of the Rome-IV positive IBS patients (293/344, 85.2%) met all the Rome III, Rome II and Manning criteria (Figure 1).

Conclusions: Rome IV criteria identified around 60% of the Asian patients labeled with IBS. Rome IV-positive IBS was a subgroup with higher severity and typical symptoms.

	Rome III	Rome II	Manning [a]
Rome IV	77.80%	74.10%	73.30%
	κ=0.49	κ=0.40	κ=0.42
Rome III		93.60%	72.60%
		κ=0.77	κ=0.27
Rome II			73.80%
			κ=0.29

[a] ≥3 Manning criteria fulfilled
κCohen's kappa coefficient

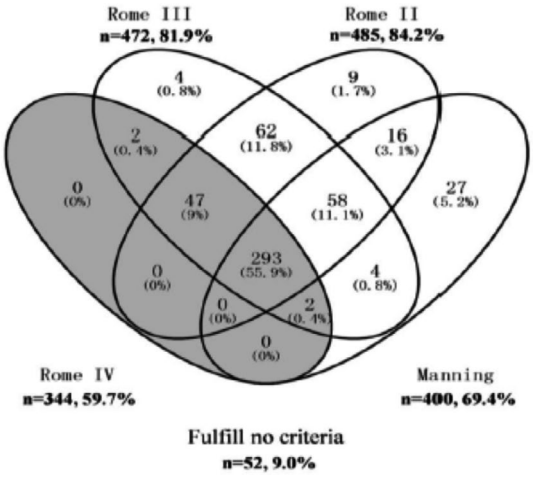

Figure 1. Distribution of symptom-based irritable bowel syndrome diagnosis and the evaluation of diagnostic consistency

3 | Rectal compliance is affected by enteric nervous system and interstitial cells of Cajal in gastrointestinal smooth muscle

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Background: Rectal compliance, contributing to fecal storage, is different from the colonic propulsive movement.
Objectives: To explore the electromechanical characteristics of rectal compliance in the murine rectum and investigate the contribution of intrinsic inhibitory neurotransmission and interstitial cells to rectal compliance.
Methods: Male C57BL/6 mice, aged 8 weeks or more were used. For in vivo experiments, the mice were anesthetized with isoflurane, and anorectal manometry was applied to measure rectal compliance through rectum. In ex vivo experiments with using murine rectum, intra-rectal pressure was measured in organ bath. The colonic migrating motor complex (CMMC) measurements and electrophysiological microelectrode recordings for membrane potential were performed using smooth muscle strips and segments. Calcium imaging was used to measure the calcium transients in the interstitial cells and smooth muscle cells within the rectum of the mice expressing a genetically encoded calcium indicator (GCaMP).
Results: The rectal compliance was significantly decreased after in vitro intraperitoneal injection and ex vivo infusion into organ bath of L-NNA and apamin, respectively (P = 0.002 vs. 0.005; P = 0.016 vs. 0.015). Rectum did not have CMMC, and after treatment of the L-NNA or apamin in the organ bath, the rectal contractions were increased, not CMMC (6.19 ± 3.98 vs. 20.35 ± 15.78 mN·min, P = 0.031), and propagation of contractions from the distal colon increased (7.20 ± 3.32 vs. 29.12 ± 20.75 mN·min, P = 0.046). In membrane potential with electric field stimulation, inhibitory junction potential significantly increased after L-NNA and apamin, respectively (17 ± 2.9 vs. 16 ± 1.6 mV, P = 0.04; 24 ± 2.5 vs. 13 ± 3.6 mV, P = 0.02). In calcium transient of smooth muscle, AUC (area under the curve) in rectum was smaller than that of colon (9.36 ± 4.57 vs. 3.49 ± 2.58 IU·min, P = 0.03), and AUC in rectum increased after L-NNA and apamin, respectively (3.79 ± 1.93 vs. 9.71 ± 4.52 IU·min, P = 0.001; 3.26 ± 1.92 vs. 8.31 ± 4.12 IU·min, P = 0.021). In calcium transient of ICC of rectal circular muscle, AUC significantly increased after L-NNA (7.39 ± 2.52 vs. 10.98 ± 3.71 IU·min, P = 0.012).
Conclusions: Murine rectal compliance was identified in the study. Enteric inhibitory neurotransmissions was related to the rectal compliance. ICC could control the rectal smooth muscle activities.

4 | Efficacy of DA-5204 for gastroesophageal reflux disease: A randomized, double-blind, placebo-controlled pilot study

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Background/Aim: Proton pump inhibitor (PPI) alone is not satisfactory for the treatment of gastroesophageal reflux disease (GERD). Therefore, we investigated the efficacy of DA-5204 (Stillen 2X®, 90 mg of *Artemisia asiatica* 95% ethanol extract per tablet) and PPI combination therapy on GERD in comparison to PPI alone.
Methods: This randomized, double-blind, placebo-controlled study randomly assigned 70 patients with endoscopically proven esophageal mucosal injury (Los Angeles classification A or B) into 2 groups: pantoprazole 40 mg once daily with DA-5204 twice daily (DA-5204 group) or pantoprazole 40 mg once daily with placebo twice daily (placebo group) for 4 weeks. The primary endpoints were endoscopically effective (normal mucosa or minimal change) or complete healing (normal mucosa) rates, and secondary endpoint was sufficient relief (≥50% reduction) of reflux symptoms using Gastroesophageal Reflux Disease Questionnaire (GerdQ) questionnaire.
Results: Final analyses included 29 patients with the DA-5204 group and 30 patients with the placebo group. At weeks 4, there was a significantly difference of the endoscopically effective healing rate between the two groups (DA-5204 group vs. placebo group; 93.1% vs. 56.7%; P = 0.001) as well as the complete healing rate (DA-5204 group vs. placebo group; 82.8% vs. 33.3%; P < 0.000). The rates of sufficient relief for reflux symptoms according to GerdQ tended to be higher in the DA-5204 group than in the placebo group, with no significant difference.
Conclusion: Our findings suggest that combined therapy with PPI and DA-5204 is more effective in treating GERD than PPI alone.
Keywords: *Artemisia asiatica*; proton pump inhibitor; gastroesophageal reflux disease; reflux esophagitis

5 | Fermentable carbohydrates induce visceral and abdominal hypersensitivity and mucus barrier dysregulation in mice through increased mucosal mast cell populations

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Objective: Irritable Bowel Syndrome (IBS) is characterized by abdominal pain, often associated with diet. Limiting the consumption of unabsorbed fermentable oligo-, di-, mono-saccharides, and

polyols (FODMAPs), fermented by the gut microbiota, improves symptoms in 70% of patients. We hypothesise this fermentation generates glycation agents, which induce the formation of advanced glycation end-products (AGEs). AGE-Receptor for AGEs (RAGE) interaction could activate mucosal mast cells (MMCs). MMC interact with afferent nerves and can induce mucin release from goblet cells (GCs). We used a mouse model to investigate whether an increased intake of dietary FODMAPs is implicated in visceral hypersensitivity and mucus barrier particularities, to study mechanisms of effect of a low-FODMAP diet.

Methods: Mice (C57BL/6♂) were treated with lactose or fructo-oligosaccharides (FOS) as FODMAP representatives for 21 days, +/− pyridoxamine co-treatment. Visceral sensitivity in response to colorectal distension in lactose-treated animals was measured using electromyography. Abdominal sensitivity in FOS-treated animals was evaluated by mechanical stimulations with von Frey filaments. MMC numbers and RAGE expression in colon were evaluated by immunofluorescence. GC numbers in empty colon, and faecal mucus barrier thickness were analysed by classical histology.

Results: Lactose treatment caused visceral hypersensitivity ($P < 0.01$). The FOS-enriched diet induced abdominal hypersensitivity ($P < 0.05$). FODMAP-treatments significantly ($P < 0.01$) increased MMC numbers and RAGE expression. Mucus production indicated by emptying GCs was increased significantly in FODMAP-treated groups ($P < 0.01$) and faecal mucus layers showed significantly ($P < 0.05$) higher Coefficient of Variations in FODMAP-treated groups. Observed effects were prevented by pyridoxamine co-treatment.

Conclusions: Our results show that increased FODMAP intake can expand colonic MMC numbers, increasing visceral and abdominal sensitivity and impacting the normal functioning of the colonic mucus barrier in mice. The prevention of these effects by anti-glycation agent pyridoxamine implies the role of glycation processes and the generation of AGEs in the origin of these effects.

6 | Effects of sildenafil on esophageal peristaltic characteristics and reserve in humans

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Objectives: Sildenafil induced smooth muscle relaxation of the esophagus by blocking type 5 phosphodiesterase that degrades cyclic guanine monophosphate. The aim of our study is to investigate the effect of sildenafil on esophageal peristalsis and function reserve in healthy adults by water perfused HRM.

Methods: Fifteen healthy adults (12 men, age 21-39, mean 27 years) participated in a randomized double blind study on 2 separate days with oral intake of either sildenafil 50 mg or placebo. All subjects underwent HRM with ten water swallows and 5 multiple rapid swallows 1 hour after oral intake of sildenafil or the placebo. The HRM parameters included esophagogastric junction contractile integral (EGJ-CI), resting lower esophageal sphincter (LES) pressure, 4-second integrated relaxation pressure (4-s IRP), distal contractile integral (DCI), resting upper esophageal sphincter pressure (UESP), and the response to MRS.

Results: Sildenafil significantly reduced ECJ-CI ($P < 0.001$), LES pressure ($P < 0.001$), 4-s IRP ($P = 0.002$), and DCI ($P < 0.001$). There was no difference in UESP ($P = 0.87$) between sildenafil and the placebo. Sildenafil induced a significant decrease in peristaltic vigor including absent peristalsis in 12 subjects and ineffective esophageal motility in 3 subjects. Peristaltic response and augmentation following MRS was significantly inhibited with sildenafil (7% vs. 100%, $P < 0.001$, and none vs. 73%, $P < 0.001$).

Conclusions: By using HRM, we have demonstrated that sildenafil inhibits EGJ barrier function, resting LES pressure, and the relaxation of LES. Sildenafil appears to inhibit esophageal contractility as well as peristaltic reserve in healthy adults. Sildenafil induces esophageal hypomotility in healthy volunteers without prior history of esophageal dysmotility.

7 | Inhibition of vitamin D catabolism and its effects on chemotherapy-induced gastrointestinal mucositis

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Background/Objectives: 5-fluorouracil (5FU) is a chemotherapy agent known to cause gastrointestinal mucositis (GM), a side effect of cancer treatment, for which there is currently no effective treatment. Vitamin D has been widely shown to have immunomodulatory and anti-inflammatory effects in the intestine, and therefore may reduce severity of GM. However, vitamin D (and analogues) has an associated risk of hypercalcaemia. The objective was to investigate the effectiveness and safety of novel competitive vitamin D catabolism inhibitor (VDCI) for reducing 5FU-induced GM, without causing hypercalcaemia.

Methods: C57Bl6 mice (n = 36) received a single intraperitoneal injection of 450 mg/kg 5-fluorouracil (5FU) or saline (vehicle control), and subcutaneous 500 ng/kg VDCI or saline (vehicle control) daily for 5 days prior and 2 days following 5FU administration. Mice were then euthanized at 48 hours following 5FU administration. Routine H&E, and transient receptor potential cation channel subfamily v member 6 (TRPV6) RT-PCR/immunohistochemistry was carried out for duodenum and colon sections. NDP.view 2 was used to quantify villi and crypt parameters. Serum levels of calcium and phosphate were measured via KONE analysis. One-way ANOVA, with Tukey's test, was used for statistical analysis.

Results: Serum calcium and phosphate levels were not altered by VDCI alone, or VDCI combined with 5FU. 5FU significantly reduced villous height (VH, $P = 0.0002$) and villous area (VA, $P = 0.0006$) in the duodenum, compared to saline. 5FU + VDCI significantly increased

VH compared to 5FU (P = 0.043), and was not significantly different to saline. TRPV6 is vitamin D dependent. 5FU treatment decreases TRPV6 RNA expression, while VDCI/5-FU doesn't upregulate TRPV6 in duodenum, suggesting a mechanism for a lack of hypercalcemia.

Conclusions: Inhibition of vitamin D catabolism alleviates intestinal damage in 5FU-treated mice. VDCI is a promising new anti-mucotoxic agent that does not cause hypercalcemia.

8 | Treatment response of high dose and standard dose rabeprazole for gastroesophageal reflux disease with extraesophageal manifestations: A single center, randomized, open-label trial

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Background/Aim: The extraesophageal manifestations (atypical symptoms) of gastroesophageal reflux disease (GERD) are more difficult to manage than the typical symptoms. A comparison of the efficacy of high dose and standard dose rabeprazole on the atypical symptoms is not well known.

Methods: This single center, randomized, open-label study included patients with GERD who were randomly assigned to 8 weeks treatment with rabeprazole 20 mg once daily (standard dose group) or 20 mg twice daily (high dose group). Patients were assessed before treatment and at weeks 4 and 8 with a 5-graded scale questionnaire consisting of 2 typical symptoms (heartburn and acid regurgitation) and 8 atypical symptoms (chest pain, cough, globus, wheezing, laryngopharyngitis, hoarseness, belching and dysphagia). Sufficient improvement of reflux symptom was defined as ≥50% reduction from the initial questionnaire score.

Results: Final analyses included 35 patients in the standard dose group and 38 patients in the high dose group. The rate of sufficient improvement for typical symptoms was significantly higher in the high dose group than in the standard dose group (100.0% vs. 84.0%, P = 0.040). For atypical symptoms, the rate of sufficient improvement tended to be higher in the high dose group than in the standard dose group (82.4% vs. 63.0%, P = 0.087). Scores of typical and some atypical symptoms (cough and globus) were improved after treatment, with significant inter-group differences in time-course changes.

Conclusion: High dose rabeprazole may be more effective for atypical symptoms as well as typical GERD symptoms than standard dose regimen.

Keywords: Rabeprazole; Proton pump inhibitor; Gastroesophageal reflux disease; Extraesophageal manifestation

9 | Fecal continence for solid and liquid stool: The function of the anal-external sphincter continence reflex and the puborectal continence reflex

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Background/Objective: The anal-external sphincter continence reflex (AESCR) and the puborectal continence reflex control fecal continence by involuntary contractions of the external anal sphincter and puborectal muscle, respectively. The effect of liquid stool on these reflexes remains unknown. The aim of this study was to investigate the effect of liquid stool on the presence of these fecal continence reflexes.

Methods: We performed anorectal function tests using high resolution manometry in 42 healthy subjects. We measured pressures at the level of the external anal sphincter and the puborectal muscle for all the tests. We used the anorectal pressure test to measure involuntary contractions. We used the balloon retention test to measure involuntary contractions, where the rectal balloon mimicked solid stool. We used the rectal infusion test to investigate the effect of liquid stool.

Results: While mimicking solid stool, the pressure at the level of the external anal sphincter increased from start to end (132 ± 54 mm Hg vs. 198 ± 69 mm Hg, P < 0.001, Figure 1A). The pressure at the level

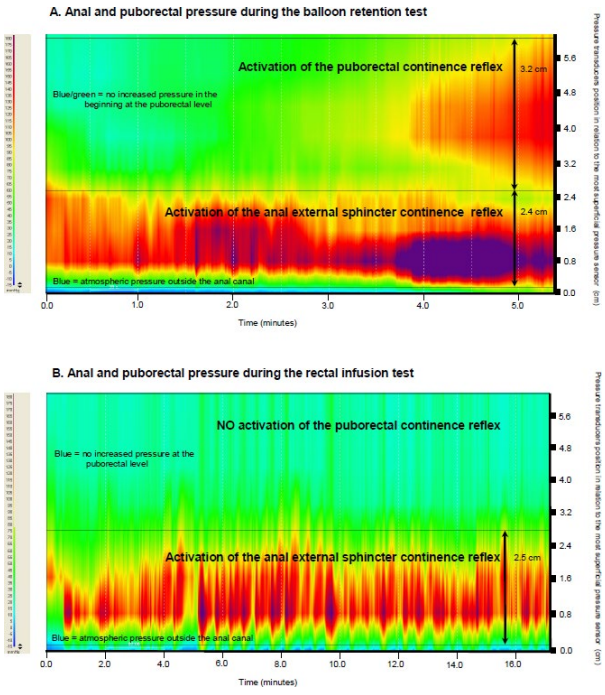

Figure 1. Activation of A) both fecal continence reflexes in presence of solid stool, and B) only the AESCR, when injecting water into the rectum

of the puborectal muscle increased simultaneously (30 ± 9 mm Hg vs. 176 ± 52 mm Hg, $P < 0.001$).

After injecting water into the rectum, we observed rapid activation of the AESCR (87 ± 32 mm Hg vs. 145 ± 36 mm Hg, $P < 0.001$, Figure 1B), while no activation of the puborectal continence reflex appeared (26 ± 9 mm Hg vs. 26 ± 7 mm Hg, $P = 0.655$).

Conclusion: The anal-external sphincter continence reflex controls fecal continence of both solid and liquid stool. Contrarily, the puborectal continence reflex contributes to solid stool continence only.

10 | Fecal continence outcomes are associated with the type, height and stage procedure of the ileal pouch-anal anastomosis

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Background/Objective: Stapled pouch-rectal cuff anastomosis is claimed to give better outcomes than pouch-dentate line hand-sewn anastomosis in ileal pouch-anal anastomosis (IPAA). The influence of the height of the anastomosis remains unclear. Considering the stage procedure, no consensus exists on which of the possible stage procedures gives the best fecal continence outcomes. We aimed to determine whether the type and height of IPAA influences fecal continence outcomes and which of the possible stage procedure gives the best fecal continence results.

Methods: Cross-sectional, retrospective study in patients who had undergone IPAA surgery at our hospital between 1992 and 2016 (n = 134). We sent questionnaires to 102 eligible patients with a 64% response rate. We used the Wexner score to assess fecal incontinence: 0 = no incontinence to 20 = complete incontinence.

Results: Patients who underwent mucosectomy with hand-sewn anastomoses (n = 11, 17%) had significantly higher median Wexner scores than patients with stapled anastomoses (10 vs. 3, $P = 0.003$, Figure 1A). Lower anastomoses correlated significantly with increasing Wexner scores ($r = -0.468$, $P = 0.001$, Figure 1B). Considering the stage procedures, multiple linear regression showed that the two-stage procedure without diverting ileostomy was significantly associated with higher Wexner scores ($B = 0.815$, $P = 0.02$), adjusted for sex ($P = 0.008$) and anastomosis type ($P = 0.002$). The three-stage procedure showed equally low complications and anastomotic leakage rates.

Conclusion: Mucosectomy with more distal, hand-sewn anastomosis during IPAA surgery was associated with poorer fecal continence outcomes and the three-stage procedure appears to give the best fecal continence results without increasing complications.

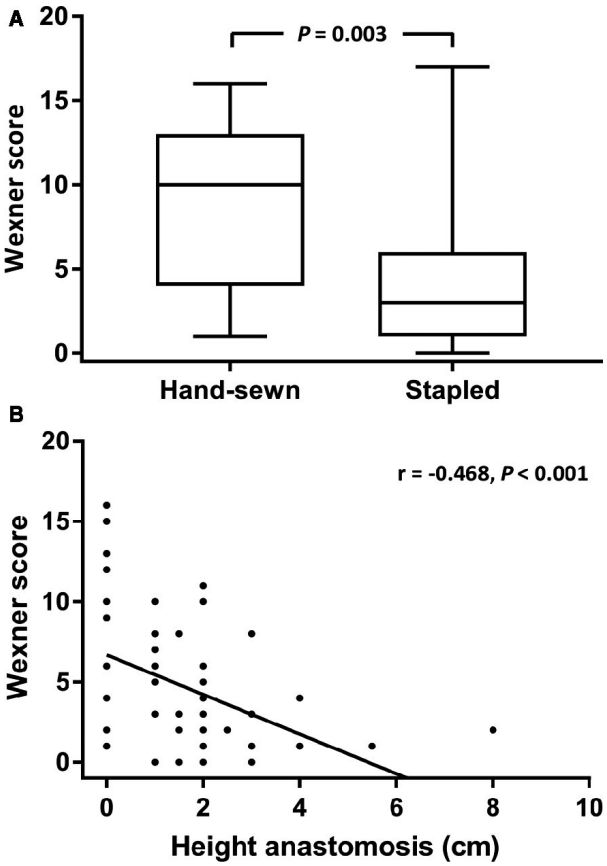

Figure 1. Association between the type, hand-sewn or stapled (A), and height in centimeters (B) of ileal pouch-anal anastomosis and severity of fecal incontinence

11 | Sigmoid volvulus in Parkinson's disease, with severe hypokalemia and hypomagnesemia causing cardiac dysrhythmia: A case report

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Parkinson's Disease (PD) is a neurodegenerative disorder presenting with classic movement impairment and gastrointestinal dysfunction, including salivary excess, dysphagia, constipation, asymptomatic megacolon, and rarely, intestinal obstruction. In the Philippines, there are no available data in the prevalence of PD, particularly those associated with gastrointestinal complications such as sigmoid volvulus. We report a case of a 64 year old Filipino female with advanced PD for 15 years, on Carbidopa/Levodopa, with a history of sigmoid volvulus that was managed conservatively, who had 2 weeks history of progressive generalized body weakness, non-tender abdominal distention, and passage of loose watery stool (≥200 mL/bout) twice per day. She had normal vital signs, with soft grossly distended tympanitic abdomen, with diffused high-pitched bowel sounds. Abdominal radiography showed a gas-filled coffee-bean-shaped colon, suggestive of sigmoid volvulus. Blood tests showed severe hypokalemia (1.43 mmol/L), and eventually, hypomagnesemia (0.61 mmol/L),

manifested on ECG as sinus bradycardia with prolonged PR and QT interval, and wide QRS, not associated with any cardiac symptoms. The patient was advised for immediate surgical intervention, but was eventually deferred due to persistence of loose bowel movement, radiographic evidence of interval decrease in bowel dilatation, and absence of signs and symptoms of complete bowel obstruction. The previously noted cardiac dysrhythmia was resolved upon correction of serum magnesium (Mg) and potassium (K). Echocardiography showed grade 1 diastolic dysfunction with unremarkable anatomical findings. Colonoscopy revealed elongated and redundant colon, with absence of bowel obstruction, suggesting spontaneous resolution of sigmoid volvulus. She was discharged after careful observation for recurrence of sigmoid volvulus, electrolyte imbalance, and cardiac dysrhythmia. She was advised for elective sigmoid resection; however, her relatives refused any surgical intervention. This report highlights the importance of early recognition and management of gastrointestinal dysfunction among patients with Parkinson's Disease, particularly sigmoid volvulus, to prevent its further complications.

13 | Duodenal eosinophilia and functional GI disorders: Is there any correlation?

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Background: Functional dyspepsia (FD) is characterized by pain or discomfort centred in the upper abdomen. Eosinophilic duodenitis can masquerade symptoms of FD but responds well to appropriate treatment. Proper diagnosis is key for accurate management. However there is paucity of data from India. We determined prevalence, clinical profile, symptom correlation of adults with eosinophilic duodenitis in this single centre study from India.
Methods: We retrospectively assessed data of patients who underwent duodenal biopsies for functional GI symptoms from 2019 January to September 2019. Patients with secondary eosinophilic duodenitis (IBD, drug induced dyspepsia, malignancy and celiac disease were excluded. Diagnostic criteria for duodenal eosinophilia were 20 eosinophils per high power field. Patient-reported symptoms and laboratory parameters were noted from chart review.
Results: Out of 362, 194 (53.6%) males aged 44.1 ± 16.5 years and 168 females aged 47.2 ± 16.1 years were identified. Patients with eosinophil count ≤20 were 270 (74.6%) and 99 (27.3%) patients had significant eosinophilia. Abdominal pain 77 (21.3%) was most common presenting symptom followed by reflux 22 (6%) and vomiting 13 (3.6%). Only one patient presented with weight loss. None of the symptoms were significantly associated with eosinophil counts. However cryptitis and crypt abscess was significantly associated with Eosinophil count >20, wherein 70.6% (12/17) of the patients with eosinophil count >20 were associated with crypt abscess (OR = 7.11, with 95% CI 2.48-20.8, P = 0.0003) as compared to cases with eosinophil count <20 (5/17). There was no significant difference in the distribution of location (LP, MM or LP) between cases with Number of eosinophils (NOE) ≤20 and >20 wherein 43.8% cases with >20 NOE were associated with LP, MM as compared to 26.6% with LP, However, the association did not reach statistical significance. Out of 362 patients 249 were treated with budesonide out of which 78.8% (78/99) were with eosinophil count >20.
Conclusion: Duodenal eosinophilia is found in almost one-fourth of patients with unexplained dyspepsia, can be effectively treated with budesonide.

15 | Sigmoid volvulus in Parkinson's disease: A systematic review of case reports

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Background/Aims: Parkinson's Disease (PD) is a neurodegenerative disorder associated with gastrointestinal dysfunction, including intestinal obstruction caused by Sigmoid Volvulus. Several cases have been reported, but its specific incidence is unknown and it still remains to be under-recognized. This review aims to systematically summarize the clinical characteristics and course of disease of PD patients reported to have Sigmoid Volvulus (SV).
Methods: A systematic literature search of all reported cases of Sigmoid Volvulus in Parkinson's disease patients was also done. Patients' characteristics and disease course were extracted, including clinical presentation, diagnostic findings, intervention, outcome, and descriptive analysis was done. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guided the methodological conduct and reporting.
Results: Eight publications from 1965 to 2018 met the selection criteria, with a total of 19 cases. Based on the Newcastle Ottawa scale, two studies were appraised as high and six as moderate methodological quality primarily due to incomplete data reported. The median age was 73 (59-87) years and, male to female ratio was 5:1. The primary symptoms upon consultation were abdominal distention (n = 12), abdominal pain (n = 10), and constipation (n = 10). The radiographic findings were suggestive of sigmoid volvulus. Eleven (69%) cases underwent successful endoscopic detorsion and decompression, but 7 among these cases eventually had recurrence of sigmoid volvulus within 6 months. Five (31%) cases underwent colectomy with colostomy, one of which initially had successful endoscopic detorsion, and eventually underwent surgical intervention. Overall, 77% (n = 10) of patients who underwent either endoscopic decompression or surgical intervention had recurrence of sigmoid volvulus.
Conclusions: Parkinson's Disease patients have increased risk to develop recurrent sigmoid volvulus, even after endoscopic decompression or surgical intervention. Early recognition and management is important to prevent its further complications.

Keywords: Parkinson's Disease, Sigmoid Volvulus, Systematic Review, Case Reports

16 | Sacral nerve stimulation inhibits the MAPK/NF-κB signaling pathway and promotes Treg-Th1/Th17 cell balance and self-renewal of enteric nervous system in TNBS-induced inflammation in rats

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Introduction: 2,4,6-Trinitrobenzene sulfonic acid (TNBS) is known to induce inflammation through triggering the MAPK/NF-κB pathway and activation of T helper cells. Recently, sacral nerve stimulation (SNS) was reported to exert an anti-inflammatory effect on TNBS-induced colitis. The aim of this study was to investigate whether the SNS anti-inflammatory effect was mediated via MAPK/NF-κB signaling pathway and/or balancing Th1/17-Treg cells. Meanwhile, we also explored if SNS could alter self-renewal of neurons in myenteric plexus.

Methods: Forty male Sprague-Dawley (SD) rats were implanted wire electrodes unilaterally at sacral nerve. One week later, the rats were administrated with TNBS intra-rectally. Five days later, 20 of the rats were treated with SNS 1 hour daily for 10 days with the optimized parameters derived from previous studies and the other 20 rats were treated with sham-SNS (exactly the same setting but SNS at 0 mA). Additional 20 rats were treated with intra-rectal injection of saline, serving as controls. Animal behaviors and various inflammatory factors were assessed by the disease activity index (DAI), macroscopic score, microscopic score, fluorescence-activated cell sorter and western blot. Longitudinal muscle myenteric plexus (LMMP) was studied by immunohistochemistry.

Results: (1) Compared with saline group, the TNBS treatment substantially induced inflammation, increased the percentage of Th1 cells ($P = 0.03$), Th17 cells ($P = 0.02$) and Treg cells ($P = 0.04$); it also increased p-ERK/ERK by 3.3 folds ($P < 0.01$) and p-JNK/JNK by 21.7 folds ($P < 0.001$) and increased nuclear translation of NF-κB p65 by 4.5 folds ($P < 0.01$); (2) compared to sham-SNS, SNS significantly decreased DAI (area under the curve: 64.3 ± 3.8 vs. 49.5 ± 3.2, $P < 0.01$), macroscopic scores (5.85 ± 0.9 vs. 2.55 ± 0.6, $P = 0.03$) and microscopic scores (4.6 ± 1.1 vs. 2.7 ± 0.8, $P = 0.04$) and normalized the colon length; (3) in colon tissues, compared with sham-SNS, SNS reduced the percentage of Th1 cells (8.87 ± 2.32% to 5.40 ± 1.39%, $P = 0.04$) and Th17 cells (12.35 ± 1.61% to 9.75 ± 1.17%, $P = 0.04$) but increased Treg cells (15.73 ± 2.81% to 20.15 ± 2.24%, $P = 0.03$); (4) SNS reduced the percentage of the phosphorylation of MAPKs compared to Sham-SNS (p-ERK/ERK: 22.5%, $P = 0.03$; p-JNK/JNK: 25.6%, $P = 0.04$) and prevented nuclear translocation of NF-κB p65 by 40.7% ($P = 0.02$, vs. sham-SNS); (5) the percentage of choline acetyltransferase (ChAT) neurons were decreased by TNBS but reversed by SNS (19.06 ± 2.07% to 25.68 ± 3.56%, $P = 0.02$). The percentage of nitric oxide synthase (NOS) neurons was increased by TNBS but decreased by SNS (17.21 ± 1.27% to 13.34 ± 1.63%, $P = 0.03$).

Conclusions: SNS is effective in inhibiting colon inflammation through the inhibition of the MAPK/NF-κB pathway, balancing of Th1/Th17-Treg cells, and also improving the LMMP neuronal self-renewal.

Keywords: Inflammatory bowel disease; sacral nerve stimulation; inflammatory cytokines; MAPK/NF-κB pathway; balancing of Th1/Th17-Treg cells; longitudinal muscle myenteric plexus; neuronal self-renewal

17 | Cannabinoid hyperemesis syndrome: Discovery, pathophysiology and innovative research

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Background and Objectives: Cannabinoid Hyperemesis Syndrome [CHS] [1][2] is characterized by a triad of chronic cannabis abuse, cyclical vomiting every few weeks or months and compulsive bathing behaviour. It occurs with both propagated and synthetic cannabis. Cessation of cannabis is curative. We will discuss our discovery of this disease in Australia, our first clinical trial, and its seminal publication in GUT in 2004 [2].

Methods: We discuss the paradoxical nature of the over 60 active cannabinoids in propagated Marijuana and the "Popcorn Effect". Compulsive bathing behaviour is analyzed. We examine the proposed Hypothalamic – Pituitary pathway due to cerebral binding and lipophilic nature of cannabinoids. We explore the effect of topical of capsicum, modulated by the CGRP antagonists and the TRPV1 vanilloid receptors, in the abatement of acute attacks of hyperemesis.

Results: Our current focus is to scrutinizing the synthetic cannabinoids JWH-018 and JWH-073, and their reproduction of the stereotypical attacks of CHS [3][4][5], as well as their potential role in locating the protein binding sites and neuromodulation of CHS. We are concurrently investigating how closely CHS correlates with similar metabolic illnesses with cyclical vomiting components, specifically Acute Intermittent Porphyria and Hyperemesis Gravidarum.

Conclusions: Structural analysis reflects that THC, JWH-018, JWH-073. HCG and Porphobilinogen are all small molecules that are architecturally very similar. They are all highly permeable across membranes. When suitable conditions arise, their levels rise, and they can be detected in the urine. Toxicity leads to stereotypical bouts of illness, potentially all modulated through the same receptor sites.

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18 | Distant effects on the colon following 6-hydroxydopamine lesioning of the medial forebrain bundle

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Background: Gastrointestinal (GI) dysfunction, including constipation, is a common non-motor symptom of Parkinson's disease (PD) that affects ~80% of patients. Defecation is under voluntary control, beginning from centres in the brain, sending signals down the spinal cord to the defecation centres. However, the mechanisms involved in PD-associated constipation remain unknown. The toxin 6-hydroxydopamine (6OHDA) is used to model PD and surprisingly causes constipation when injected into the medial forebrain bundle (MFB). We investigated the downstream effects of 6OHDA lesioning of the MFB.
Methods: Male Sprague-Dawley rats were unilaterally injected with 6OHDA in the MFB. Amphetamine rotation testing was performed 2 weeks later and motor function was assessed using the ledged beam test. Colon motility was assessed using the bead expulsion test and quantified by the stimulation of the spinal lumbo-sacral defecation centres with a ghrelin receptor agonist. Functional deficits in colon motility were also correlated to enteric nervous system (ENS) neuropathy.
Results: 6OHDA-lesioned rats rotated significantly more when compared to sham animals indicating damage to dopaminergic neurons. As expected, 6OHDA rats developed motor dysfunction. These rats also developed deficits in colon motility, shown by a significantly increased bead expulsion time ($P < 0.05$) compared with shams. Administration of capromorelin increased the number of contractions and colorectal propulsion in both groups compared to baseline, however, the number of contractions and propulsion of contents by 6OHDA rats was significantly reduced in comparison to shams ($P < 0.05$), indicating that 6OHDA animals have reduced responsiveness of the defecation circuits. Interestingly, enteric neuropathy was observed in the distal colon, indicating lesion of the MFB has downstream effects at the cellular level, remote from the site of 6-OHDA administration.
Conclusions: The results indicate that there are likely trans-synaptic effects in pathways from the brain that send signals down the spinal cord to the defecation centers and then to the colorectum.

20 | Direct activation of tuft cells by transient receptor potential subfamily M member 5 (TRPM5) agonists increases colonic motility and secretion through induction of a Th2 cytokine response and mobilization of non-neuronal acetylcholine

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Transient receptor potential subfamily M member 5 (TRPM5) is a Ca^{2+}-activated, monovalent cation channel selectively expressed on chemosensory tuft cells within the gastrointestinal tract. Expression of TRPM5 in tuft cells significantly contributes to the expulsion of parasitic helminths, a process driven by a Th2 cytokine response involving IL-25 and IL-13. Recent evidence, including our own data, has also detailed the co-expression of TRPM5 and cholinergic acetyltransferase (ChAT) in tuft cells, implicating them as potential sources of non-neuronal acetylcholine (ACh). We asked whether direct activation of TRPM5 could induce tuft cells to initiate Th2 immune responses and the non-neuronal release of ACh. To achieve this, we generated potent and selective small molecule activators of TRPM5 that are orally available and pharmacokinetically suitable for in vivo study. Treatment of TRPM5 agonists in mice by oral gavage led to rapid, dose-dependent increase in IL-5 and IL-4 cytokine protein levels both systemically and locally in colonic tissue, as measured by the Meso Scale Discovery (MSD) electrochemiluminescence platform. Increased gene expression of the Th2 cytokines IL-25, IL-13, IL-4, and IL-10 was also detected in colonic tissue by qRT-PCR. The compounds had no effect in TRPM5 knockout mice. In addition, TRPM5 agonists elicited a dose-dependent increase in fecal pellet output (FPO) with elevated water content in the feces suggesting the activation of secretion which is a key component of mechanism of parasite expulsion. The stimulation of secretion was further confirmed by Ussing Chamber experiments on mucosal epithelium which demonstrated a rapid and dose dependent activation of short circuit currents (I_{sc}) with compound addition. Finally, TRPM5 agonists were applied to Caco-2 cell lines overexpressing TRPM5, resulting in a concentration dependent release of ACh as measured by liquid chromatography-mass spectrometry (LC-MS). Taken together, these results show for the first time that direct agonism of TRPM5 activates tuft cells, leading to increased GI motility, secretion, and a Th2 immune response in vivo. The underlying drivers of IL-25 and non-neuronal ACh shed new light into the mechanisms of parasite expulsion and may also provide new insights into chronic inflammatory and motility disorders.

21 | Swallowing function evaluation using high-resolution impedance manometry in patients undergoing anterior cervical discectomy and fusion: A case series study

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Background: Postoperative dysphagia is the most common complication after anterior cervical discectomy and fusion (ACDF). However, methods used in relevant studies, including questionnaire and videofluoroscopic swallowing studies, have not been able to identify the muscles affected during surgery. Some structures along the pharyngeal region have attracted attention, including the recurrent laryngeal nerve at C5 to T1 level or the cricopharyngeus muscle at C5 to C6. High-resolution impedance manometry (HRIM) can assist in identifying the affected muscle group. This prospective case series study hypothesized that pharyngeal pressure decreases after ACDF and identified the effects of ACDF on swallowing ability through HRIM.

Methods: Eight patients receiving elective ACDF were enrolled, and a dysphagia short questionnaire was administered to them. All patients received a swallowing function test through HRIM before their surgery on postoperative days (PODs) 1 and 7. Furthermore, pressure flow analysis was performed using HRIM to calculate pharyngeal parameters.

Results: The highest surgical levels among the eight patients were as follows: two patients had C3/4, two had C4/5, three had C5/6, and one had C6/7. All patients except one had postoperative dysphagia symptoms noted on POD 1. Mean pharyngeal peak pressures were significantly reduced at POD 1 (66.06 mm Hg) compared with before surgery (95.57 mm Hg) and POD 7 (90.29 mm Hg). The upper esophageal sphincter (UES) peak and basal pressures exhibited no significant differences between the three time points. Moreover, no significant differences were found in the UES integrated relaxation pressure and bolus presence time.

Conclusions: After ACDF, the patients exhibited significantly reduced mean pharyngeal pressure on POD 1 but levels before surgery and on POD 7 were comparable. HRIM provided objective data for identifying muscles related to swallowing dysfunction after ACDF.

22 | Age distribution and pathophysiological analysis of chronic constipation (CC) in JAPAN-from infants to elderly people

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Background: Overall prevalence of chronic constipation (CC) is approximately 20% in Japan and recent studies have demonstrated that 25% of children with functional constipation (FC) continued to experience symptoms at adult age. Our hospital has opened a CC and IBS outpatient clinic for all ages from 0 to 98 years old since 2011.

There is an overlap between Irritable Bowel Syndrome-Constipation (IBS-C) and FC as defined by Rome IV. The Appendices Committee of the Japanese Gastroenterology Society published guidelines for the clinical practice of CC and defined CC as FC not excluding IBS-C. CC is composed of anorectal disorders (defecation disorder (DD)) and bowel disorders. The main treatment for DD is physical therapy, whereas the main treatment for bowel disorders is medication.

Knowing the mechanism of CC (DD or bowel disorders) and the age distribution is very important in choosing effective treatment option and early treatment from younger age.

Objectives and Study: 415 CC patients (age 0-98, child 128 (male 63, female 65), adult 287 (male 69, female 218), 2017 Jan-Dec) were recruited. Defecation disorder (DD) was estimated from the rectal stool with X-ray with or without decreased rectal sensation.

Results: The patient's age distribution (total 38.6 + 27.8, male 35.2 + 32.8, female 40.7 + 25.0, $P < 0.05$) is trimodal (age 3, 50 and 80) and onset age is younger in female (male 25.3 + 22.8, female 18.6 + 17.3 $P < 0.05$). In children (<16 y.o.), there is no gender difference of onset age (male 2.8 + 3.8, female 2.2 + 3.2, n.s.). In adult, onset age is younger in female (male 42.3 + 25.8, female 23.5 + 16.8, $P < 0.005$), and 20.2% of male and 42.2% of female are childhood onset.

In 77.5% of child and 23.0% of adult, the existence of DD was inferred from X-ray and interview.

Onset age of adult DD (male 41.4 + 32.9, female 33.9 + 19.8) was older than that of the whole.

Conclusion: There was no gender difference in onset age of childhood CC. Adult CC, especially women had onset in younger age. DD was frequent in child CC, not in adult CC. Onset age of adult DD was older than that of the whole. Evaluation of pathophysiology of CC contributes to effective treatment from younger age.

24 | Distribution of TRPV1-immunoreactivity in the esophagus and dorsal root ganglia of rats

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Background/Aim: The development of gastroesophageal reflux disease leads to the activation of acid-sensitive TRPV1-sensory nerves in the esophagus. However, the distributions of TRPV1-sensory nerves in the esophagus and their origin, TRPV1-sensory neurons in the dorsal root ganglia (DRG), are not well known. Therefore, we examined the distribution of TRPV1-sensory nerves throughout the esophagus and the frequency of appearance of TRPV1-sensory neurons projecting to the esophagus from the DRG.

Materials & Methods: Wholemount preparations of the tunica mucosa and the outer muscle layer with the myenteric plexus of the rat esophagus were prepared for TRPV1 immunohistochemistry combined with SP, CGRP, or ASIC3 following fixation. In addition, after a fluorescent tracer, Fast Blue (FB), was injected into the abdominal esophagus, cryostat sections of the fixed thoracic (T) and lumbar (L) DRG were made and similarly processed for immunohistochemistry.
Results & Discussion: (1) Most (>90%) of the TRPV1-positive nerve fibers in both the mucosa and myenteric plexus were positive for SP or CGRP, and they were significantly more frequent in the cervical and abdominal esophagus than in other esophageal portions. This suggests that the cervical and abdominal segments of the esophagus are more sensitive to acid than the other portions. (2) FB-labeled sensory neurons were found in DRG from T1 to L3, with the highest number at the T9 level; approximately 70% of them were TRPV1 positive, implying that sensitivity to acid in the abdominal esophagus may be predominant at the lower levels of the thoracic DRG. (3) In the DRG, TRPV1-sensory neurons projecting to the abdominal esophagus showed different rates of co-expression of SP (52%), CGRP (59%) and ASIC3 (24%), suggesting that the abdominal esophagus is innervated by such different types of acid-sensitive TRPV1 neurons in the DRG, which may transmit distinct signals depending on the noxious stimuli, including acid.

25 | Sex differences in colonic ion secretion in response to stress hormones

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Background and objectives: Biological sex plays an important role in irritable bowel syndrome (IBS) subtypes. Constipation-predominant IBS (IBS-C) is more prevalent in women, whereas diarrhea-predominant IBS (IBS-D) is more common in men. Stress increases intestinal secretion and exacerbates symptoms of IBS. The majority of previous preclinical studies investigating effects of stress on gut function were performed in male animals. The present study aimed to determine: (1) if baseline colonic ion secretion differed between males and females; and (2) effect of stress hormones on colonic ion secretion in males and females.
Methods: Adult wild-type and $CRF_2^{-/-}$ mice of both sexes were used. Mucosa/submucosa preparations from the proximal colon were mounted in Ussing flux chambers for measurement of short-circuit current (I_{sc}) as an indicator of intestinal ion secretion.
Results: Proximal colons from male mice demonstrated a significantly higher (1.23 folds) baseline I_{sc} than those from female mice. Treatment with 1 μmol/L of stress hormone corticotrophin releasing factor (CRF), urocortin 1 (UCN1), UCN2, or UCN3 caused a greater increase in colonic I_{sc} (ΔI_{sc}) in males than females. Colonic I_{sc} response to the selective CRF_1 agonist, stressin I (1 μmol/L), was similar in male and female mice. In male mice, the selective CRF_2 agonists, UCN2 and UCN3, caused significantly greater ΔI_{sc} than CRF and stressin I at each concentration tested. We found that UCN2 is the most potent whereas UCN3 is the most efficacious stress hormone in stimulating intestinal ion secretion. Importantly, UCN2- and UCN3-evoked ΔI_{SC} responses were significantly reduced in colonic preparations pretreated with antisauvagine-30, a selective CRF_2 antagonist, and in the $CRF_2^{-/-}$ mice.
Conclusions: Male mice demonstrated a significantly higher baseline colonic ion secretion than females. CRF_2 receptors play a predominant role in the regulation of colonic ion secretion in male mice. The greater baseline ion secretion and responses to stress hormones that are also secreted from the gut may contribute to the higher prevalence of IBS-D in males.

26 | Efficacy of *Dolichos lablab* L. on irritable bowel syndrome: Open-label prospective pilot trial

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Objectives: Pathophysiology of irritable bowel syndrome (IBS) is poorly understood and management of IBS remains a challenge. *Dolichos lablab* L. (DL), a bean species, has traditionally been used for treating gastrointestinal disorders in China and Korea. However, no studies have investigated its use for treating IBS. The aim of this study is to examine the efficacy of DL on the symptom relief of IBS.
Methods: Twenty adult patients with IBS were enrolled. Eligible patients satisfied Rome IV criteria for diagnosis of IBS. After a 2 week observation period, all participants received DL extract capsules for 8 weeks. Primary endpoint was the mean change of abdominal pain from baseline assessed by visual analogue scales (VAS) score for 8 weeks of treatment. Secondary endpoints were the changes in abdominal pain from baseline as week 4, patient-reported symptom improvement including stool frequency and consistency, and IBS-quality of life (IBS-QoL) at week 8.
Results: The VAS scores of abdominal pain at week 8 were significantly decreased ($P < 0.0001$). Overall symptomatic improvement was observed in 13 (65%) participants at week 4 and 17 (85%) participants at week 8. Compared to baseline, the participant's IBS-QoL and frequency of defecation were significantly lower at 8 weeks after the administration of DL. Adverse events were observed in 2 participants and no drug-related severe adverse event was observed.
Conclusions: Treatment with DL was associated with relief of abdominal pain, overall symptomatic improvement and decreased frequency of defecation. The results showed the potential therapeutic application of DL in the treatment of IBS. Future studies with greater statistical power are needed to clarify the possible effects of DL in the treatment of IBS patients.

27 | Step-wise algorithm of pharmacologic, dietary, and psychology treatments for irritable bowel syndrome with diarrhea: A value-based framework using budget impact and cost-effectiveness analysis

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Background: Irritable bowel syndrome (IBS) is one of the most common and expensive gastrointestinal disorders. We developed a value-based treatment model and step-wise treatment algorithm for IBS with diarrhea (IBS-D).

Methods: A decision analytic model was constructed to compare costs and health outcomes measured using quality-adjusted life years (QALY) with guideline-recommended or Food and Drug Administration-approved drugs, over-the-counter supplements, low FODMAP strategy, and cognitive behavioral therapy (CBT) from payer and patient perspectives. Model inputs were derived from systematic reviews of clinical trials and other US national datasets.

Results: The cost of untreated IBS-D was $6844.87 from a societal perspective, $1039.87 from a payer perspective, and $5805.00 from a patient perspective per year. The QALY-gained/year with untreated IBS was 0.73. To minimize budget impact to payers, tricyclic agents (TCA) were the least expensive therapies, followed sequentially by low FODMAP or CBT, rifaximin, and eluxadoline (Table 1). To maximize cost-effectiveness to payers, low FODMAP or CBT were first-line followed sequentially by TCA, rifaximin, and eluxadoline. Both rifaximin and eluxadoline had incremental cost effectiveness ratios exceeding $1 million/quality-adjusted life year gained compared to no treatment. From a patient perspective, eluxadoline was the least expensive IBS-D treatment strategy due to costs of food with low FODMAP and repeated appointments with CBT. Costs and prices were the most important factor determining payer treatment preferences. Impact of treatment on missed work-days and need for repeated appointments to complete treatment were the most important determinants of treatment preference to patients. Peppermint oil and fiber were preferred from a patient perspective, among treatments with weak supporting evidence.

Conclusions: We constructed a decision analytic model to optimize treatment choice for IBS-D. Low FODMAP and CBT are the most cost-effective IBS-D treatments to payers. Factors influencing treatment preference are dissimilar between patients and payers.

Table 1 Step-wise IBS-D management algorithms from a payer perspective

Algorithm to minimize budget impact	Algorithm to maximize cost-effectiveness
TCA	Low FODMAP or CBT
↓	↓
Low FODMAP or CBT	TCA
↓	↓
Rifaximin	Rifaximin
↓	↓
Eluxadoline	Eluxadoline

28 | Changes in fecal short-chain fatty acids (SCFAs) following fecal microbiota transplantation (FMT) in patients with irritable bowel disease (IBS)

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Objective: We have recently investigated the efficacy of FMT in IBS, using a single super-donor, in a randomized, double-blind placebo-controlled study. Abnormalities in the fecal SCFAs of IBS patients have been reported and are believed to play a role in the pathophysiology of IBS. In the present study, the fecal SCFAs were analyzed at the baseline and 1 month after FMT in the IBS patients that were included in our previous study.

Methods: A total of 143 IBS patients out of the 164 patients that participated in our previous study were included. They belonged to three groups: placebo group (49 patients), the group that received 30 g fecal transplant (50 patients) and the group that received 60 g fecal transplant (44 patients). The patients completed IBS-SSS, and Fatigue Assessment Scale (FAS) questionnaires at the baseline and 1 month after FMT. They delivered further a fecal sample at the baseline and 1 month after FMT. The concentrations of total SCFAs, acetic, propionic, iso-butyric, butyric, iso-valeric, valeric, iso-capronic, and capronic acids were determined by vacuum distillation followed by gas chromatography.

Results: Fecal levels of butyric acid were significantly increased after FMT in both 30-g and 60-g groups (P =< 0.001, in both). In the 60-g group, the concentrations of total SCFAs, iso-butyric, iso-valeric, and valeric acids increased after FMT. Butyric acid levels correlated significantly with IBS-SSS and FAS scores in 60-g group (P = 0.005, r = −0.3, in both). Furthermore, SCFA concentration correlated with the IBS-SSS score (P = 0.005, r = −0.4). There was no difference in the concentrations of SCFAs in the placebo group after FMT.

Conclusions: FMT increases the concentrations of fecal SCFAs in IBS patients. The increase in butyric acid level is correlated to the improvement of symptoms in IBS patients following FMT. SCFA might play a significant role in the pathophysiology of IBS.

29 | De novo neurogenesis in the post-embryonic zebrafish enteric nervous system from Schwann cell precursors rather than resident cell types

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Background & Aims: The enteric nervous system (ENS) is essential for normal gastrointestinal motility, and defects in its function define several difficult to treat conditions. While the developmental origin of the ENS from neural crest cells is well understood, there is conflicting evidence regarding postnatal enteric neurogenesis and neuronal homeostasis. Using zebrafish as a model due to its simplified ENS which is amenable to live-imaging, we sought to explore the origin of enteric neurons that arise in post-embryonic life in both normal development and upon injury, and tested effects of the 5-HT_4 receptor agonist, prucalopride, in this process.
Methods: To assess enteric neurogenesis, we photoconverted enteric neurons within the intestines of a Phox2b-kaede line such that all resident neurons turned from green to red. We then performed live time-lapse imaging to look for the emergence of new, green-only, neurons. In other experiments, resident enteric neurons were removed using two-photon laser ablation. To follow the potential contribution of neural crest-derived cells to the gut, lineage tracing was performed with neural tube injections of a lipophilic dye as well as with an inducible Sox10-Cre transgenic line. Lastly, post-embryonic zebrafish were exposed to prucalopride to test this drug's effect on enteric neurogenesis both during normal development and after injury.
Results: Our results suggest that the post-embryonic zebrafish intestine lacks resident neurogenic precursors, and indeed appears to have no enteric glia. Despite this, enteric neurogenesis persists post-embryonically both during normal development and after injury. Our data suggest that new enteric neurons arise from trunk neural crest-derived Schwann cell precursors that migrate along nerves to the intestine. Prucalopride increases enteric neurogenesis in normal development as well as after injury if exposure occurs prior to injury.
Conclusions: Enteric neurogenesis persists in the post-embryonic period in both normal development and injury, appears to arise from gut-extrinsic Schwann cell precursors, and is promoted by prucalopride treatment.

30 | Defining sites of hold-up in oral-anal transit using radionuclear gastrointestinal transit studies in children with intractable constipation using a 48-hour protocol

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Background/Objectives: Radionuclide gastro-intestinal transit studies (RN-GITs) follow radioactive tracer from mouth to anus. Studies in adults run for 72-96 hours. In children, to reduce the number of visits and the burden to families, our centre stops RN-GITs at 48 hours.
Objective: To determine if gastric, small bowel, colonic and rectosigmoid transit can be determined from 48-hour data from RN-GITs.
Methods: Reports of RN-GITs performed in children with intractable chronic constipation in a tertiary paediatric hospital 2012-2015 were retrospectively reviewed. Laxatives were stopped for 5 days before, ^{67}Gallium-citrate was ingested (in liquid), gamma camera images were acquired at 0, 0.5, 1, 2, 6, 24, 30 and 48 hours and analysed qualitatively and quantitatively. High-dose laxatives were prescribed after the last image if needed.
Results: 328 diagnostic studies were available and analysed. The transit pattern through the stomach, small bowel and colon was obtained with delayed gastric emptying in 15%, delayed small bowel transit in 25%, normal colonic-transit in 16%, rapid colonic-transit in 34% and slow colonic-transit in 48%. At 48 hours, rectosigmoid transit could not be determined in 45% of patients as not enough tracer had reached the rectosigmoid. The rectosigmoid pattern was obtained in 94% of patients with rapid, 87% with normal but only 13% with slow colonic transit.
Conclusion: Using a 48-hour RN-GITs in children with intractable chronic constipation, we were able to assess gastric, small bowel and colonic motility, but could only assess rectosigmoid function in half. Longer studies are required to assess rectosigmoid emptying in patients with slow colonic transit, but diagnostic decisions can be made based on colonic patterns for most patients.

31 | Altered intestinal permeability and effect of mosapride and glutamine on postoperative ileus in a guinea pig model

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Background/Aims: The alteration of permeability and accumulation of inflammation seems to play an important role in the pathophysiology of POI. This study was performed to characterize the alteration of intestinal permeability regarding the development of POI. Moreover, we investigated whether mosapride and glutamine have

an anti-inflammatory effect and have an effect on the intestinal permeability in the POI model.

Methods: An experimental POI model with guinea pig was created. Fluorescent markers (FITC-dextran) were passed through the harvested intestinal membranes in the Ussing chamber and checked for differences in optical translucency in the ileum and the proximal colon. To characterize the alteration of intestinal permeability, we compared intestinal permeability of control group with POI group which 3 and 6 hours after surgical manipulation in the ileum and the proximal colon. We also compared inflammatory grade represented by leukocyte counts and the expression of calprotectin between control and POI group. To investigate influence of mosapride and glutamine on POI, we compared permeability and inflammatory grade of POI group with mosapride (0.3 and 1 mg/kg, s.c.) and glutamine (500 mg/kg, p.o.) administration group.

Results: The ileum and proximal colon showed increased permeability in the POI group compared with control group. There was a significant increase in the leukocyte counts and calprotectin level in the POI group compared with the control group in the ileum and proximal colon. Elevated leukocyte counts were significantly decreased by mosapride and glutamine in the proximal colon. Elevated calprotectin level was significantly decreased by mosapride and glutamine in the ileum and proximal colon. Increased permeability of POI group was significantly decreased after mosapride and glutamine administration in the ileum and proximal colon.

Conclusions: Increased inflammatory grade and intestinal permeability were observed in the POI guinea pig model. Mosapride and glutamine have an anti-inflammatory effect and modulate altered gut permeability.

Keywords: Postoperative ileus, intestinal permeability, inflammation, mosapride, glutamine

32 | Epigenetic alterations of inflammatory markers in diabetic and idiopathic gastroparesis

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Gastroparesis is described as delayed emptying of gastric contents in the absence of mechanical obstruction that contributes to poor blood glucose control and causes significant morbidity. A number of studies have shown that macrophages are critical in the pathophysiology of gastroparesis whereas not much is known about the epigenetic modifications throughout this disease process. In the current study, we evaluated the inflammatory mediators and epigenetic changes in relation to the alterations in the interstitial cells of Cajal (ICC) in human gastric antral smooth muscle obtained from both diabetic gastroparetic (DG) and non-diabetic idiopathic gastroparetic (ID) patients. Freshly harvested human gastric antral smooth muscle biopsies were obtained from Type 1 and Type 2 diabetic gastroparetic patients, idiopathic gastroparetic patients as well as non-diabetic non-gastroparetic control patients requiring surgical interventions. Samples were processed for protein extraction as well as for the immunohistochemical analysis. Western blot analysis to investigate the inflammatory mediators were conducted using antibodies for interleukin 1 (IL1)-β, toll-like receptor 4 (TLR4). To analyze the histone deacetylases (HDACs)-mediated epigenetic mechanisms, class I HDACs comprising HDAC1, 2, 3 and 8 as well as histone 3 lysine 9 acetylation (H3K9ac), and high-mobility-group A2 (HMGA2) were studied. For immunohistochemical studies, HDAC2, HMGB1 and association of methyl binding protein-2 (MeCP2) with enteric neuronal dysfunction were analyzed. Our results indicate that there is an increase in H3K9ac and a decrease in HDAC2 expression as well as alterations in macrophage marker expression CD163 and CD68 in the smooth muscle of the gastric antrum of DG patients. To further evaluate gastric pathophysiology in both etiologies of gastroparesis, we compared the inflammatory mediators interleukin 1 (IL1)-β, toll-like receptor 4 (TLR4), TLR4 ligand high mobility group box 1 (HMGB1), HMGA2 and examined their association with HDAC2 and H3K9ac. We observed that MeCP2 expressed throughout the GI tract and the levels were proportional to the abundance of neuronal content. This study demonstrated that alterations in epigenetic mechanisms could lead to increased cell signaling and inflammation in the antral muscle in DG compared to ID patients which could result in a loss of ICC, ultimately leading to gastroparetic etiology.

33 | The effect of ilaprazole in patients with non-erosive reflux disease gastro-oesophageal reflux disease

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Objective: Patients with gastroesophageal reflux disease without esophagitis show varying responses to proton pump inhibitors (PPIs). The aim of this study was to objectively evaluate the effect of a new PPI, ilaprazole, on patients with heartburn but without reflux esophagitis.

Methods: This prospective study was performed on 20 patients with heartburn but without reflux esophagitis. All patients underwent upper endoscopy and 24-hr combined multichannel intraluminal impedance and pH esophageal monitoring (MII-pH). They were then treated with ilaprazole (20 mg) once daily for 4 weeks. The GerdQ

questionnaire, histologic findings, and inflammatory biomarkers were used for assessment before and after ilaprazole.

Results: Among the 20 patients, 13 (65%) showed GerdQ score ≥8. Based on MII-pH results, patients were classified as true nonerosive reflux disease (n = 2), hypersensitive esophagus (n = 10), and functional heartburn (n = 8). After treatment, patients showed a statistically significant improvement in GerdQ score ($P < 0.001$). Among histopathologic findings, basal cell hyperplasia, papillary elongation, and infiltration of intraepithelial T lymphocytes improved significantly ($P = 0.008$, $P = 0.021$, and $P = 0.008$; respectively). Expression of TNF-α, IL-8, TRPV1, and MCP-1 decreased marginally after treatment ($P = 0.049$, $P = 0.046$, $P = 0.045$, and $P = 0.042$; respectively).

Conclusions: Daily ilaprazole (20 mg) is efficacious in improving symptom scores, histopathologic findings, and inflammatory biomarkers in patients with heartburn but no reflux esophagitis.

34 | Long non-coding RNA THRIL controls TNF-A related inflammatory process in stomach gastrointestinal manifestation of systemic disease

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Objective: Recently, long non-coding RNAs (lncRNAs) have emerged in association with regulation of the mucosal immunology. We investigated the roles of lncRNA THRIL (TNFα and heterogeneous nuclear ribonucleoprotein L [hnRNPL] related immunoregulatory lncRNA) in TNF-a related inflammation in stomach.

Methods: 1. Cell lines: Human gastric normal epithelial cell line GES-1, and 4 differenent gastric cancer cell lines including MNK28, MKN74, MKN45, AGS and KATOIII 2. Infectious agents and bacteria: Escherichia coli lipopolysaccharides (LPS), H. pylori strains 8822 (cytotoxin-associated gene A [CagA]-) and 60190 (ATCC 49503, CagA+), and ΔCagA (an isogenic mutant of 60190 lacking CagA) 3. Total RNA extraction, reverse transcription and quantitative real-time PCR (qRT-PCR):The expression level THRIL, hnRNPL, NF-kB1 (p50), TNFα and IL-8 was determined by qPCR using iQ SYBR Green Supermix (Applied Biosystems) and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). 4. Enzyme-linked immunosorbent assay (ELISA), Western blot for secreted TNFα, IL-8 5. Knock-down of THRIL by two different siRNAs and shRNA 6. Immunofluorescence and Western blot for NFkB cascade including NF-κB p65, IkBα,phospho-IkBα, IKKα, IKKß, and phospho-IKKα/ß.

Results: The amount of THRIL expression in the GES-1 cells was relatively smaller than that in the gastric cancer cell lines. siRNAs and shRNA for THRIL not only significantly reduced TNFα basal excretion but also atteuated TNFα secretion by H. pylori 60190 and ΔCagA strains. NF-κB1 (p50) mRNA level in the GES-1 cells was upregulated by H. pylori 60190 infection, however shRNA for THRIL block induction of NF-κB1. The phosphorylation of IKKα/ß was reduced by shRNA for THRIL in contrast to that with control. Finally knockdown of THRIL attenuated NF-κB nuclear translocation by H. pylori 60190.

Conclusions: We found that THRIL modulates H. pylori-induced inflammation in stomach.This finding also implies the importance of lncRNA as a regulatory factor involved in the pathogenesis of inflammations or infectious diseases in humans stomach.

35 | Psychosocial aspects of functional gastrointestinal disorders: Experiences of multidisciplinary medical-psychiatric outpatient care

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Objectives: This study is aimed to compare the psychosocial characteristics among patients with functional gastrointestinal disorders (FGID), adults with functional gastrointestinal symptoms and normal people, and investigate the factors related to therapeutic needs for FGID.

Methods: 99 patients diagnosed with FGID in multidisciplinary medical-psychiatric outpatient care (Brain-Gut Stress Clinic) of Wonkwang University Hospital were selected as a FGID patient group, 87 adults who complained functional gastrointestinal symptoms were selected as a FGID-positive group and 79 adults were selected as a normal control group based on the Rome IV criteria. Demographic factors and psychosocial factors were evaluated using the Korean-Beck Depression Inventory-II, Korean-Beck Anxiety Inventory, Korean-Childhood Trauma Questionnaire (CTQ) and Connor-Davidson Resilience Scale (CD-RISC). Student *t*-test, one-way ANOVA and logistic regression analysis were used to compare differences among groups.

Results: In comparison of the FGID group (FGID patient + FGID-positive group) to the normal control group, the odd ratio (OR) of the FGID group was 8.047 in the high risk of anxiety group (95% CI: 2.37-27.28). In the high risk of emotional neglect of CTQ subscale, the OR of FGID was 2.023 (95% CI: 1.09-3.72). In resilience, there were differences in the sum of optimism ($t = 3.209$, $P < 0.001$). In comparison of the FGID patient to the FGID-positive group, the OR of the FGID patient group was 3.944 in the depression high risk group (95% CI: 1.68-9.20). Childhood trauma differed in the sum of physical neglect ($t = -4.086$, $P < 0.001$). In the high risk of emotional neglect of CTQ subscale, the OR of FGID patient group was 3.27 (95% CI: 1.75-6.08). In resilience, there were differences in the sum of CD-RISC ($t = 4.333$, $P < 0.001$), hardiness ($t = 2.881$, $P < 0.01$), persistence ($t = 4.196$, $P < 0.001$), optimism ($t = 6.071$, $P < 0.001$), support ($t = 2.726$, $P < 0.01$), and spiritual in nature ($t = 3.039$, $P < 0.01$).

Conclusions: The FGID patients have distinctive psychosocial factors compared to the both FGID-positive and normal control group.

Therefore, the active interventions for psychosocial factors are required in the treatment of patients with FGID.

36 | LRRK2 is associated with gastrointestinal motility and enteric neurogenesis

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Background/Objectives: Leucine rich-repeat kinase 2 (LRRK2) is a protein kinase that plays a key role in Parkinson's disease (PD). We have revealed that LRRK2 is expressed in enteric neurons and related to gastrointestinal motility in vivo. In this study, to clarify the role of LRRK2 in the enteric nervous system, we performed a gut motility assay using spatiotemporal maps and whole-mount immunostaining.
Methods: Gut preparations were obtained from LRRK2-knockout (KO), LRRK2-G2019S-mutant, and wild-type (WT) mice after cervical dislocation. Gut motility was recorded using a video camera following equilibration in an organ bath. Data were converted to spatiotemporal maps using a software package provided by. Bornstein (Melbourne University). Longitudinal muscle myenteric plexus (LMMP) obtained from each mouse was used for immunofluorescence analysis.
Results: Spatiotemporal mapping revealed that KO mice had more fragmented and partial colonic migrating motor complexes (CMMCs) with irregular intervals between the CMMCs, even though the total number of CMMCs was comparable to that in WT mice. NOS inhibition alleviated the CMMC abnormality in KO mice, accompanied by an increase in the number of CMMCs. Immunofluorescence analysis of LMMP showed that the proportions of major subtypes of enteric neurons were comparable among the mice. The proportion of neurons expressing both Sox2 and Hu C/D was increased in KO mice relative to WT mice.
Conclusions: Sox2 is known to be a key protein in the regulation of neuronal differentiation as well as a glial marker. Given that new neurons generated from glia express Sox2, LRRK2 appears to be associated with neurogenesis from glia to neuron. Further study will be necessary to clarify the role of LRRK2 in enteric neurogenesis and the mechanism of colonic dysmotility in LRRK2-KO mice.

37 | Low prevalence of diabetic gastroparesis but high prevalence of functional disorders and psychological comorbidities in patients evaluated for gastroparesis

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Background: Diabetes is the most common known cause for gastroparesis. The clinical presentation of gastroparesis overlaps significantly with functional dyspepsia (FD). Diagnosis can be established by the FDA -approved and validated, non-radioactive, 13C-octanoic acid breath test.
Purpose: To evaluate the demographics and clinical characteristics of patients referred for a 13C octanoic acid gastric emptying study as well as its clinical yield.
Methods: Medical records of consecutive patients referred for gastric emptying study in the Tel Aviv Medical Center between 4.2017-9.2019 were retrospectively reviewed. Patient's demographics, study results and relevant clinical data were collected. Postoperative patients (upper GI surgeries) and patients with missing records were excluded.
Results: 145 subjects were included, 94 (64.8%) females, mean age and BMI was 55.4 ± 17 years, and 25.2 ± 5.2 respectively. 29 (20%) had diabetes (predominantly type 2). 31% performed at least one additional functional study such as pH monitoring/manometry/defecography. According to their gastric emptying and clinical data 96 (66.2%) had FD, 21 (14.5%) FD and IBS, 12 (8.3%) GERD, and 2 (1.4%) could not be classified. Only 14 (9.7%) were found to have gastroparesis and only 3 of them (2%) had concurrent diabetes. 50 (34.5%) used psychotropic medications and additional 17 (11.7%) exhibited overt anxiety or depression. Patients with a normal study, were more likely to be taking psychotropics or exhibit anxiety\depression (50% vs. 14%, $P = 0.012$).
Conclusions: In our cohort diabetic gastroparesis was found to be a rare diagnosis (2%), probably due to under-referral of complicated diabetic patients. FD, with or without IBS, was found to be by far the most prevalent diagnosis, with half exhibiting psychological comorbidities. This referral bias is leading to a low diagnostic yield (<10%) of the study and low benefit for the patients.

38 | Efficacy of ramosetron on irritable bowel syndrome like symptoms in patients with inactive inflammatory bowel disease – A randomized, double-blind, placebo-controlled study

Toshihiko Tomita; Sumire Mori; Yoshihito Inoue; Hirotsugu Eda; Kumiko Nakamura; Ken Hara; Akio Tamura; Tomohiro Ogawa; Takashi Kondo; Tomoaki Kono; Masashi Fukushima; Katsuyuki Tozawa; Tadayuki Oshima; Hirokazu Fukui; Hiroto Miwa
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Background and Aim: Recent studies report that patients with inflammatory bowel disease (IBD) may develop irritable bowel syndrome (IBS)-like symptoms such as diarrhea and abdominal pain even though they have achieved clinical remission or mucosal healing as demonstrated by the lack of intestinal inflammation. There is no consensus to date as to the appropriate treatment of these patients. Therefore, the aim of this study was to assess the effects of ramosetron in improving global IBS-like symptoms and bowel movement in patients with inactive IBD who developed symptoms associated with IBS with diarrhea (IBS-D) in a placebo-controlled study.

Subjects and Methods: We conducted a randomized, double-blind placebo-controlled study. Japanese patients with inactive IBD (ulcerative colitis, Crohn's disease) who met the Rome III diagnostic criteria for IBS-D symptoms were randomly assigned to receive either ramosetron (n = 35) or placebo (n = 35). Ramosetron (5 μg) was administered orally once daily for 4 weeks, and general improvement of IBS-like symptoms were evaluated at the 4-week time point (response rate). Improvements in abdominal pain and discomfort (response rate at 4 weeks) and bowel movement were evaluated at the same time.

Results: Four patients were lost to follow-up (incomplete or unreturned questionnaire), and a total of 66 patients (male 32: female 34) were included in the analysis. All 4 patients who were excluded from the analysis were in the ramosetron group; thus, the final analysis included 31 and 35 patients in the ramosetron and placebo groups, respectively. A significantly higher proportion of patients in the ramosetron group experienced global improvements in IBS-like symptoms (response rate at 4 weeks), with 35.4% (11/31) and 11.4% (4/35) in the ramosetron and placebo groups, respectively (P = 0.037). Ramosetron significantly improved bowel movement compared to placebo (38.7% vs. 14.2%, P = 0.028). There were no statistical differences in the rates of improvement for abdominal pain and discomfort (29.0% (9/31) and 14.3% (5/35)) in the ramosetron and placebo groups, respectively (P = 0.227).

Conclusion: Our placebo-controlled study demonstrated that ramosetron significantly improved global improvements and bowel movement in patients with inactive IBD.

Clinical Trials Registry no: UMIN000023399

40 | Effect of surgical pyloroplasty on results of gastric emptying in drug-refractory gastroparetic patients

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Background: There is limited evidence for accelerating gastric emptying (GE) with prokinetic agents or by Gastric Electrical Stimulation (Enterra Device). This unmet need triggered our interest to consider pyloroplasty (PP) as a procedure to accelerate gastric emptying. Therefore, the aim of our study was to investigate the changes in GE in gastroparetic (GP) drug-refractory patients undergoing a surgical pyloroplasty.

Methods: Overall 50 GP patients, 36 diabetics (DM) with mean DM duration of 17 (3-50) years, and 14 idiopathic (ID) patients underwent the Heineke-Mikulicz pyloroplasty all performed at one academic center by a single surgeon. GE scintigraphy test was performed using a standardized 4-hour protocol. This test was completed before surgery and at the last follow up visit which took place from 6 to 86 months after PP surgery.

Results: Overall 50 patients, mean age 44 years (range 20-78); 37 F; with mean 5.7 (1-20) years of GP symptoms were enrolled. Following surgical pyloroplasty the mean GE retention was significantly (P < 0.01) reduced at 2 and 4 hours from 73.3% to 45.6% and from 45.7% to 16.8% respectively (Table 1), 30 (60%) patients normalized their GE at 2 hours, and 19 (40%) at 4 hours (<10% retention). Interestingly, 39% of diabetics and 28% of Idiopathic patients achieved this normalization of their GE.

Conclusion: (1) Severe drug-refractory GP patients who underwent surgical pyloroplasty showed significant long-term acceleration of GE; (2) normalization of GE at 4 hours was achieved in 40% patients, with diabetics having a more predictable response to PP than idiopathic. Surgical pyloroplasty addresses the specific pathophysiological changes identified in the pyloric smooth muscle of severe GP patients, thus resulting in a successful therapeutic outcome.

Table 1

	1 hour	2 hours	3 hours	4 hours
Baseline	85.2	73.3	59.0	45.7
Follow Up	64.2	45.6	29.5	16.8
P value	<0.001	<0.001	<0.001	<0.001

41 | Pyloric pathophysiology is key explanation for refractory gastroparesis

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Background: One factor identified in the pathophysiological of impaired gastric motility in GP is the depletion of the interstitial cells of Cajal (ICC) identified in antral gastric tissue samples. Recently the focus on GP pathophysiology has shifted to the pylorus. The aim of this study was to compare the prevalence of ICC-opathy and fibrosis affecting antral and pyloric smooth muscle in drug refractory GP patients.

Methods: Full thickness antral and pyloric biopsies were obtained during the surgical implantation of a gastric electrical stimulation (GES) system and a pyloroplasty (PP) in 36 patients, mean age 43.2 (range 20-67 years.) 27 females, 20 diabetic (DM) mean DM duration 17 years. (3-50) and 16 idiopathic (ID); who had failed all available medical therapies. Tissues were stained with H&E, trichrome for fibrosis and C-Kit for ICC presence. Depletion of ICC was defined as less than 10 cells /HPF based the mean 20 HPF in the antral and pyloric smooth muscle based on data from "control" patients at our center.

Results: Overall 36 patients with a mean of 6 years (1-20) of GP symptoms provided antral and pyloric tissue samples. The mean number of antral ICC was 12 ± 5 and mean number of pyloric ICC was 7 ± 5 (P < 0.05). Eleven of 36 patients (30%) showed depletion of antral ICC and 25 (70%) of GP patients had less than 10 ICC/HPF in their pyloric samples. Antral smooth muscle fibrosis was present in 10 (27%) of patients, while 25 (70%) had fibrosis present in pyloric

biopsy samples. Overall 9 (25%) patients (6 DM) had significant loss of ICC (<10/HPF) in both regions.

Conclusions: (1) Reduction of pyloric ICC accompanied by smooth muscle fibrosis is present in 70% of severely symptomatic GP patients, compared to 30% having the same changes in their antral smooth muscle. This study extends our knowledge of the pathophysiology of GP and emphasizes the dominant role of the pylorus, thus providing more specific treatment strategies in severe GP patients.

43 | Neonatal adversity in female rats disrupts the epigenetic regulation of stress-induced visceral pain in adulthood

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Background: We discovered that elevated histone acetylation in the central nucleus of the amygdala (CeA) underlies visceral pain in female rats, exposed to unpredictable early life stress (uELS). Furthermore, we have shown that chronic stress in adulthood exacerbates uELS-induced visceral hypersensitivity. Here, we employed a dual hit model of uELS and adult stress to investigate central epigenetic mechanisms that drive abnormal stress-induced pain vulnerability.

Methods: Female rats were exposed to either uELS (n = 18) or odor only (n = 18) *(single hit)* from postnatal days 8-12. In adulthood (day 90-120), indwelling cannulae were positioned on the dorsal margin of the CeA. Subsequently, 500 nL of garcinol (histone acetyltransferase (HAT) inhibitor targeting PCAF and p300, 1 ng/nL) or vehicle (50% DMSO-50% ACSF) was microinjected into the CeA for 7 days. In another cohort of rats, garcinol or vehicle was microinjected into the CeA after exposure to water avoidance stress (WAS, 1 hour/d for 7 days, *dual hit)*. On day 8, visceral sensitivity was assessed in freely moving rats by quantifying the number of abdominal contractions during graded pressures of isobaric colorectal distension (0-60 mm Hg). A two-way ANOVA with Bonferroni post-hoc analysis tested statistical significance.

Results: Exposure to uELS *(single hit)* increased colonic sensitivity (uELS = 33 ± 8 vs. odor only = 16 ± 4 abdominal contractions, $P < 0.0001$). Garcinol microinjections into the CeA reversed colonic hypersensitivity to levels resembling odor only controls (19 ± 6 abdominal contractions, $P = 0.0030$). In the dual hit model, neonatal stress and WAS in adulthood increased colonic sensitivity (uELS: 45 ± 3, $P < 0.0001$; odor only: 30 ± 6 abdominal contractions $P < 0.0001$). In the dual hit model, garcinol microinjections into the CeA of uELS females reduced, but did not normalize colonic sensitivity (uELS from 45 ± 3 to 28 ± 5, $P < 0.0001$).

Conclusion: Elevated histone acetylation in the CeA underlies visceral pain after uELS. However, in a dual hit model combining uELS and adult stress, the inability of garcinol to normalize colonic hypersensitivity, suggests that two hits activate garcinol-insensitive HATs that contribute to stress-induced visceral pain.

45 | Fat sensing in the gut

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Background/Objectives: The presence of fat in the gut elicits immediate dopamine release in the brain. This rapid brain response indicates the existence of a fat sensor in the gut with direct connections to the central nervous system. The sensor cell of the gut is the enteroendocrine cell, of which a subset synapse with the vagus nerve – these synaptically connected cells are called neuropod cells. Our objective was to test whether neuropod cells rapidly transduce fat signals to the vagus nerve.

Methods: To test whether fatty acid receptors are expressed on neuropod cells, we sequenced FAC sorted dissociated duodenal epithelial cells from CckGFP mice (n = 5). Differential gene expression between GFP positive and negative cells was performed using DESeq2. Whole nerve vagal recordings were used to test whether fat in the duodenum stimulates vagal firing. Intralipid (6%) long-chain fatty acid solution was infused through the duodenum of wild-type (n = 9) mice while electrophysiological activity was measured in the cervical vagus. To test whether vagal firing rate depends on neuropod cells, CckCRE_Halorhodopsin-YFP mice (n = 3) were duodenally infused with Intralipid in the presence of 532 and 473 nm light. Finally, to test if fat elicits glutamate release, glutamate receptor inhibitors AP-3 (1 mg/kg) and kynurenic acid (150 µg/kg) were delivered to wild-type mice (n = 5) immediately before Intralipid (6%) infusion.

Results: Duodenal fat elicited significant increase in vagal firing within 100 seconds of the infusion. Transcripts for fatty acid receptors were significantly enriched on CCK-positive enteroendocrine cells (Ffar2, Ffar3, Ffar4, and Gpr119), indicating enteroendocrine cells are the primary gut fat sensor. Accordingly, the vagal response to fat was significantly attenuated with optogenetic silencing of CCK gut cells. Finally, fat-elicited vagal firing was blocked in the presence of glutamate receptor inhibitors.

Conclusions: These data support a role for fast neurotransmitter release from duodenal neuropod cells to rapidly communicate the presence of fat in the gut to the brain to drive future behaviors.

47 | Abnormalities of whole gut transit in newly diagnosed coeliac disease and effect of gluten free diet treatment demonstrated by magnetic resonance imaging

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Background/Objectives: Coeliac disease (CD) is an autoimmune disease which primarily affects the small bowel mucosa. The only treatment is a life-long gluten free diet (GFD), which should result in reversal of the enteropathy. Limited evidence suggests disordered gut transit in untreated CD whilst the effect of GFD on transit is unclear.

This study in newly diagnosed CD patients aimed to investigate the change in gut transit before and after 12 months of GFD treatment using magnetic resonance imaging (MRI).

Methods: Thirty-six CD patients were studied at baseline and 12 months post GFD treatment. Thirty-six matched healthy controls (not following GFD) were also studied.

Participants had an MRI scan 24 hours after ingesting 5 transit marker capsules. A whole gut transit score was calculated according to each capsule's position, yielding a Weighted Average Position Score (WAPS) for each participant.

Results: In the patients the mean ± SEM WAPS at baseline was 2.23±0.26, significantly delayed compared to 1.34 ± 0.17 for healthy controls (N = 36, $P < 0.02$). Extrapolating the WAPS to transit time in hours gave a baseline mean transit time of 70 hours for the CD patients compared to 44 hours for the controls. Following a 12 month GFD treatment, the mean WAPS for CD reduced to 1.76 ± 0.23 (N = 29, $P < 0.05$).

Conclusions: Our data confirm delayed transit in the newly diagnosed coeliac patients which demonstrates partial recovery after 12 month GFD treatment.

This is the first study using MRI to provide new insights into the pathophysiology of whole gut transit in CD and the effect of GFD treatment. The imaging is quick, non-invasive and acceptable and could help monitoring and follow up.

48 | Esophageal dysfunction in patients with Sjögren's syndrome

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Background/Purpose: Sjögren's syndrome is an autoimmune disease which may cause inflammation of exocrine glands include salivary glands. Some patients with Sjögren's syndrome also accompany with gastrointestinal disease such as gastroesophageal reflux disease. Previous studies showed there is association between Sjögren's syndrome with esophageal motility diseases. But the esophageal dysfunction in Sjögren's patients is not well-known. We aim to clarify the clinical symptoms and esophageal motility function in patients with Sjögren's syndrome.

Methods: 71 patients with suspected esophageal reflux or motility disease received esophagogastroduodenoscopy and esophageal manometry were enrolled. 13 patients were diagnosed with Sjögren's syndrome according American-European Consensus Criteria. After excluding 15 patients with achalasia and motility disease, 43 patients remained as controls. We retrospectively evaluated their clinical symptoms, endoscopic and esophageal manometry findings.

Results: Acid regurgitation was more prevalent in patients with Sjögren's syndrome (53.8%, control: 11.6%, *P*-value: 0.003) but endoscopic finding of reflux esophagitis was similar in both groups (Sjögren's patients: 38.5%, control: 39.5%, *P*-value: 0.945). There is more tendency of Barrett's esophagus in Sjögren's patients (15.4% vs. 0%, *P*-value: 0.051). Sjögren's syndrome patients had decreasing length of upper esophageal sphincter (2.38 ± 0.65 cm vs. 3.07 ± 0.74 cm, *P*-value: 0.003) but there was no significant difference in upper esophageal sphincter pressure, body peristalsis, lower esophageal sphincter length and pressure.

Conclusions: Patients with Sjögren's syndrome had more acid regurgitation but no significantly reflux esophagitis. Sjögren's syndrome patients had decreasing length of upper esophageal sphincters.

49 | Ginger enhances gastric motility in patients with diabetic gastroparesis

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Background/Purpose: Diabetic gastroparesis is the diseases due to delay gastric emptying that influence the quality of life and sugar control, and there is no satisfactory medical treatment. According our previous study, ginger stimulated gastric emptying and antral contractions in patients with functional dyspepsia. It is unclear about the effect of ginger on the patients with diabetic gastroparesis. We

plan to study whether ginger enhance gastric emptying and improve gastric symptoms in the patients with diabetic gastroparesis.

Materials and Method: Eleven diabetic patients with gastroparesis were studied twice in a randomized double-blind cross-over manner. The patients ingested three ginger capsules (total 1200 mg) or placebo. Antral area, fundus area and diameter, and the frequency of antral contractions were measured by ultrasound. Gastrointestinal sensations were scored using visual analog questionnaires. Serum glucagon-like peptide-1, motilin, gastrin and ghrelin were measured in regular time.

Results: There was significant different in antral area (P = 0.02, repeated measure ANOVA) and more antral contractions (P = 0.01, repeated measures ANOVA) after ginger than placebo, but fundus dimensions did not differ, and there was no significant difference in any gastrointestinal symptoms. There were no different serum peptides (motilin, gastrin, ghrelin and GLP-1) between two different days.

Conclusions: Ginger may increase antral contractions and enhance gastric emptying in diabetic gastroparesis patients, but there was no effect on relieving gastrointestinal symptoms.

50 | Extracorporeal shockwave increases gastric contraction and emptying in a rat model

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Background and Aim: Delayed gastric emptying occurs in more than 50% of chronic diabetic patients and this is associated with significant impairments in quality of life. Traditional therapy for delayed gastric emptying has focused on supportive treatment and there is no significant effective therapy. The effect of low-energy shockwave on gastric motility is never studied. We investigated low-energy shockwave on gastric motility in a diabetic rat model.

Methods: Twenty-eight male Wistar rats were studied and separated in three groups in randomized order as: control, diabetic rats received shockwave, and diabetic rats received the sham procedure. Antral area and motility were recorded using the trans-abdominal ultrasound. Blood was taken for measurement of gastric motility peptides. Subjects were sacrificed for immunohistochemical stain analysis of enteric plexus of the stomach.

Results: We successfully induced 20 diabetic rats and set ultrasound for measuring rat gastric contract and emptying model and demonstrated that 6 weeks of low-energy shockwave could promote gastric contraction (13.60 ± 0.20: 11.40 ± 0.70 times/5 min, P = 0.02), and emptying in diabetic rats (7.60 ± 1.33: 4.08 ± 1.09%, P = 0.02). Moreover, we demonstrated that shockwave could increase defecation, feces and decrease serum cholesterol and triglycerol. However, no effect on glycohemoglobin and gastric motility peptides was recorded. In the immunohistochemical staining, shockwave increased expression of gastric myenteric neuron plexus (P < 0.05, by ANOVA).

Conclusion: Low-energy shockwave can increase gastric contraction and emptying by activating axonal regeneration and increasing myenteric plexus, but not related with motility peptides.

51 | Gastric emptying scintigraphy in evaluation of Korean patients with upper gastrointestinal symptoms who visited a tertiary referral center

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Background and Aim: Many patients with dyspepsia and negative findings on endoscopy continue to experience symptoms despite acid suppression and/or prokinetics. These patients can be difficult to manage. In a meta-analysis of 868 patients with dyspepsia and 397 healthy individuals as controls, gastric emptying was substantially delayed in almost 40% of the overall patients. The aim of this study was to assess the frequency of impaired gastric emptying in patients referred with unexplained upper gastrointestinal symptoms.

Methods: We reviewed consecutive patients with upper gastrointestinal symptoms who had been referred to the Gastroenterology Motility Clinic at Soonchunhyang University Seoul Hospital and undertook gastric emptying scintigraphy using solid meals between July 2017 and June 2018. Demographics and results of scintigraphic gastric emptying were assessed.

Results: A total of 282 patients were identified. The mean age of overall patients was 54. 6 ± 17.8 years and they were 70.2% female. A normal gastric emptying was observed in 71.3% of the overall patients (Figure). A delayed gastric emptying and rapid emptying were found in 24.5% (n = 69) and 4.3% (n = 12), respectively. There was no significant difference in sex proportion among patients with rapid, normal or delayed gastric emptying. The median age of patients with delayed gastric emptying was lower than that of patients with normal gastric emptying (55 years vs. 63 years, adjusted P value = 0.026). The frequency of diabetes mellitus was significantly different among patients with rapid (33.3%), normal (15.4%) and delayed gastric emptying (46.4%) (P < 0.001). However, the prevalence of rheumatologic diseases or Parkinson's disease was similar among them.

Conclusion: This large study is first to evaluate the prevalence of abnormal gastric emptying in Korean patients with upper gastrointestinal symptoms. Gastric emptying scintigraphy using solid meal

alters management in approximately 30% of Korean patients with unexplained upper gastrointestinal symptoms.

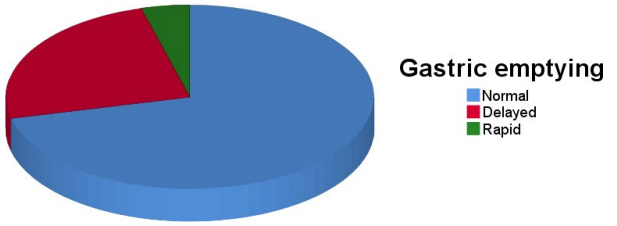

Figure. The proportion of impaired gastric emptying among Korean patients with unexplained upper gastrointestinal symptoms

52 | Evaluating the quality of the internet information for functional constipation on Korean websites: Qualitative study

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Background: The internet is one of the most frequently used tools for patients to obtain information about their disease. Despite increasing use of internet, little is known about the quality of the web sites especially on functional constipation (FC). We aimed to evaluate the quality of the internet information for the Korean patients with FC.

Methods: Members of Constipation Study Group in the Korean Society of Neurogastroenterology and Motility developed Quality Evaluation Instrument (QEI) based on the recent guidelines of FC in Korea. Through searching of the terms 'constipation' and 'functional constipation' in the three most renowned search engines in South Korea, 50 websites were selected in this study. Two gastroenterologists rated these web sites according to QEI that awarded sites points (0-68), 5-point global quality score (GQS) and the validated DISCERN instrument (15-75).

Results: The median QEI score was 7 ([min-max] 0-47; [IQR] 4.0-14.25) and the median GQS was 1 ([min-max] 0-4; [IQR] 1-2). When evaluated by DISCERN, 14% (7/50) were rated as excellent to good and 86% (43/50) as fair to very poor. In spearman correlation analysis, QEI score showed good relationship not only DISCERN instrument (r = 0.82, P < 0.001) but also GQS (r = 0.75, P < 0.001). Based on analysis using DISCERN instrument, the institutional sites scored best, whereas alternative medicine, mess media and support sites scored significantly lower than other sites. There was no significant difference among search engines in regards to mean DISCERN score, QEI and GQS.

Conclusion: The quality of the Korean web sites providing health information on FC showed substantial variation. In order to provide adequate health information to patients with FC, it is necessary to establish an assurance system to control quality of health information.

53 | Acute colitis following chronic experimental traumatic brain injury in mice induces persistent systemic inflammation and dysautonomia

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Background: Disruptions in the bidirectional communications of the brain-gut axis are increasingly implicated in the onset and progression of a variety of disorders, diseases and injuries. We have found that intestinal inflammation (dextran sodium sulfate; DSS) following chronic experimental TBI in mice (>28 days) persistently exacerbated deficits in fine motor coordination, social behavior and anxiety-like behavior in TBI-injured mice. The aim of this study was to determine the potential involvement of the neural and immunological pathways of the brain-gut axis in DSS-induced exacerbation of TBI-associated neurocognitive dysfunction.

Methods: Male C576Bl/6 mice were randomized into naïve (anesthetic), sham (craniotomy), or moderate-to-severe controlled cortical impact (CCI) groups. At 28 days after injury, mice from each group were treated for 7 days with 3% DSS to induce colitis, followed by return to regular water for 28 days. Prior to, during and following induction of CCI and colitis, mice underwent electrocardiography for heart-rate variability (HRV) analyses. Mice were euthanized at 0, 7 and 28 days post-DSS and the spleen and mesenteric lymph nodes (MLN) were weighted and processed for immune characterization via flow cytometry.

Results: A sustained increase in spleen and MLN weights occurred after 7 days in DSS groups. Myeloid cells were increase in spleen while myeloid cells, as well as, T-cells were elevated in MLN. CCI transiently elevated sympathetic tone (↑LF/HF) at 24 hours returning to baseline levels by 7 days. Sham+DSS and CCI+DSS mice exhibited increased sympathetic tone during both DSS injury and recovery phases.

Conclusions: These data show that intestinal inflammation following TBI results in a sustained local and systemic immune response which is also associated with persistent alteration of autonomic balance. These findings suggest that disruption of the cholinergic anti-inflammatory via alteration of the vagal-spleen axis contributes to colitis-induced exacerbation of TBI-associated neuropathology and cognitive dysfunction.

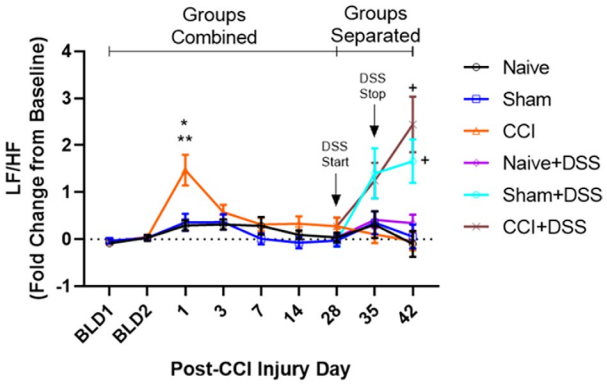

Figure 1. Heart-rate variability (HRV) results expressed as fold change from baseline low-frequency (LF) / high-frequency (HF) ratios. RM-Two Way ANOVA: + significant vs CCI, Sham, Naïve+DSS; ** significant vs Naïve, * p<0.05 vs Sham

54 | An altered composition of gut microbiota, organic acids, and the effect of probiotics in the guinea pig model of postoperative ileus

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Background and Aim: Postoperative ileus (POI) is the paralysis of intestinal motility caused by abdominal surgery. The aim of this study is to identify the altered composition of gut microbiota, organic acid, and the effect of probiotics in guinea pig model of POI.

Methods: A laparotomy with cecal manipulation was performed to induce POI in guinea pigs. Fecal samples were collected at 1, 3, and 5 days after POI procedure. Extracted fecal DNA was amplified and sequenced using Illumina MiSeq sequencing system. After pretreatment of probiotics (Mixture of *Enterococcus faecalis*, *Bacillus mesentericus*, *Clostridium butyricum*) and placebo via oral route for 7 days prior to POI procedure, 16S RNA PCR with species specific primers and ELISA were used to evaluate the effect of probiotics on specific species known to be beneficial to intestinal permeability, organic acids, and bowel movement at each day.

Results: As in humans, four major bacterial phyla were *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* in guinea pig. Community alpha-diversity of fecal microbiota was not significantly different between control and POI model. However, Beta-diversity measured by Bray-Curtis dissimilarity in POI model was significantly different from that of control ($P < 0.01$). The taxonomic relative abundance of lactic-acid producing bacteria including *Bifidobacterium* and *Lactobacillus* is significantly decreased in POI model. The relative abundance of *Clostridium butyricum*, *Bifidobacterium longum*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum* were significantly decreased at 1 day or 3 days after POI procedure compared to control group. In probiotics group, the abundance of *Bifidobacterium longum* and *Bifidobacterium bifidum* were significantly increased compared to control or placebo group. Fecal butyrate level was decreased after POI procedure, and was significantly increased in probiotics group. Bowel movements assessed by daily fecal number and weight after POI procedure were significantly increased in probiotics group than control group.

Conclusions: POI induces gut bacterial dysbiosis and reduction of bacterial metabolites. Pretreatment of probiotics recovers several beneficial species, as well as butyrate production and bowel movement. These data may help treat and prevent of POI.

Keywords: postoperative ileus, gut microbiota, intestinal permeability

55 | Development and validity evaluation of a self-evaluated questionnaire for functional dyspepsia

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Background/Aims: There is uncertainty about how to measure patient self-reported outcomes in functional dyspepsia (FD). The aim of this study was to development of instruments and to determine of the respondent definition for patient reported outcomes to evaluate the efficacy of therapeutic agents in FD patients.

Methods: Self-reported questionnaire for upper gastrointestinal symptoms (SEQ-UGIS) was developed through structural processes and translation with cultural validation. Two-week reproducibility was evaluated and construct validity was assessed by correlating subscale scores with SEQ-UGIS, Patient-Assessment of Gastrointestinal Disorders (PAGI-SYM), Nepean dyspepsia index-Korean version (NDI-K) and validated NDI-K quality of life (QOL) questionnaires. Finally, discriminative validity was performed by comparing the changes of SEQ-UGIS score after 4 weeks' treatment with proton pump inhibitor and prokinetic drug.

Results: A total of 121 Korean patients were included (mean age 48.8 ± 13.3 years, female 67.8%). Internal consistency of 11 item SEQ-UGIS was good (Cronbach's alpha = 0.53-0.85); test-retest reliability was acceptable (intra-class correlation coefficient = 0.70-0.74). The SEQ-UGIS score was expected to be highly correlated

with the subscale of dyspepsia domain of the PAGI-SYM (Pearson correlation coefficient r^2 = 0.651, P < 0.001), NDI-K dyspepsia subscale (r^2 = 0.784, P < 0.001) and NDI-K QOL subgroups (r^2 = −0.324 to −0.431, all P < 0.001). Both drug responder and non-responder showed significant decrease in SEQ-UGIS scores after treatment, and significant difference in changes of mean difference between the two groups (−9.17 ± 9.15 vs. −4.58 ± 7.79, P = 0.030).

Conclusions: This study support that SEQ-UGIS for functional dyspepsia has the reliability, validity and can be useful tool to evaluate therapeutic response in patient with FD.

Keywords: Functional dyspepsia; Patient reported outcome measures; Surveys and questionnaires; Validation studies

57 | Attenuation of stress-induced activation of supraspinal pain circuits contributes to the anti-nociceptive effects of linaclotide

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Background: Linaclotide, a peripherally restricted guanylate cyclase-c (GC-C) agonist, relieves abdominal pain in irritable bowel syndrome in part through an inhibition of afferent signaling at the level of the spinal cord. However, it remains to be determined if linaclotide affects supraspinal nociceptive signaling. Here, we test the hypothesis that linaclotide attenuates stress-induced visceral hyperalgesia by altering neuronal activity within key brain nuclei involved in pain modulation.

Methods: Female rats were exposed to water avoidance stress (WAS) or sham stress (1 hour/d for 10 days; n = 8/group). On days 5-11 rats received oral linaclotide (3 μg/kg, q.d.) or vehicle (n = 4/group). On day 11, rats received three isobaric colorectal distensions (CRD), each 60 mm Hg, and colonic sensitivity was assessed via a visceromotor response (VMR). Following euthanization the brain was isolated for the immunohistochemical quantification of phospho-ERK (pERK) immunoreactive neurons in the prefrontal cortex (PFC), paraventricular nucleus of the hypothalamus (PVN), central amygdala (CeA), periaqueductal gray (PAG), and rostroventral medulla (RVM). Two-way analysis of variance (ANOVA) was used to analyze changes in VMR and pERK expression.

Results: Vehicle-treated rats exposed to WAS showed an increased VMR to CRD compared to sham stress (****P < 0.0001). Furthermore, WAS increased the CRD-evoked neuronal activation, as assessed via quantification of $pERK^+$ neurons, in each brain region under investigation (*P < 0.05-****P < 0.0001). In a separate cohort, linaclotide inhibited the VMR to CRD in WAS-exposed rats compared to vehicle treatment (††††P < 0.0001). Additionally, linaclotide decreased the number of CRD-evoked $pERK^+$ neurons in the PFC, PVN, PAG, and RVM, but not the CeA, compared to vehicle treatment in WAS rats (††P < 0.01-††††P < 0.0001). Linaclotide had no effect on the VMR or pERK immunoreactivity in sham stress-exposed rats compared to vehicle.

Conclusions: Here we provide evidence that linaclotide inhibits stress-induced colonic hypersensitivity by altering neuronal activation within the supraspinal pain modulation circuitry. Thus, this study further highlights a pivotal role of GC-C mediated mechanisms in visceral pain processing. IBS.

59 | Expression of tight junction proteins according to functional dyspepsia subtype and sex

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Background/objectives: To investigate whether the expression of tight junction proteins (TJPs) are different depending on subtype of functional dyspepsia (FD) and sex.

Methods: Control (n = 95) and FD group (n = 165) based on ROME III criteria were prospectively enrolled. Gastric mucosal mRNA expression levels of various TJPs (claudins, CLDN 1, 2 and 4; zonula occludens-1, ZO-1; occludin, OCLN) were assessed by RT-PCR. *H. pylori* was evaluated by histology, CLO test and culture. Questionnaire was analyzed.

Results: FD group showed higher CLDN2 mRNA expression than those of controls (P = 0.048). mRNA expressions of CLDN2 and CLDN4 in *H. pylori* (+) FD group were higher than those of *H. pylori* (+) controls (P = 0.013 and 0.032, respectively). In case of CLDN2 of FD group *H. pylori* (+) patients showed marginally higher expression than those of *H. pylori* (−) (P = 0.092). Female postprandial distress syndrome (PDS) group showed lower mRNA expression of CLDN1 than that of male PDS (P = 0.025) and female mixed type showed lower CLDN4 mRNA expression than that of male (P = 0.010). FD group showed higher anxiety score than controls (P = 0.004). In terms of quality of life, female FD group showed lower physical and social domain scores compared to male FD group (P = 0.035 and 0.016, respectively).

Conclusions: TJPs affected FD depending on subtype and sex. *H. pylori* may be involved in the change of TJPs and affect gastric mucosal permeability in FD patients.

60 | Differential effects of oxidative stress on mouse jejunal and colonic mechanosensitive afferent.

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Oxidative stress is implicated in the pathogenesis of many gastrointestinal conditions, such as inflammatory bowel disease (IBD), ischemia and colon cancer. How diverse regions of the gut react to, and handle, elevated levels of reactive oxygen species (ROS) is unclear. Here, we investigated the effect of oxidative stress on sensory signalling in the small intestine and colon.

Methods: Sensory afferent nerve firing in response to distension was recorded in-vitro from jejunum and colon of male C57BL/6J. Data are presented as mean ± SEM with n = 5-6. Statistical analysis was performed using Student's *t*-test and ANOVA (two-way) as appropriate. $P < 0.05$ was considered as significant. Epithelial cells were isolated from each region and RNA were extracted for expression analysis, n = 3. RNA was used to determine differential gene expression in jejunum and colon by microarray analysis.

Results: Ramp distension of segments of intestine evoked a biphasic increase in afferent discharge, which represents the activation of low threshold (LT), wide dynamic range (WDR) and high threshold (HT) mechanosensitive afferent fibres. To examine the regional difference in response to oxidative stress, H_2O_2 (100 & 500 μmol/L) and mitochondrial inhibitor antimycin A (20 μmol/L) were bath applied to the jejunum and colonic segments. In the jejunum, the response to H_2O_2 (100 & 500 μmol/L) was observed as an increase in spontaneous nerve firing while antimycin A (20 μmol/L) had no effect. Neither had any effect on mechanosensitive responses. However, in the colon, nerve firing in response to distension was significantly enhanced by both H_2O_2 (100 & 500 μmol/L) and antimycin A (20 μmol/L), mainly in the WDR & HT component ($P = 0.02$). Moreover, baseline nerve activity increased from 14.43 ± 4.3 spike/s to 23.08 ± 5.7 spike/s, $P = 0.02$. Microarray analysis showed that mucosal genes encoding antioxidant mechanism were significantly higher in the jejunum than colon.

Conclusion: Our result suggests differential sensitivity to oxidative stress by jejunum and colonic afferents. Difference in response could be due to variance in expression of genes involved in ROS generation and antioxidant mechanism.

61 | The role of esophageal baseline impedance levels in differential diagnosis of Korean patients with GERD-like symptoms, and within-day variability of the levels

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Background/Objectives: Lower esophageal impedance levels are associated with GERD. The aim of this study was to investigate the role of esophageal baseline impedance levels in differentiating Korean patients with GERD-like symptoms, and within-day variability of the levels.

Methods: Data of 24 hours esophageal MII-pH monitoring in consecutive endoscopy-negative patients with GERD-like symptoms were analyzed. Baseline impedance was measured in the proximal esophagus (15 and 17 cm channels) and the distal esophagus (3 and 5 cm channels) at 5 minutes before meal (time 1), 5 minutes after meal (time 2), 30 minutes after meal (time 3), 30 minutes before sleep (time 4), 30 minutes after sleep (time 5), and 60 minutes after sleep (time 6).

Results: A total of 104 patients were included in the study. The levels of the proximal esophagus at time 6 were significantly lower in the reflux hypersensitivity group ($P = 0.021$), compared with the functional symptom group. The levels at other time points did not significantly differ among the groups. The levels of the distal esophagus were significantly lower at all time points in the pathologic reflux group, while those in the reflux hypersensitivity group did not significantly differ, compared with the functional symptom group. The levels at time 2 and 3 were significantly lower in the proximal ($P < 0.001$) and distal esophagus ($P < 0.001$), compared with those at time 1. Significantly lower levels were observed at time 5 ($P < 0.005$) and 6 ($P < 0.005$) in the proximal esophagus, compared with those at time 4, but it was not observed in the distal esophagus. When a cut-off value of 2375Ω in the distal esophagus, the sensitivity and specificity for the diagnosis of pathologic reflux were 0.762 and 0.677, respectively. When a cut-off value of 2375Ω in the proximal esophagus, the sensitivity and specificity for the diagnosis of reflux hypersensitivity were 0.688 and 0.609, respectively.

Conclusions: Meal ingestion and sleep can affect the impedance levels of the proximal or distal esophagus. The baseline impedance levels of the distal or proximal esophagus appear to be useful for differentiating Korean patients with GERD-like symptoms.

62 | Sequential symptom evaluation during hydrogen breath tests with the validated, test-specific adult carbohydrate perception questionnaire (aCPQ): Comparison of symptom timelines after fructose- and lactose-ingestion

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Carbohydrate ingestion can cause symptoms such as pain, nausea, meteorism, flatulence and diarrhea in sensitive patients; these are not always associated with malabsorption. Whether ingestion of various carbohydrates induces different symptoms is currently a matter of debate. The aCPQ allows a valid, test-specific characterization and quantification of carbohydrate induced gastrointestinal symptoms.

Aims: To characterize and compare the symptomatic response to lactose- and fructose loads during breath hydrogen tests (BHT) with a validated, test-specific symptom questionnaire.

Methods: Patients with functional abdominal disorders (age ± SEM: 45 ± 15 years; 39% male) swallowed 25 g fructose (n = 422) or 50 g lactose (n = 466). Symptoms during BHT were ascertained regularly up to 9 hours on a 100 mm VAS-scale. An increase of breath-hydrogen concentration ≥20 ppm over baseline was considered as malabsorption. Intolerance was defined as increase of at least one symptom ≥20 mm over baseline in the VAS scale. Significant symptom increase over baseline ($P < 0.01$) is reported, duration of symptoms after lactose and fructose are compared by student's *t*-test ($P < 0.05$), NS = not significant.

Results: 148/422 patients were fructose malabsorbers, 202 were intolerant to fructose (of whom 96 (48%) malabsorbed fructose). 199/466 patients were lactose malabsorbers, 298 were intolerant to lactose (of whom 159 (53%) malabsorbed lactose). Hydrogen peaks occurred significantly earlier after fructose (median: minute 60) than after lactose ingestion (minute 90) ($P < 0.001$). Pain increased significantly (vs. baseline, $P < 0.01$) in fructose intolerant patients from minute 30 to minute 120, pain resolved thereafter and did not recur. In lactose intolerant patients, pain lasted from minute 30 to 9 hours ($P = 0.05$ vs. fructose). Meteorism lasted from 30 minutes to 9 hours (fructose) and 60 minutes to 9 hours (lactose) respectively (P = NS). Flatulence: 60 minutes to 9 hours (fructose) vs. 120 minutes to 9 hours (lactose) ($P = 0.003$). Diarrhea: 6 to 9 hours (fructose) vs. 120 minutes to 9 hours (lactose) ($P = 0.04$). Significant increase in nausea symptom scores was only induced by fructose ingestion from 30 to 120 minutes but not by lactose ingestion (P = NS).

Conclusion: There are distinct differences in symptom quality and timelines of symptoms after fructose and lactose ingestion in intolerant patients. The adult carbohydrate perception questionnaire (aCPQ) is a valuable tool for an unbiased symptom assessment.

63 | Colonic dysmotility associated with high fat diet-induced obesity: Role of glial adenosine A2B receptors

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Background/Objectives: The contribution of adenosine A_{2B} receptors (A_{2B}Rs) to the modulation of enteric glia (EG) activity, and thereby their involvement in intestinal dysmotility occurring in obese patients is largely unknown. We evaluated the role of A_{2B}Rs on EG activity in the colonic neuromuscular compartment of obese mice.

Methods: C57BL/6 mice were fed with standard (SD) or high fat diet (HFD) for 8 weeks. Colonic longitudinal muscle strips (LMS) were set up in organ baths with Krebs solution containing guanethidine, L-NAME and atropine to record contractile responses driven primarily by tachykinins. BAY 60-6583 (BAY; A_{2B}Rs agonist), MRS1754 (MRS; A_{2B}Rs antagonist) and the gliotoxin fluorocitrate (FC) were tested on colonic contractions. A_{2B}Rs, glial fibrillary acid protein (GFAP) and SP distribution were examined by immunohistochemistry. To mimic a HFD-induced obesity scenario, in vitro experiments were performed exposing EG cells to palmitate (PA) and/or LPS, with or without A_{2B}R ligands. Glial cell-derived neurotrophic factor (GDNF), interleukin-1β (IL-1β) and SP levels were assayed in the supernatants of EGC culture. Toll-like receptor (TLR)-4 expression was assessed by western blot analysis.

Results: MRS enhanced the electrically induced NK_1-mediated tachykininergic contractions in LMS from HFD mice. BAY decreased the ES-evoked colonic tachykininergic contractions, acting with higher efficacy in preparations from HFD mice. The effects of A_{2B}R ligands on colonic contractions were blunted upon incubation with FC. HFD mice showed an increased expression of colonic A_{2B}Rs, GFAP and SP as compared with SD mice. In cultured EG, upon pre-exposure to PA, LPS increased TLR-4 expression as well as IL-1β, GDNF and SP release. BAY reduced TLR-4 expression as well as IL-1β, GDNF and SP release. Such effects were blunted by MRS.

Conclusions: A_{2B}Rs, expressed on EG, modulate the enteric inflammation and abnormal tachykininergic responses associated with obesity. In vitro experiments showed a modulatory action of A_{2B}Rs on SP release from EG via GDNF pathway.

64 | Enteric α-synuclein accumulation impairs intestinal epithelial barrier through inflammasome activation before the onset of brain pathology in a transgenic mouse model of Parkinson's disease

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Background/Objectives: Enteric α-synuclein inclusions, impaired intestinal epithelial barrier (IEB) and bowel inflammation could represent early events in Parkinson's disease (PD) and contribute to bowel motor dysfunctions. This study examined the correlation among enteric α-synuclein accumulation, altered IEB and enteric inflammation in a transgenic model of PD.

Methods: A53T transgenic (Tg) mice develop a progressive PD-like neurological and motor deficiency after 9 months of age. Animals were sacrificed at 3 months of age to evaluate enteric α-synuclein inclusions, alterations of the IEB and enteric inflammation since the early phases of PD, before brain pathology. Blood was collected to evaluate circulating lipopolysaccharide (LPS) levels. Distal colon was processed for the evaluation of α-synuclein and zonulin-1 protein

expression, interleukin-1beta (IL-1β) levels and caspase-1 activity. THP-1 cells were treated with LPS and α-synuclein, in the presence or absence of the caspase-1 inhibitor YVAD for 24 hours. Then, supernatant was collected and, after assessment of IL-1β levels (ELISA), it was used to treat CACO-2 epithelial cells. After 72 hours, CACO-2 cells were lysed and zonulin-1 protein expression was assessed.

Results: Tg mice displayed an increase in α-synuclein expression in colonic tissues. Colonic zonulin-1 was decreased and circulating LPS levels were increased. Colonic IL-1β levels and caspase-1 activity were elevated. Co-treatment with LPS/α-synuclein promoted IL-1β release in the supernatant of THP-1 cells. The THP-1-derived supernatant decreased significantly zonulin-1 expression when applied to CACO-2 cells. Such an effect was abrogated when THP-1 cells were incubated with YVAD. The incubation of LPS and/or α-synuclein did not modify zonulin-1 expression in CACO-2 cells.

Conclusion: In the early stages of PD before brain pathology, enteric α-synuclein accumulation compromises IEB through inflammasome activation. These changes could contribute to the pathogenesis of intestinal motor dysfunctions in PD patients.

65 | The additional measurement of methane during breath tests does not alter the diagnosis of carbohydrate malabsorption in a Central European paediatric population

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Introduction: The importance of methane-measurement to detect malabsorption in hydrogen non-producers is controversial. Moreover, the need for a correction factor (CO_2) is disputed. Combined detection of hydrogen with other gases increases cost and complexity of the test but is recommended by guidelines.

Aims: To evaluate the usefulness of methane and CO_2 correction in breath tests for the diagnosis of carbohydrate malabsorption.

Methods: 132 paediatric patients (59 males, 73 females; mean age ± SEM: 11.3 ± 3 years, weight: 41.2 ± 15 kg) underwent fructose- (n = 54) or lactose- (n = 78) breath tests for evaluation of carbohydrate malabsorption. 1 g fructose/kg (25 g maximum) or 2 g lactose/kg (50 g maximum) were applied. Breath samples were collected at 30-minute intervals for three hours and analysed for hydrogen, methane and CO_2. Malabsorption was diagnosed by an increase in: A) hydrogen ≥20 parts per million (ppm) over baseline; B) methane ≥12 ppm; C) hydrogen plus methane ≥15 ppm. The diagnosis was made before and after use of a correction factor (5.5% CO_2 as numerator). McNemar's test for paired proportions was calculated, *P*-values < 0.05 were considered significant.

Results: Before applying the correction factor, hydrogen detection led to a diagnosis of 58 malabsorbers/74 absorbers. None of absorbers exhaled relevant methane that would have changed diagnosis (neither methane ≥12 ppm nor hydrogen+methane ≥15 ppm). After CO_2 correction, malabsorption (hydrogen ≥20 ppm) was diagnosed in 3 patients that would have not been diagnosed without correction; in contrast, 5 patients lost their diagnosis of malabsorption (hydrogen ≥20 ppm) after CO_2 correction ($P = 0.7$). None of absorbers exhaled relevant methane (≥12 ppm) that would have changed diagnosis. Combination of corrected hydrogen+methane detected 2 patients who were not detected by hydrogen alone ($P = 0.5$).

Conclusion: Addition of methane measurement did not improve detection of carbohydrate malabsorbers in this Central European paediatric population. The use of CO_2 correction alters the diagnosis of malabsorption in a minority of patients, and does not significantly change the test results overall. In summary, the inclusion of the measurement of methane in breath tests does not improve the quality of malabsorption detection; the use of a correction factor might benefit individual patients.

	Hydrogen ≥20 ppm over baseline, with CO_2 correction	
	Absorption	**Malabsorption**
Hydrogen ≥20 ppm over baseline, no correction		
Absorption	69	5
Malabsorption	3	55

66 | Enteric β-amyloid accumulation, bowel inflammation and intestinal dysmotility precede brain pathology in a spontaneous model of Alzheimer's disease

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Background/Objectives: Alzheimer's disease (AD) patients are characterized by β-amyloid (Aβ) accumulation and enteric inflammation that could contribute to intestinal dysmotility since the earliest stages of the disease. This study examined fecal Aβ accumulation, enteric inflammation and in vitro colonic motor activity in a spontaneous model of AD before the development of brain pathology.

Methods: Senescence-accelerated mouse prone 8 (SAMP8) mice were employed as model of AD. Animals at 4, 6 and 8 months of age underwent Morris water maze test as well as assessment of fecal output, to evaluate cognitive functions and colonic transit from the early phases of AD up to its full development. Animals were sacrificed at 6 months and colonic longitudinal muscle preparations were set up in organ baths and connected to isometric transducers. Neurogenic motor responses were evoked by electrical stimulation (ES) in Krebs solution containing guanethidine (10 μmol/L), N^{ω}-nitro-L-arginine

methyl ester (L-NAME, 100 μmol/L) and L-732,138 (NK_1 receptor antagonist, 10 μmol/L) to record cholinergic contractions. Myogenic contractions elicited by carbachol in the presence of tetrodotoxin were also recorded. Fecal Aβ1-42 and colonic interleukin (IL)-1β levels were examined by ELISA. Colonic nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome, caspase-1 and adaptor protein ASC expression were assessed by western blot.

Results: SAMP8 mice displayed cognitive dysfunctions and decreased fecal output since 6 months of age. These animals displayed reduced neurogenic colonic cholinergic contractions, as compared with controls, while colonic cholinergic myogenic responses were unchanged. Fecal Aβ1-42 protein and colonic IL-1β levels were increased. An increased active caspase-1 expression was observed, while NLRP3 and ASC expression did not vary.

Conclusions: In the SAMP8 AD model, cognitive deficiencies are associated with an impaired colonic transit. A decreased cholinergic motor activity, fecal Aβ1-42 accumulation and colonic inflammation could contribute to colonic dysmotility.

67 | Secretory products from two commensal bacterial strains evoke excitation in intrinsic and extrinsic neurons in Sprague Dawley rats

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Background/Objectives: Mounting evidence implicates the vagus nerve as a signalling conduit between microbes in the gut lumen and the central nervous system. The mechanism, however, by which bacteria signal to their hosts across an intact barrier is only beginning to be elucidated. The study aim was to investigate mechanisms by which two commensal bacterial strains modified the host peripheral nervous system.

Methods: The neurostimulatory actions of the secretory products of polyunsaturated fatty acids (PUFAs)-producing *Bifidobacterium Breve* NCFB2258 and GABA-secreting *Lactobacillus brevis* DPC6108 were evaluated using immunofluorescent labelling, calcium imaging and extracellular electrophysiology on tissue from Sprague Dawley rats.

Results: When applied to colonic mucosa, both *Bifidobacterium Breve* NCFB2258 and *Lactobacillus brevis* DPC6108 stimulated increased nuclear expression of the early transgene, cFos and a robust increase in neuronal $[Ca^{2+}]_i$ in underlying submucosal neurons. The calcium response evoked by PUFA-secreting *Bifidobacterium Breve* NCFB2258 was reduced by the PPARα antagonist, GW6471. The calcium response evoked by GABA-secreting *Lactobacillus brevis* DPC6108 was inhibited by bicuculline, a $GABA_A$ receptor antagonist and to a lesser extent by the $GABA_B$ receptor antagonist, phaclofen. Increased firing in vagal afferents evoked by *Bifidobacterium Breve* NCFB2258 was also attenuated by the PPARα antagonist whereas bicuculline suppressed the stimulatory effect of *Lactobacillus brevis* DPC6108 on vagal firing.

Conclusions: These findings illustrate that both PUFA and GABA secreted by different bacterial strains have the capacity to modulate intrinsic and extrinsic neurons across an intact gut barrier in a healthy rodent model. Supernatants induced activation of submucosal neurons, which locally regulate gut absorption and secretion and also, increased vagal afferent firing, which has been implicated in the microbiota-gut-brain signalling axis. These findings begin to elucidate how the host central nervous system is informed about changes in the external environment of the gut lumen.

68 | Neuronal signaling to stem cells via adrenergic receptors in gut organoids and intestinal homeostasis

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Neuro-epithelial crosstalk is an understudied but emerging mediator of intestinal homeostasis. The extrinsic nervous system of the GI tract comprises a complex network of sensory and autonomic neuron populations that densely innervate the intestinal tissue, including the epithelial lining of the gut, and release neurotransmitters and neuropeptides that act on surrounding cells. The molecular logic of how neurons signal to gut epithelial cell progenitors is not well understood. In addition, very little is known about the molecular mechanisms underlying how neuro-epithelial crosstalk affects the tissue more broadly at homeostasis and during inflammation. To identify potential neuro-epithelial signaling axes, we analyzed small intestine and colon single-cell RNAseq data of epithelial cell types to characterize neurotransmitter and neuropeptide receptor expression. We identified ten candidate receptors expressed by intestinal epithelial cell populations comprising four major receptor classes: adrenergic, nicotinic, muscarinic, and neuropeptide receptors. Utilizing intestinal organoid monolayers and high-throughput imaging, we performed a screen to characterize how endogenous neuropeptides, neurotransmitters and receptor agonists affected the frequency of stem cell, enterocyte, goblet cell, Paneth cell and tuft cell lineages during differentiation. We found that treatment of gut organoid monolayers with the α2 adrenergic receptor medetomidine hydrochloride resulted in a significantly increased number of proliferative Lgr5+ intestinal stem cells. This finding is consistent with the selective expression of the α-2a adrenergic receptor (Adra2a), but not other α2 receptors, in intestinal stem cells. We have confirmed this finding in three-dimensional intestinal organoids, where treatment with α2 but not β2-specific adrenergic receptor agonists results in a significantly increased proportion of Lgr5+ stem cells. Moreover, treatment with the endogenous receptor ligand norepinephrine leads to significantly more Lgr5+ stem cells in a dose-dependent manner. Future

work will explore the role of sympathetic neurons and Adra2a signaling in intestinal stem cell maintenance and proliferation in vivo as well as the molecular mechanism underlying this phenomenon.

73 | The role of sympathetic nerves in modulation of guinea pig colon motility

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Background/Objectives: The sympathetic nervous system acts on the gut to inhibit motility, however the effect of sympathetic activity manipulation in vivo on faecal output is unknown. In our pilot study, we examined the outcomes of surgical sympathectomy of the distal colon on faecal pellet output followed by in-vitro motility experiments.

Methods: Adult guinea pigs underwent a laparotomy under inhalational anaesthesia according to our institutional animal ethics guidelines. All para-vascular nerves associated with the inferior mesenteric artery (IMA), majority of which are sympathetic efferents, were sharply divided and the artery swabbed with aqueous phenol for further chemical ablation. Pre- and post-operatively the daily faecal pellet output (FPO) was recorded for 7 days. Two stress-tests were also performed on each animal pre- and post-operatively during which the FPO was recorded.

Control animals (n = 4) and sympathectomy animals (n = 2) were euthanized and motility characterised in vitro. Spatiotemporal maps and suction electrodes recorded motility and smooth muscle electrical activity during spontaneous emptying and artificial pellet propulsion. Ring electrodes were used to stimulate the para-vascular nerves of the IMA. Degeneration of sympathetic fibres in the colon was confirmed by immunohistochemistry for tyrosine hydroxylase.

Results: Electrical stimulation of the IMA nerves in the sympathectomy animals had no effect on pellet propulsion compared to controls, where stimulation stopped pellet propulsion. There was a trend towards a higher FPO in the first 24 hours postoperatively compared to preoperative numbers.

Conclusion: Activation of sympathetic fibres in-vitro inhibits distal colon motility. Potential compensatory mechanisms in-vivo may counteract the effects of surgical sympathectomy.

74 | Role of dopaminergic neurons in regulating peristalsis of rat proximal colon

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Background/Objectives: Constipation is a commonly seen defecation disorder in the patients with Parkinson's disease in whom a loss of dopaminergic neurons in the enteric nervous system has been demonstrated. However, the causal relationship between the loss of dopaminergic enteric neurons and constipation remains to be elucidated. Here, we aimed to investigate the roles of dopaminergic neurons in regulating colonic peristalsis.

Methods: Isolated segments of rat proximal colon were cannulated at both ends, and luminally perfused with degassed 0.9% saline, while extraluminally perfused with warmed (36°C), oxygenated Krebs solution. Colonic movements were monitored by a video camera, and transformed into maps of circumferential gut diameter using the spatio-temporal mapping technique. Fluorescent immunohistochemistry was also carried out to identify the distribution of dopamine receptors.

Results: In the colonic segments, oro-anal propagation of peristaltic waves was periodically generated. The peristaltic waves were prevented by neural blockade with tetrodotoxin (0.6 μmol/L), and thus driven by neuronal activity. Bath-applied SCH-23390 (20 μmol/L), a D1-like dopamine receptor antagonist, largely reduced the frequency of the peristaltic waves or abolished their generation. Upon the inhibition of the peristaltic waves, non-propagating, tetrodotoxin-insensitive contractions were generated. Bath-applied sulpiride (10 μmol/L), a D2-like dopamine receptor antagonist, decreased the frequency of peristaltic waves but did not prevent their generation. Unexpectedly, bath-applied dopamine (3 μmol/L) abolished the peristaltic waves associated with a colonic dilation in a manner of being blocked by SCH-23390 (5 μmol/L). D1 dopamine receptor immunoreactivity was detected in enteric neurons as well as non-muscle cells in the muscle layers.

Conclusions: Endogenous dopamine appears to act predominantly on D1-like dopamine receptors expressed in enteric neurons and/or intramuscular interstitial cells of Cajal to facilitate the colonic peristalsis. In contrast, exogenous dopamine prevents the peristalsis via the activation of D1-like dopamine receptors that are presumably expressed in a different population of cells.

75 | A randomized double-blind placebo controlled trial on the effect of stimulative laxative and osmotic laxative in patients with chronic constipation

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Background and Aim: No reported randomized controlled studies have investigated the efficacy of anthraquinones stimulant laxative for chronic constipation of, which have frequently been used in Japan. We conducted a double-blind placebo-controlled trial of the effects of stimulant laxatives and osmotic laxative on constipation symptoms, stool shape, and quality of life (QOL) in patients with chronic constipation.

Patients and Methods: The subjects were 90 patients with functional constipation who met the Rome IV criteria. They were randomly assigned 30 each to a stimulant laxative group (sennoside), an osmotic laxative group (magnesium oxide: MgO), and a placebo group. Stimulant laxative capsules (0.25 g/capsule), osmotic laxative capsules (0.25 g/capsule), or placebo capsules (0.25 g/capsule) were given to the respective groups two at a time, three times a day after each meal, for 28 days. Subjects were asked to keep a stool diary throughout the trial. The primary endpoint was overall improvement of symptoms. Secondary endpoints were spontaneous bowel movement (SBM) or complete spontaneous bowel movement (CSBM), stool shape, abdominal symptoms, and QOL (Japanese version of the Patient Assessment of Constipation Quality of Life [JPAC-QOL] questionnaire).

Results: The final analysis was performed with 30 patients in each group. The responder rate of overall improvement was 11.7% in the placebo, 69.2% in the Sennoside, and 68.3% in the MgO ($P < 0.0001$). The change in SBM was significantly greater in the Sennoside and the MgO than in the placebo ($P < 0.001$). The change in CSBM was also significantly greater in the Sennoside and the MgO than in the placebo ($P < 0.001$). The change in stool shape was significantly greater in the Sennoside and the MgO than in the placebo, with softening of the stool ($P < 0.001$). On the JPAC-QOL, significant improvements were seen in the Sennoside and the MgO compared with the placebo (Sennoside: $P < 0.005$, MgO: $P < 0.005$).

Conclusion: Anthraquinone stimulant laxative and MgO significantly improved the frequency of bowel movements, stool shape, QOL score, and are thought to be effective drugs for the treatment of constipation.

76 | Short term treatment of achalasia by dark chocolate

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Background: Pneumatic dilatation or myotomy for achalasia are usually efficient. Pharmacological treatment (Ca channel blockers or nitrates) is usually minimally helpful and accompanied by side effects.

Aim: To test the efficacy of dark chocolate (containing flavonol – a nitric oxide donor) in improving achalasia symptoms and esophageal physiology.

Methods: Consecutive naive patients diagnosed as suffering from primary achalasia (diagnosed by gastroscopy and/or barium meal plus manometry). All patients were randomized into 2 groups – treatment arm used dark chocolate, 75% cacao solids and compared to placebo arm – patients using "white" chocolate. All underwent repeat manometry 15 minutes after chewing and melting 21gr of chocolate to test the immediate effects of the chocolate on esophageal function. All patients were discharges with dark chocolate for a period of 2 weeks and were instructed to chew and melt 7gr of chocolate 3 times a day, 15 minutes before each meal. All patients filled a daily symptom diary and health related quality of life questionnaire (HRQL).

Results: 27 patients were recruited for the study. 4 omitted because of subsequent refusal. 19 agreed to undergo 2 manometries before and after chocolate consumption (10 study group, 9 controls) all patients were followed by symptom and HRQL questionnaires. No significant changes in LES pressure before and after treatment were observed for each group and no significant differences between the 2 groups (+6.8 mm Hg vs. −1.38 mm Hg). Esophageal body pressures declined more in the study group, however, not statistically significant. After 2 weeks of treatment, statistically significant changes were observed in the sense of food limitation, solid food dysphagia and regurgitations ($P < 0.01$). No significant changes were observed for liquid dysphagia, vomiting, nausea, chest pain and heartburn.

Discussion: In this small pilot study, chocolate was able to modify some symptoms related to dysphagia, however, it did not show efficacy in changing physiological parameters.

78 | Enriched environment housing alleviates post-colitis visceral hypersensitivity in male and female rats

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Background/Objectives: Adverse psychosocial events contribute significantly to the relative risk of developing post-infectious irritable bowel syndrome. We modeled adverse psychosocial factors by single housing and adverse biological factors by the combination of neonatal and adult colonic inflammation. Our objectives are to evaluate the effects of differential housing conditions on development of post-inflammatory visceral hypersensitivity (VHS) and gene expression in various brain nuclei in male and female rats.

Methods: Ten-day old neonatal rats received colon infusion of trinitrobenzene sulfonic acid (TNBS) and were either single housed (SH) or placed in enriched environment housing. Controls received saline and were conventionally housed after weaning. At 8 weeks, EH and SH rats received colonic infusion of TNBS; 3 weeks later colonic sensitivity was measured The conditioned place preference (CPP) test was used to measure the magnitude of analgesic-induced relief (intracolonic 2% Lidocaine) from ongoing pain.

Results: Females displayed significantly higher VHS than males in all three experimental conditions. Within females, single housed rats were significantly more sensitive compared to control $P < 0.001$, and to enriched environment housed rats, $P = 0.01$. Within males, single housed rats were significantly more sensitive compared to control, $P < 0.001$ and to enriched environment housed rats, $P = 0.003$. In the CPP test, both male $P < 0.001$ and female $P < 0.001$ SH rats displayed significantly greater changes in preference for the lidocaine-paired chamber compared to control rats. EH significantly reduced change

in preference in both male $P = 0.035$ and female rats, $P = 0.001$ compared to SH rats. We found significant increases in BDNF protein expression in both male and female SH rats in the nucleus accumbens (NAc) compared to their respective controls and EH housed rats. Knockdown of BDNF expression in the NAc significantly reduced CPP in post colitis single housed males and female.

Conclusions: Single housed rats displayed evidence of an aversive state and hypersensitivity to colorectal distension following recovery from colitis. These conditions were significantly ameliorated by enriched environment housing or by knockdown of BDNF expression in the NAc.

79 | Symptoms elicited by a rapid drink challenge test in patients referred to esophageal manometry

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Introduction: Association between motor disturbances detected during esophageal manometry, and the symptoms referred by patients during the exploration is poor.

Aim: To evaluate the symptoms elicited by a rapid drink challenge test (RDC) in patients referred to high resolution esophageal manometry (HREM), and to correlate these symptoms with the pressure responses to the test.

Methods: We studied 1312 consecutive patients (856 F, 465 M, mean age 58 ± 15 years) referred to esophageal manometry. In each patient, a 200-ml RDC was performed after the standard protocol of HREM, and patients were asked to score (from 0 to 3), the symptoms (dysphagia, chest pain and regurgitation) elicited by the RDC. Association between pressure responses and symptoms were analyzed.

Results: The RDC was successfully performed and analyzed in 1302 patients, elicited symptoms in 601 (46%) patients, and 81% of these symptoms were recognized by patients as their customary symptoms. Dysphagia was the most common symptom (referred by 26% of patients), followed by regurgitation (13%) and chest pain (10%). According to previously described patterns of pressure responses to the RDC (Neurogastroenterol Mot 2016; 28:543-553), 74% of patients had a normal pressure response, 13% had a brief hyperpressive non-obstructive pattern, and 13% had an obstructive pressure pattern, with an excellent association between the specific pressure pattern and the manometric diagnose according to Chicago 3.0 ($P < 0.001$). Likewise, we found a significant association between the pressure pattern in response to the RDC, and the intensity of dysphagia ($P < 0.001$), and regurgitation ($P < 0.001$) elicited by the test, but not with the intensity of chest pain ($P = 0.125$).

Conclusion: The RDC may elicit customary symptoms in patients with suspected esophageal motility disorders. Dysphagia and regurgitation are associated to obstructive pressure responses during the test, whereas chest pain is not. This test could be useful to demonstrate an association between obstruction across the esophagogastric junction and dysphagia/regurgitation during esophageal manometry.

80 | Changes in enteroendocrine cells in response to high fat diet

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Background and Objectives: Enteroendocrine cells (EEC) contained in the gut are an emerging system of interest in the treatment of obesity. Changes in EEC populations and the enteroendocrine peptides they secrete have been reported in obesity in rodent and human studies. It is not clear which peptides change and whether changes precede or follow weight gain. This study aimed to characterize changes in EEC populations in jejunum and stomach in response to a high fat diet (HFD).

Methods: 10-week-old male C57Bl6 mice were fed a control diet or HFD (10.7% and 59.5% energy from fat, respectively) for 16 weeks. Body composition was measured weekly and oral glucose tolerance was measured after 16 weeks. EEC in jejunum and stomach were assessed using immunohistochemistry. EEC immunoreactive for serotonin, secretin, ghrelin, GIP, CCK, oxyntomodulin, somatostatin, neurotensin, and PYY were counted in jejunal samples. Stomach samples were analysed for EEC immunoreactive for serotonin, somatostatin, and ghrelin.

Results: HFD fed mice gained varying degrees of weight. Overall, HFD fed mice exhibited significant total weight and body fat gain, and slightly impaired glucose tolerance compared to controls. EEC immunoreactive for all peptides tested were observed at higher densities in the jejunum of HFD fed mice. Significant ($P > 0.05$) increases in EEC density were noted for ghrelin, GIP, oxyntomodulin, neurotensin, and PYY. There was a strong correlation ($r < 0.6$) between EEC density and body fat percentage. No significant changes in tested EEC populations in the corpus were observed between diet groups.

Conclusions: Our results suggest a correlation between body fat percentage and EEC density in the jejunum, but not stomach. Jejunal EEC are exposed to advanced products of digestion, unlike gastric EEC. These results may suggest that high fat diet exerts a direct effect on EEC that influences their peptide production. This is being investigated further.

81 | Disparities in gastric emptying and postprandial glycaemia between Han Chinese and Caucasians with type 2 diabetes

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Aims: Gastric emptying is a major determinant of postprandial glycaemia in both health and type 2 diabetes (T2DM); the potential impact of ethnicity on gastric emptying is unclear. We compared the rate of gastric emptying of a standardised meal and the associated glycaemic response in Han Chinese and Caucasian patients with T2DM.

Methods: Fourteen Han Chinese and 14 Caucasian T2DM patients, managed by diet and/or metformin monotherapy, underwent concurrent measurements of gastric emptying and blood glucose for 240 minutes after a 99mTc-calcium phytate-labelled mashed potato meal.

Results: Han Chinese patients were slightly younger ($P < 0.05$), and had a lower BMI ($P < 0.05$), than Caucasians. There were no differences in either HbA1c or fasting blood glucose between them. Gastric half-emptying time (T50) was shorter ($P < 0.05$) and the postprandial blood glucose increment greater ($P < 0.05$) in Han Chinese than Caucasian patients. Both the increment in blood glucose from baseline at 60 minutes and peak blood glucose were related inversely to T50 ($P < 0.05$ each).

Conclusions: Han Chinese with relatively well-controlled T2DM have more rapid gastric emptying compared to Caucasians, which is associated with a greater postprandial glycaemic excursion. These differences may inform the choice of management, e.g. Han Chinese may particularly benefit from therapies that slow gastric emptying.

Keywords: ethnicity; gastric emptying; postprandial glycaemia; type 2 diabetes

83 | Analysis of chemical coding reveals multiple distinct classes of 5-HT containing enteroendocrine cells

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Background and Objectives: 5-HT containing enteroendocrine cells (EEC), referred to as enterochromaffin cells (EC), have for many years been considered to be a single type of hormone-secreting cell, distinct from EEC that contain peptide hormones. However, 5-HT that originates from EC has a very wide range of actions, and limited expression data suggest that there are subtypes of these cells that also contain peptide hormones. The objective was to produce a reporter mouse in which all EC were marked and to use immunohistochemistry to investigate subtypes of EC.

Methods: We generated a tryptophan hydroxylase (TPH1)-bacTRAP-GFP mouse to locate 5-HT cells and a NeuroD1- bacTRAP-GFP mouse to label all enteroendocrine cells (EEC). Fixed tissue from all gut regions was investigated. Tissues were double or triple stained to determine the chemical coding of the EC cells.

Results: 5-HT cells were a high proportion of all EEC, ranging from 55% to 72% of EEC in the duodenum, jejunum, proximal and distal colon. There was a substantial population of 5-HT/CCK cells in the duodenum. Of 5-HT/CCK cells, 80% had ghrelin immunoreactivity, 40% contained oxyntomodulin, and fewer than 10% contained detectable GIP or neurotensin. A major jejunal cell group expressed 5-HT and tachykinins. In this region, 59% of 5-HT or TK cells contained both hormones, 25% had only tachykinin immunoreactivity and 16% had only 5-HT. There was also widespread colocalization of 5-HT and secretin. In the duodenum, about 35% of 5-HT cells were immunoreactive for secretin, which represents about 85% of secretin containing cells. 5-HT/secretin cells were also discovered in the proximal colon. 5-HT cells had prominent basal processes, some several 100 μm long, in the distal colon, but not in the stomach or small intestine.

Conclusions: 5-HT cells, defined by colocalization with peptide hormones, form multiple subclasses. The different functions of the chemically-defined subclasses are yet to be determined.

84 | Clinical assessment after peroral endoscopic myotomy for the treatment of esophageal achalasia and esophageal motility disorders

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Background: Recently, peroral endoscopic myotomy (POEM) has been performed as an initial method in place of laparoscopic myotomy or balloon dilatation as a treatment for achalasia and other major motility disorder. We describe our clinical assessment after POEM procedures performed at our institution.

Methods: Patients who underwent POEM for achalasia and major esophageal motility disorders at tertiary medical center were included. Various follow-up exam data including immediate endo-luminal functional lumen imaging probe (Endo FLIP), high resolution manometry (HRM), esophagogram, esophageal transit time scan and subjective symptom using Eckardt score were analyzed retrospectively.

Results: Sixteen patients (44.2 ± 17.1 years, 62.5% male) were performed POEM. The Eckardt score was significant lower after POEM (7.1 ± 2.52 vs. 1.2 ± 0.9, $P < 0.001$). Although not all patients

underwent postoperative follow-up examinations, immediately intraoperative pre and post Endo FLIP (n = 4, distensibility index at 50 mL, 2.57 ± 0.86 vs. 6.50 ± 2.43, *P* = 0.02), integrated relaxation pressure on HRM (n = 9, 25.87 ± 9.83 vs. 12.88 ± 7.27, *P* = 0.004), maximal diameter on esophagogram (n = 16, 3.43 ± 1.47 vs. 2.64 ± 1.19 *P* = 0.008) were significantly improved. However, retention rate on esophageal transit time scan or diameter of esophagogastric junction did not show any significant change.

Conclusion: Along with the Eckardt score, which confirms with subjective symptoms, several objective post-POEM evaluation tests have been proposed recently. Although a small number of analysis results, the intraoperative Endo FLIP data, follow-up HRM and esophagogram tests can be helpful for predicting clinical success. Further well designed study is needed to confirm this usefulness.

85 | Alterations of gut microbiota profiles in patients with functional abdominal bloating/distension

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Background/Objectives: The pathophysiology of functional abdominal bloating/distention (FABD) is not fully understood yet, and fecal microbiota of those who have FABD compared to healthy individuals have not been analyzed. The aim of the study was to evaluate whether fecal microbiota diversity and composition are altered in FABD patients, compared with healthy individuals.

Methods: Gut microbiota diversity and composition of fecal samples from 33 subjects were prospectively studied. The 33 subjects consisted of 12 healthy controls (HC) and 21 patients diagnosed with FABD by the Rome IV criteria. FABD patients were further divided into two groups according to results from the hydrogen breath test for the evaluation of small intestinal bacterial overgrowth (SIBO). Fecal microbiota analysis was performed by 16S rRNA amplification sequencing.

Results: The hydrogen breath test results showed that 9 patients were SIBO negative and 12 patients were SIBO positive. Microbiota diversity was significantly lower in the bloating group than the healthy control group in both Shannon (4.764 ± 0.166 vs. 3.350 ± 0.181, *P* < 0.001) and inversed Simpson index (0.932 ± 0.008 vs. 0.780 ± 0.025, *P* < 0.001). The proportion of Proteobacteria (FABD vs. HC, 10% vs. 2%, *P* = 0.007) was significantly higher in FABD than in HC, and the proportion of Actinobacteria (0.3% vs. 9%, *P* < 0.001) was significantly lower in FABD than in HC. The proportion of Faecalibacterium (3.13% vs. 12.87%, *P* = 0.002) was significantly higher in FABD than in HC. The proportions of Prevotella (HC vs. SIBO (+), 4.46% vs. 21.23%, *P* = 0.024) and Faecalibacterium (3.13% vs. 16.05%, *P* = 0.002) were significantly higher in SIBO (+) than in HC.

Conclusions: Gut microbiota profiles in patients with FABD differ from those in healthy individuals. Those alterations in gut microbiota may play a role in the pathogenesis of the FABD.

87 | Multifactorial anti-inflammatory activity of STW5-II towards murine intestinal organoids

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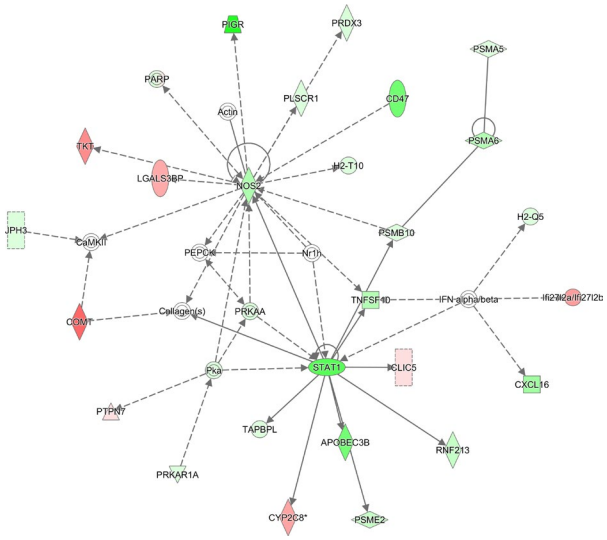
Background: Intestinal inflammation is a key player in the pathogenesis of many functional and organic gastrointestinal diseases. Inflammatory bowel diseases such as ulcerative colitis and Crohn's disease are characterized by high grade inflammation, while irritable bowel syndrome is associated with low-grade inflammation.

Aim: To establish organoids-based model of intestinal inflammation and to investigate the molecular mechanisms of the herbal medicinal product STW 5-II.

Methods: Mouse intestinal organoids were cultured in Matrigel domes. Organoids were treated with increasing doses of STW 5-II for 24 hours followed by inflammation induction. A cocktail of pro-inflammatory cytokines (IFNγ, TNFα, IL-1β, IL-6) and bacterial components (lipopolysaccharide LPS-B5, flagellin) was used to induce intestinal inflammation. The exact mechanisms of STW 5-II were elaborated using microarrays analysis, qPCR, western blot, immunohistochemistry and immunofluorescence. We have investigated the expression of many pro-inflammatory genes such as STAT1, NFκB, NOS2, TNFα and IFNγ in addition to some anti-inflammatory mediators such as SIRT1 and IL10.

Results: Microarrays analyses highlighted multiple inflammatory pathways that could be affected by STW 5-II. It decreases the expression of pro-inflammatory mediators such as STAT1, NFκB, NOS2, TNFα and IFNγ, while it upregulates the anti-inflammatory mediators such as SIRT1 and IL10. These findings were further confirmed by qPCR. Corresponding protein expression was confirmed by immunoblotting, IHC and immunofluorescence. STW 5-II was effective in a dose dependent manner in attenuating the cytokines-mediated inflammation within the organoids.

Conclusion: STW 5-II shows very potent anti-inflammatory activity with multiple mechanistic targets. STW 5-II could be a promising therapy to alleviate intestinal inflammation associated with IBS, UC and Crohn's disease.



Microarrays analyses showed some affected proinflammatory targets upon treatment with STW 5-II.

91 | Inability to Belch described by community-based participatory research and impedance-manometry

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Background/Objectives: Patient-initiated social media research bypasses academic-centered scientific process and involves community members in design and implementation. Inability to belch is rarely reported in the medical literature and there are not diagnostic criteria. The objective of this study was to utilize a patient-doctor research partnership to evaluate symptom experience and determine physiologic characteristics of a case example.

Methods: Using the Reddit social-media website, patients with shared symptom experience of inability to belch created an electronic survey with iterative refinement based on community member recommendations. The survey was open, voluntary, and anonymous, collecting data in January 2019.

Due to inadequate available information in traditional clinical and scientific resources a gastroenterologist partnered with the survey community to identify shared goals and create a research plan.

Results: Survey participants (n = 789) were from 36 countries with equal gender distribution and 83% between 25 and 44 years. Inability to belch was life-long (85%) and typically worsened throughout the day. Bloating (89%), gurgling (88%), and excessive flatulence (81%) were common symptoms. Themes identified included avoiding triggers of carbonated or alcoholic beverages and self-induced "air vomiting" for acute symptom relief. Despite significant social, romantic, and professional impairment, only 32% brought concerns to a general physician and 3% to a specialist. Few patients seen by a general physician felt concerns were taken seriously (20%) and very few were offered treatment (6%).

The example case was a 16-year-old female referred with lifelong inability to belch and bothersome chest gurgling without dysphagia or weight loss. Esophageal impedance-manometry did not identify a major motor disorder. Resting upper esophageal sphincter (UES) pressure and UES relaxation with wet swallows was normal. Esophageal impedance-manometry (Fig 1) demonstrated gastric belch associated with UES contraction, and antegrade clearance of esophageal air only after swallow.

Conclusions: Community-based participatory research on inability to belch allowed symptom validation, focus on patient-reported outcomes, and a data-driven partnership with the medical community. Efforts to improve awareness, identify physiologic mechanisms, and optimize treatment are needed.

92 | Effects of lixisenatide versus liraglutide on esophageal functions and motility in patients with type 2 diabetes

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Background/Objectives: GLP-1 receptor agonists (GLP-1 RAs) are increasingly used in patients with Type 2 diabetes mellitus (T2DM) and are known to influence gastrointestinal motility. Short-acting GLP-1 RAs (e.g. Lixisenatide) markedly delay gastric emptying whereas long-acting GLP-1 RAs (e.g. Liraglutide) have only little effects on gastric motility. Whether GLP-1 RAs influence esophageal motility is unclear. Therefore, the current study aims to assess the effects of GLP-1 RAs on esophageal motility.

Methods: 57 people with T2DM were randomized to a 10-week treatment with liraglutide or lixisenatide in a double-blind manner. High-resolution esophageal manometry (HRM) was utilized to assess esophageal function at baseline and after treatment. Endpoints were changes in resting pressure and relaxation of the lower esophageal sphincter (LES), mean and maximum distal pressure amplitude and distal contractility integral (DCI).

Results: HRM was successful in 23 participants of the liraglutide and 19 of the lixisenatide group. Baseline measurements of HRM were not different between the groups. Neither lixisenatide, nor liraglutide led to a significant change in the endpoints. In the subgroup of patients with a reduced distal contractile integral (DCI) of <450 at baseline, a significant increase of the DCI was observed in both groups (P = 0.031 in liraglutide and P = 0.012 in lixisenatide).

Conclusions: Lixisenatide and liraglutide do not exert major impact on the motility of the LES and esophageal motility in T2DM. DCI may be influenced by GLP-1 RAs, but further studies need to verify the present results.

(NCT number: NCT02231658).

93 | Epidemiology and management of achalasia in Korea based on nationwide 5009 patient dataset: Adoption of high-resolution manometry

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The incidence of achalasia has increased since the advent of high-resolution manometry (HRM) in Western countries. However, the precise epidemiology of achalasia in Korea is still unknown. Therefore, we aimed to determine the incidence, epidemiological features, and treatment modalities of achalasia in the entire population of Korea using information from nationwide dataset. We evaluated data from the Korean Health Insurance Review and Assessment Service Database for all patients diagnosed with primary achalasia (ICD-10 code: K22.0) between 2008 and 2016. The demographics and treatments were also reviewed. A total of 5009 achalasia cases (53.6% of whom were females) were identified for the period from 2008 to 2016. The mean incidence per 100 000 individuals over the study period in Korea was 1.1 (95% CI 1.03 to 1.16). We observed a steadily increasing trend in the overall incidence rate from 1.03/100 000 in 2008 to 1.27/100 000 in 2016 (P for the trend 0.014; Figure 1). This increase in incidence coincides with the beginning of the widespread use of HRM in Korea in 2011. The incidence of achalasia increased with age (Spearman rho, 0.77; P = 0.0003). During the study period, 1350 patients (26.9%) underwent balloon dilatation and 130 patients (1.9%) underwent surgical myotomy. Achalasia diagnosis in Korea appears to have increased over the past 10 years similarly to Western countries, possibly due to the improved diagnostic modality of HRM.

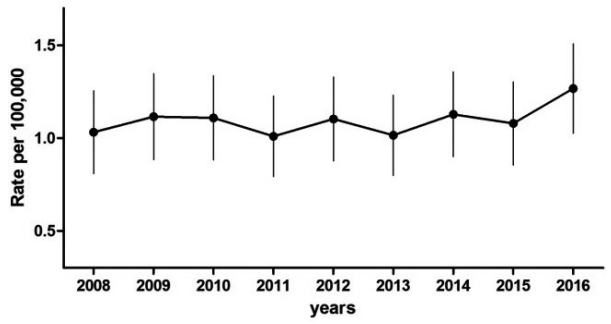

Figure 1. The incidence of achalasia in Korea between 2018 and 2016. (P = 0.014)

†P value was estimated by Cochran-Armitage trend test.

95 | Enteric neuropathy in animal models of Parkinson's disease

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Background: Parkinson's disease (PD) is associated with nerve damage in the brain and gut, but the nature of intestinal (enteric) nerve damage and gut dysfunction in PD progression remains unclear. This work aims to identify structural and functional changes in the enteric nervous system (ENS) of commonly used mouse models of PD that are known to exhibit central neuropathy and a gut phenotype.

Methods: Enteric neuropathy was assessed in MPTP-treated mice (15 mg/kg, IP, 3 doses, 3 hours apart at 16 weeks, taken 19 weeks), mice with 6OHDA administered into the substantia nigra (3 µg, at 16 weeks, taken 18 weeks) and rotenone-treated (30 mg/kg, oral, for 28 days, taken 8 weeks later) wild-type and A53T transgenic mice expressing human mutant α-synuclein. Enteric neuropathy was assessed in the ENS by immunohistochemistry for the pan-neuronal marker Hu and neuronal NOS (nNOS). Hu translocation to the nucleus, an indication of ENS stress, was quantified.

Results: MPTP treatment had no effect on the number of Hu+ neurons in colon or ileum but was associated with an increase in Hu+ nuclear translocation in colon (P < 0.0001), and a decrease in the proportion of nNOS+ neurons. 6-OHDA lesion had no effect on the number of neurons or degree of Hu+ translocation; however, it significantly reduced the proportion of nNOS neurons in colon. No change in the number of Hu+ neurons in colon was found when comparing WT vs. A53T mice at 32 weeks, however a reduction in the number of Hu+ neurons was found following rotenone administration in WT mice (P < 0.06).

Conclusions: All mouse models of PD exhibit gut dysfunction, but our study showed that the extent and nature of enteric neuropathy differs across models. These findings suggest that the extent and type of damage to the ENS is dependent on the model and the stage of disease and that functional changes in GI motility may not be directly attributable to changes in enteric neurons.

96 | Dopamine/ghrelin receptor heteromers and their roles in lumbo-sacral defecation centres

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Background and Objectives: Both dopamine and ghrelin activate the lumbo-sacral defecation centres and cause propulsive contractions of the colorectum. Dopamine, but not ghrelin, is found in nerve terminals in the defecation centres and dopamine could possibly be a neurotransmitter of the defecation pathways. There is evidence that dopamine D2 receptors (DRD2) and ghrelin receptors (GhrR) form heteromers in lipid membranes and transfected cells. Our studies were undertaken to determine whether dopamine, acting at DRD2/GhrR heteromers could have a physiological role in defecation.

Methods: Physiological studies were conducted in anaesthetized and conscious rats. DRD2 localization was investigated in reporter mice and by immunohistochemistry in rats and mice. GhrR was localized in reporter mice and by in situ hybridization histochemistry in rats. Dual expression was investigated in neurons of the mouse and human spinal cord.

Results: Ghrelin receptor agonists applied systemically or directly (intrathecally) to the defecation centres caused colorectal propulsion and defecation. Similar effects on colorectal function were observed in response to dopamine (intrathecal) or DRD2 agonists (IT or systemic). For both agonists, responses to IT application were prevented by cutting the nerve connections from the spinal cord to the colorectum. Behaviourally induced defecation in rats (water avoidance test) was reduced by both the DRD2 antagonist, sulpiride, and the GhrR antagonist, YIL781, suggesting the physiological involvement of both receptors. The effect of a DRD2 agonist, quinpirole, applied directly to the defecation centres of rats in vivo, was antagonized by systemic YIL781, indicating that activation of DRD2 directly or indirectly activates GhrR. GhrR was found in neurons of the defecation centres, but not in down-stream neurons. Neurons in the defecation centres of mice and human were revealed by in situ hybridization to express both receptors.

Conclusions: DRD2 and GhrR are in the same neurons of the defecation centres and are functionally involved in the defecation control pathways. Our pharmacological and localisation studies are consistent with these receptors forming heteromers and with dopamine being the physiological activator of the heteromeric DRD2/GhrR complex. The complex can be activated by both dopamine and ghrelin, but only dopamine has a physiological role.

97 | Amino acid uniporter LAT4 is under diurnal and dietary control in the small intestine of mice

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Food entrainment and circadian rhythms are important regulators of digestive function, including nutrient transport. Here we investigated influences on the intestinal L amino acid transporter LAT4 (SLC43A2), a sodium-independent uniporter that has been suggested to act as a gatekeeper for amino acid absorption. We have characterized two phosphorylation sites on LAT4 – Ser274 and Ser297 and discovered that (de-)phosphorylation regulates LAT4 function during food anticipation. To investigate whether LAT4 shows circadian and dietary regulation we tested its expression, localization and phosphorylation in small intestine of food-entrained mice administered different diets. Intestinal villi were isolated and treated with potential regulators of LAT4. In contrary to multiple nutrient symporters and antiporters, there was no circadian regulation of LAT4 mRNA expression. However, we observed intestinal segment-specific differences in LAT4 protein expression and phosphorylation. During food anticipation (ZT12) and feeding (ZT16), the duodenum was the only segment showing an increase in LAT4 protein expression, whilst in jejunum we detected a decrease in phosphoSer274 and increase in phosphoSer297, suggesting upregulation of LAT4 function. Ileum showed the strongest LAT4 regulation, with near absence of Ser274 phosphorylation at ZT16, accompanied with strong phosphorylation of mTORC1 target S6 in both crypts and villi. Decreasing dietary protein content from 18% to 8% led to internalization of LAT4 and increased Ser274 phosphorylation, suggesting a major regulatory decrease in transport capacity. Surprisingly, when dietary protein content was increased to 40%, we observed only a slight increase in jejunal LAT4 expression, without clear change in phosphoSer274 levels. In isolated intestinal villi, protein kinase A stimulation led to an increase in Ser274 phosphorylation, whereas treatment with insulin and amino acids increased phospho-Ser297 levels. In conclusion, amino acid uniporter LAT4 is under circadian and dietary control and is potentially regulated within PKA and mTORC1 pathways.

98 | Identifying patterns of motor activity in the gastrointestinal tract of silver perch (*Bidyanus bidyanus*)

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Background: Silver perch, a fresh water fish, is capable of surviving in a wide temperature range (2-38°C), with their growth increased above 20°C. Using spatio-temporal mapping our aim was to quantify the myogenic and neurogenic origin of intestinal motility and determine the effects of temperature change upon the intestinal motility.

Methods: The entire intestine was removed from euthanised fish (n = 10) and cut in half. Proximal and distal regions were studies separately in organ baths with oxygenated Krebs maintain at either 15°C (n = 5) or 25°C (n = 5). In one fish the entire intact intestine was studied at 25°C. Motility was analysed during rest, after oral infusion of Krebs solution and after application of hexamethonium (100 μmol/L) and tetrodotoxin (TTX) (0.6 μmol/L).

Results: Antegrade and retrograde propagating contractions (PC) were recorded in all preparations. In the proximal intestine, at 15 and 25°C, retrograde PCs occurred at 2.68 [1.68-4.45] and 3.13 [1.61-6.46] times the frequency of antegrade PCs, respectively. These differences were not seen in the distal intestine. Hexamethonium did not inhibit PC. In contrast, in the proximal intestine at 15°C, hexamethonium caused an antegrade PC frequency 4.17 [2.04-9.17] times greater than during the infusion period. The warmer temperatures had no effect on the frequency of the PC, but did increase the length and speed of propagation. TTX abolished all contractile activity (Fig 1A). In the one fish in which the entire intact intestine was studied, cyclic retrograde activity originated at the anal end and propagated along the entire length (300 mm; Fig 1B).

Conclusions: Both antegrade and retrograde PC occur throughout the Silver perch intestine at both 15 and 25°C. These neurogenic motor patterns do not require enteric nicotinic transmission.

99 | Targeted gastric ablation as a method for modulating gastric slow wave pacemakers

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Background/Objectives: Slow-wave dysrhythmias have been associated with gastric motility disorders, offering an under-explored potential therapeutic target. Gastric ablation has demonstrated potential to induce conduction blocks in healthy tissue. However, the effects of targeted ablation on slow-wave pacemaker sites are currently unknown. This study aimed to utilise gastric-ablation to disrupt pacemaker sites and to evaluate feasibility of this technique to modulate slow-wave dysrhythmias.

Methods: In vivo experiments were performed in weaner pigs (n = 6, 42 ± 3 kg). Following laparotomy, high-resolution electrical mapping was performed on the gastric serosa to define the baseline slow-wave activation profile and locate normal and dysrhythmic gastric pacemaker sites. Radiofrequency ablation was then performed at the pacemaker sites (temperature-control mode, 70°C), followed by further high-resolution mapping of the post-ablation slow-wave profiles.

Results: High-resolution mapping enabled pacemaker site localisation in all pigs, and ablation successfully eliminated the pacemaking activity in all cases. Mean slow-wave amplitude decreased from 1.42 ± 0.15 mV to an undetectable amplitude at the ablation site. In 2 pigs, a dysrhythmic ectopic pacemaker was located and eliminated. An example case is shown in Figure 1, whereby antegrade propagation emerged from a pacemaking region proximal to the ablated ectopic site. In the other 4 cases, the natural pacemaker was targeted and eliminated, with a new pacemaker emerging immediately distal to the ablation site.

Conclusions: This study demonstrates the feasibility of gastric ablation to modify normal and dysrhythmic slow-wave propagation, guided by high-resolution electrical mapping. Targeted ablation of slow-wave initiation abnormalities is a potential future therapy for gastric dysmotility.

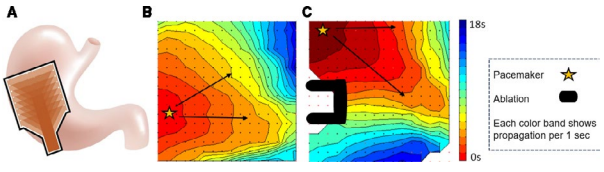

Figure 1. (A) Electrode array on the mapping location. (B) Ectopic pacemaker recorded before ablation. C) Ablation eliminated the ectopic activation pattern

100 | High prevalence of esophageal dysmotility in patients after brainstem stroke: A high resolution manometry study

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Objective: Using High resolution manometry (HRM) to evaluate the swallowing motility in esophageal stage in patients with brainstem stroke.

Methods: Eighteen patients and 10 healthy subjects were included in the study. There was no significant difference in age, gender, weight and body mass index (BMI) between two groups. All subjects underwent a high-resolution esophageal manometry to assess esophageal motility and diagnosed by Chicago classification ver.3.0 (CC).

Results: Among 18 patients with brainstem stroke, the proportion of abnormal esophageal dynamics was 77.8%, which was higher than that in the healthy control group (10%), but the difference was not statistically significant (χ^2 = 11.873, P = 0.001). Motility abnormality in patients manifested as Absent contractility, Jackhammer esophagus, EGJ outflow obstruction and distal esophageal spasm. Compared with the control group, patients' UES resting pressure was significantly lower, while the UES residual pressure, integrated relaxation pressure of LES were higher and the UES relaxation time were significantly shorter (P < 0.05). LES resting pressure, distal contractile integral, distal latency had no significant differences between two groups.

Conclusion: This study found that abnormal esophageal motility was highly prevalent in patients with brainstem stroke, which should be paid more attention to. In the future, evaluation of esophageal deglutition function in brainstem stroke patients should be further standardized.

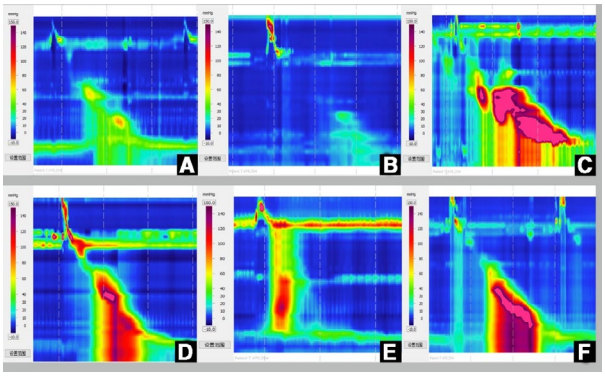

Figure 1. HRM abnormality in patients group

(A) EGJ outflow obstruction; (B) absent contractility; (C) Jackhammer esophagus; (D) distal esophageal spasm; (E) ineffective UES relaxation; (F) shortening of UES relaxation duration

101 | Allosteric modulation of the delta opioid receptor improves dysmotility in preclinical models of irritable bowel syndrome

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Introduction: Allosteric modulation is a therapeutically important conceptual advancement in the G protein-coupled receptor (GPCR) field. Despite being extensively studied in the CNS, allosteric modulation of GPCRs in the context of the enteric nervous system (ENS) and as a potential pharmacological approach for treating gastrointestinal (GI) disorders is largely unexplored.

Objectives: To examine allosteric modulation in the ENS using the delta opioid receptor (DOR) as a model GPCR, and to determine whether allosteric modulation is effective for treating GI symptoms in preclinical models of irritable bowel syndrome (IBS).

Methods: The DOR positive allosteric modulator BMS-986187 was characterized in the ENS by pharmacological analyses of neurogenic contractions of the mouse colon, and by DOR internalization assays using myenteric wholemount preparations from mice that express an enhanced green fluorescent protein tagged to the C terminus of DOR (DOReGFP). The ability of BMS-986187 to influence GI motility was examined both in vitro and in vivo.

Results: BMS-986187 enhanced the inhibitory effect of distinct DOR ligands on neurogenic contractions, whereas the potency and efficacy of mu and kappa opioid receptor agonists were unaffected. BMS-986187 augmented DOReGFP internalization to colonic

distention and to the partial DOR agonist ARM390, demonstrating modulation at the neuronal level. BMS-986187 alone reduced the number of motor patterns generated in the isolated colon, confirming that DOR-expressing myenteric neurons influence motility at the endogenous level. Finally, BMS-986187 reduced fecal output and diarrhea onset in the novel environment stress and castor oil models of IBS symptoms, respectively.

Discussion: Collectively, we report that BMS-986187 is an effective modulator of DOR in the ENS and ameliorates motility disturbances in preclinical models of IBS. This study supports allosteric modulation of GPCRs as a therapeutic approach for treating GI dysmotility.

102 | Effects of intragastric vs. intraduodenal administration of the bitter compound, quinine, on the glycaemic response to, and slowing of gastric emptying of, a mixed-nutrient drink, in healthy men

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Background/Objective: Preclinical studies have demonstrated that bitter compounds have potent effects on gluco-regulatory hormones (e.g. GLP-1, insulin) and gastrointestinal motor function (e.g. gastric emptying), which are key to the regulation of postprandial blood glucose. These effects are triggered by activation of bitter receptors located on enteroendocrine cells. There are substantial variations in the regional distribution of bitter receptor subtypes (i.e. a greater density in the duodenum than the stomach); therefore, delivery to the duodenum may be more effective in stimulating these functions to lower postprandial blood glucose. We compared the effects of intragastric (IG) vs. intraduodenal (ID) administration of quinine hydrochloride (QHCl) on the glycaemic response to, and gastric emptying of, a mixed-nutrient drink in healthy men.

Methods: 12 healthy lean men (age: 25 ± 2 years) received, on 4 separate occasions, 600 mg QHCl or control, either IG or ID, 60 minutes (for IG, to allow significant gastric emptying to occur) or 30 minutes (for ID) before a mixed-nutrient drink (500 kcal, 74 g carbohydrates), in randomised, double-blind fashion. Plasma glucose and C-peptide were measured at baseline, in response to quinine alone, and for 2 hours after the drink. Gastric emptying of the drink was measured for 2 hours (^{13}C-acetate breath test).

Results: QHCl alone stimulated C-peptide (AUC/min; control: 252 ± 29, QHCl: 357 ± 51; $P < 0.05$), with no difference between IG and ID QHCl, nor any effect on fasting plasma glucose. In response to the drink, C-peptide was greater ($P < 0.05$), and gastric emptying slower ($P < 0.05$), with a reduction in postprandial plasma glucose (mean at 30 minutes; control: 6.7 ± 0.3, QHCl: 5.4 ± 0.3 mmol/L, $P < 0.05$), after QHCl compared with control, with no differences between IG and ID QHCl.

Conclusions: IG and ID QHCl have comparative effects to stimulate insulin, slow gastric emptying and diminish postprandial glycemic excursion in healthy men.

103 | Embryonic development of motility: A bottom-up approach to the workings of the intestine

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A newborn baby spontaneously gravitates towards its mother's nipple and starts sucking on it with preternatural ease. Much as it will later learn to walk, see, talk and ride a bike, it *has* already learned to eat and digest, hidden from public sight, in its mother's womb. In this talk, I will present how the digestive reflex – intestinal motility – comes about, focusing on a particular species: the chicken. Different cell types involved in motility become active at different time points in development, allowing for a detailed understanding of the role of each of them in the orchestra of intestinal movements. In particular, we have shown that the very first digestive movements at E6-E7 (6-7 days of development, ~6-7 weeks in humans) are driven by mechanosensitive calcium waves that propagate across gap junctions in the circular smooth muscle layer. We found that these phasic contractions along with circular smooth muscle tone are essential in guiding anisotropic lengthening of the proliferating intestine, revealing a link between early intestinal motor function and organ morphogenesis. We further demonstrated by calcium imaging that interstitial cells of Cajal become active around E13 and that they increase the rhythmicity and the speed of contractile waves, indicating a possible developmental transition from trigger to phase waves. We finally presented evidence that enteric nervous system activity sets in at E14-E16, is inhibitory (it relaxes the circular smooth muscle), is stimulated by longitudinal smooth muscle contraction and is rostro-caudally polarized. These properties give rise to two essential, emerging attributes of the adult gut: the migrating motor complex and the asymmetric barometric reflex first described by Bayliss & Starling in 1898. Bridging the gap between fetal and adult motility will yield a deeper understanding of the dynamics of the mature intestine.

104 | Intraluminal capsaicin is a concentration-dependent stimulator of rectal chemosensitivity and reduces rectal mechanosensitivity in healthy volunteers

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Introduction: Capsaicin, the pungent ingredient of chili pepper, has evolved as a tool in the study of chemonociception in the upper gastrointestinal tract, where, capsaicin induces symptoms via a pathway not involving mechanoreceptors, probably by stimulating the capsaicin receptor TRPV1.

Aims: To study the effect of intraluminal capsaicin on rectal perception, tone and mechanosensitivity.

Methods: A barostat balloon was positioned 5-15 cm from the anal verge for distension and measurement of tone in 15 healthy subjects (age: 26 ± 4 years, 10 female). Pressure controlled random distensions were performed before and after rectal perfusion with 10, 50 or 100 μg/mL capsaicin. Infusions at a rate of 0.5 mL/min were performed until discomfort was reported or for 60 minutes maximum. Rectal tone was determined during the 15 minutes before and during capsaicin perfusion. Graded questionnaires evaluating quality and intensity of abdominal and rectal symptoms were filled out during distensions and capsaicin perfusion. Student's *t*-tests and linear regression analyses were performed, *P*-values <0.05 were considered significant, data are given as mean ± SEM.

Results: The table shows concentration-dependent latency (in minutes after start of capsaicin infusion) to first perception and discomfort, time until recovery, and aggregate perception scores in the rectum 10 minutes after start of capsaicin infusions. Capsaicin perfusion induced rectal symptoms of gas, stool, urgency, warmth and abdominal symptoms comparable to rectal distension: e.g., at time of discomfort urgency scores were 4.7 ± 0.5 vs. 4.6 ± 0.6 and scores for warmth were 2.5 ± 1.8 vs. 1.5 ± 1.6 during capsaicin and distension, respectively ($P > 0.05$). Distension induced discomfort at a balloon pressure of 34 ± 2.8 mm Hg before and 24 ± 2.5 mm Hg after capsaicin ($P < 0.05$) and a balloon volume of 178 ± 10.3 mL before and 140 ± 13.9 mL after capsaicin infusion ($P = 0.05$), respectively. Rectal tone did not change significantly during capsaicin infusion ($P > 0.05$).

Conclusion: Rectal perfusion with capsaicin dose-dependently induced symptoms comparable to mechanical distension and increased sensitivity to consecutive rectal distensions, but did not alter rectal tone. Capsaicin may be used as a diagnostic tool for the evaluation of chemosensitivity of the rectum.

106 | Development of a three-stage gut-brain culture system

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Background/Objectives: The interplay between our gut and brain is well documented and is commonly referred to as the gut-brain axis. It has been shown that an increase in gut permeability induces systemic inflammation that is associated with impaired brain function. However, the mechanisms behind this link are poorly understood. To investigate how gut-derived inflammatory signals are transmitted to the brain the development of a three-stage gut-brain culture system was necessary.

Methods: Intestinal epithelial cells in a dual environment model of the gut were exposed to increasing doses of lipopolysaccharide (LPS) (stage 1). Following incubation, the conditioned media was added to a blood-brain barrier model (stage 2). The impact of LPS (stage 1) or conditioned media (stage 2) on barrier integrity and cytokine production of the gut and blood-brain barrier, respectively, was determined. Finally, the conditioned media of the blood-brain barrier model will be added to a neuron model (stage 3) to investigate whether this results in secretion of neuroinflammation biomarkers by the neurons.

Results: Preliminary results show a decrease in barrier integrity of intestinal epithelial cells exposed to LPS in a dose-response manner (stage 1). The addition of conditioned media from intestinal epithelial cells treated with 1 mg/mL LPS but not from cells treated with control media decreased the barrier integrity of the blood-brain barrier model after approximately 73 hours (stage 2). This loss in barrier integrity was partially recovered over the next 5 hours. Results of all three stages of the gut-brain culture system will be presented.

Conclusions: Results of this research will provide insights into the immune-mediated mechanisms behind the gut-brain axis. Ultimately, the three-stage gut-brain culture system can be utilised to identify compounds such as food ingredients or probiotics that improve brain function by reducing gut permeability and associated inflammation.

Table 1 Dose dependency of perception thresholds induced by rectal capsaicin

Capsaicin concentration	First sensation (min)	Discomfort (min)	Recovery time (min)	Perception score after 10 min
10 μg	29 ± 25	52 ± 13	6 ± 1	5 ± 3
50 μg	8.6 ± 1	29 ± 15	7 ± 2	9 ± 5
100 μg	5 ± 1	14 ± 5	5 ± 1	14 ± 2
P-value	0.04	0.001	0.5	0.02

107 | The rapid drink challenge test in patients with dysphagia and normal manometry according to Chicago 3.0

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Introduction: Previous studies have shown that the rapid drink challenge test (RDC) can demonstrate obstruction across the esophagogastric junction (EGJ).
Aim: To determine if the RDC demonstrates obstruction across the EGJ, and elicits dysphagia, in patients complaining of dysphagia and normal manometry according to Chicago 3.0 criteria.
Methods: We studied 241 consecutive patients (176 F, 65 M, mean age 59 ± 13 years) referred to esophageal manometry for dysphagia. In each patient, a 200-ml RDC was performed after the standard protocol of HREM, and patients were asked to score (from 0 to 3) if dysphagia was elicited by the RDC. The pressure responses elicited by the RDC were analyzed as previously described (Neurogastroenterol Mot 2016; 28:543-553).
Results: The RDC was successfully performed and analyzed in 238 patients. Only 13 (5%) patients developed an obstructive pressure pattern during the RDC. By contrast, 50% of patients referred dysphagia during the RDC. However, most patients referred mild-moderate dysphagia, and only 12 (5%) patients referred severe dysphagia during the RDC. Overall, there was positive association between the pressure responses elicited by the RDC and the dysphagia referred during the test (P = 0.019). However, when the 12 patients who referred severe dysphagia were analyzed, only 2 developed an obstructive pressure pattern during the RDC, whereas the remaining 10 patients had a normal pressure response to the RDC. By contrast, 7 of the patients who had an obstructive pattern during the RDC referred dysphagia during the RDC.
Conclusion: Most patients complaining of dysphagia and normal manometry have a normal pressure response to a RDC that correlates with the intensity of dysphagia elicited by the RDC. In a minority of patients, the RDC detects obstruction across the EGJ. This test could be useful for discrimination between patients with dysphagia due to an obstruction across the EGJ not detected by the standard protocol, and patients with functional dysphagia.

108 | Wnt/R-spondin/frizzled signaling – Following bread crumbs to understand postnatal enteric progenitor regulation

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Background/Objectives: Neural progenitor cells from the postnatal enteric nervous system are a potential source for cell-replacement therapies in enteric neuropathies. Yet, we know little about the molecular mechanisms regulating this cell pool in postnatal life, let alone specific markers to identify neural progenitors in the ENS, especially in the human intestine. Here, we hypothesize that the canonical Wnt/R-Spondin-pathway plays a central role in ENS-progenitor proliferation and that the Wnt-receptor Frizzled-4 is expressed by human ENS-progenitor cells.
Methods: We investigated the influence of Wnt-signaling on the proliferation of isolated postnatal ENS-progenitors from murine and human intestine using gene-chip analysis, BrdU-incorporation assays, immunohistochemistry, western blot, and RT-PCR experiments. We used FACS analysis, immunohistochemistry, and patch-clamping to characterize Frizzled-4-expressing cells from human intestine.
Results: Here we present sound evidence that the activation of the canonical Wnt pathway by Wnt3A and R-Spondin1 increases the proliferation of enteric neural progenitors and leads to a higher yield of differentiated neurons in vitro, both in humans and mouse models. We identified the Wnt-receptor Frizzled-4 as a novel marker expressed on human postnatal ENS-progenitor cells: Frizzled-4^{positive} cells gave rise to neurosphere-like bodies, expressed Nav, Kv and BK channels, and, hence, differentiated into functional neurons. In Frizzled-4^{negative} cultures, we did not detect any neural cells.
Conclusions: The canonical Wnt/R-Spondin pathway plays a central role in the regulation of homeostasis of ENS progenitor cells in humans and in various mouse models. The deep integration of the Wnt cascade in the regulatory control network of human ENS becomes evident by our findings, which define the Wnt receptor Frizzled-4 as a newly discovered surface marker useful for the isolation of ENS progenitors.

109 | Effect of probiotics on Gulf War illness with irritable bowel syndrome

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Background: Gulf War (GW) Veterans with irritable bowel syndrome (IBS) are more likely to report fatigue, joint pains, headache, and other somatic symptoms; common clinical symptoms of GW illness (GWI). There is evidence that probiotics by restoring normal intestinal microbiota may improve symptoms of IBS. We evaluated the effect of probiotics on IBS and other symptoms of GWI.
Methods: Sixty-two GW Veterans with IBS (Rome-III criteria) and two or more symptoms of GW illness were enrolled in the study. After a 2-week run-in period, Veterans were randomized (1:1) to receive either probiotic (De Simone Formulation; formally known as VSL#3 and Visbiome) or placebo once daily for 8-weeks in a double-blind study. The symptoms assessed were: abdominal pain, bloating, stool frequency, stool consistency, severity of diarrhea, severity of constipation, satisfaction with bowel habits, IBS affecting

or interfering with life. Quality of life (QOL) was assessed using IBS-QOL. The somatic symptom checklist was used to detect extra-intestinal symptoms commonly present in GWI. The symptom data were compared after 8 weeks of treatment.

Results: Data were analyzed from 53 Veterans who completed the study (47 (89%) males; age range 42-73, mean ± SD (55 ± 8) years). Nine patients dropped out before the end of the study. There was three-fold greater improvement in diarrhea severity in the treated group, although it failed to achieve statistical significance ($P = 0.13$). There was four-fold greater satisfaction with the bowel habits in the treated group compared to the control and it was borderline significant ($P = 0.09$). There was no significant improvement in IBS-QOL (Fig) and in symptoms of GWI.

Conclusion: There was no change in symptoms of GWI including IBS with the probiotics. There was no improvement in IBS-QOL.

Lack of response of the probiotics may suggest that besides gut microbiome, gut virus and fungi may have a role in causing the symptoms of GWI. It is also possible that the bacteria present or the dose of the probiotic was not sufficient to improve symptoms.

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111 | Artificial intelligence complements evaluation of baseline impedance from pH-impedance studies and predicts outcome in gastro-esophageal reflux disease (GERD)

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Background/Objectives: Mean nocturnal baseline impedance (MNBI) complements evaluation of GERD from pH impedance studies, and together with acid exposure time (AET), predicts symptom response from GERD therapy. Artificial intelligence (AI) has potential to streamline acquisition of baseline impedance (BI) from pH-impedance studies, and to facilitate interpretation. Our aims were to determine feasibility of AI in BI extraction (AIBI), and to correlate total, upright and recumbent AIBI with mean nocturnal BI (MNBI), AET, and GERD treatment outcome.

Methods: Raw data from pH impedance studies were acquired from symptomatic patients studied off therapy at two international tertiary care centers (St. Louis, USA and Padua, Italy). Data were uploaded into a dedicated prototypical AI software program designed specifically to extract AIBI. Manual corroboration of 3 test studies by two investigators (BDR, CPG) demonstrated >80% accuracy of the software program in identification of swallows, reflux episodes, and belches; these events, meal periods and artifacts were discarded while calculating AIBI. Differential upright and recumbent AIBI, and the upright: recumbent AIBI ratio were calculated. Patients completed self-report validated questionnaires before and after medical GERD therapy consequent to pH impedance monitoring, and ≥50% symptom improvement on these questionnaires constituted GERD treatment response. AIBI values were compared to AET and MNBI, and to symptom response.

Results: Of 117 patients (53.1 ± 1.2 years, 66% F) meeting inclusion criteria, 57 reported ≥50% treatment response. Recumbent AIBI was similar to MNBI regardless of AET or treatment outcome (P = ns), but upright and total AIBI were different from MNBI, both at 3 and 5 cm above the lower esophageal sphincter ($P < 0.05$ for each comparison). In addition to AET and MNBI, AIBI, and the upright: recumbent AIBI ratio were significantly different between responders and non-responders to medical GERD management ($P < 0.03$ for each comparison, Table).

Conclusions: Artificial intelligence demonstrates high potential in refining interpretation of pH-impedance studies. While recumbent AIBI resembles MNBI, the differential between upright and recumbent AIBI augments BI evaluation, and predicts outcome from medical GERD management.

112 | Effects of intraduodenal administration of the fatty acid, lauric acid (C12), and the amino acid, L-leucine (Leu), alone and combined, on antropyloroduodenal motility, plasma cholecystokinin and energy intake in healthy men

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Background/Objective: Intraduodenal infusion of C12 and Leu, at loads of 0.4 and 0.45 kcal/min for 90 minutes, respectively, stimulate gut hormones, including plasma cholecystokinin, and pyloric pressures, associated with suppression of energy intake. In our recent study, combination of C12 with L-tryptophan substantially reduced energy intake, at loads that individually did not affect energy intake, associated with much greater stimulation of cholecystokinin. We have now investigated whether combined administration of C12 and Leu, at loads that individually suppress energy intake, would have the capacity to further enhance the intake-suppressant effects of each nutrient, associated with greater modulation of upper-gut functions.

Methods: 16 healthy, lean males (age: 23 ± 1 years) received 90-minute intraduodenal infusions of control (saline), C12 (0.4 kcal/min), Leu (0.45 kcal/min) or C12+Leu (0.85 kcal/min) in randomised, double-blind fashion. Antropyloroduodenal pressures were measured continuously (high-resolution manometry), and plasma cholecystokinin (radioimmunoassay) at 15-minute intervals. Energy intake from a standardised buffet-meal was quantified immediately post-infusion.

Results: All nutrients stimulated plasma cholecystokinin compared with control ($P < 0.05$), and C12 and C12+Leu compared with Leu ($P < 0.05$) (mean concentration, pmol/L; control: 2.3 ± 0.3, C12: 3.8 ± 0.3; Leu: 2.7 ± 0.3; C12+Leu: 4.0 ± 0.4). Moreover, all nutrients reduced antral pressure waves ($P < 0.05$), and C12 and C12+Leu, but not Leu, reduced duodenal pressure waves ($P < 0.01$). There was

a treatment x time interaction for the number of isolated pyloric pressures ($P < 0.05$); while mean values were greater with C12 and C12+Leu, post-hoc analysis revealed no differences between treatments. There were no differences in energy intake between the three nutrient treatments (kcal; control: 1398 ± 84, C12: 1226 ± 80; Leu: 1260 ± 92; C12+Leu: 1208 ± 83).

Conclusions: Combination of C12 and Leu, at the loads given, did not have the capacity to reduce energy intake beyond their individual effects, possibly because maximal effects, particularly by C12, on gut functions, including pyloric and CCK stimulation, had been evoked.

113 | Co-morbidities of IBS: Role of voltage-gated sodium channels in mouse vaginal afferent mechanosensation and pain

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Background and Objectives: Endometriosis and vulvodynia affect ~8-10% of women and are common co-morbidities for women with irritable bowel syndrome (IBS). Nonetheless, the mechanisms underlying chronic visceral pain associated with these disorders remain unknown. This study aims to determine: (1) whether sensory neurons innervating the mouse vagina express voltage-gated sodium (Na_v) channels, which are recognized to be involved in pain sensation, and (2) whether pharmacological modulation of Na_v channels can regulate the function of sensory fibers innervating the mouse vagina and prevent pain evoked by vaginal distension.

Methods: Retrogradely tracing from the mouse vagina was used to identify vagina-innervating neurons within the lumbosacral dorsal root ganglia (DRG). Na_v channels expression in vaginal-innervating DRG neurons was measured by single cell RT-PCR. In vitro whole-cell patch clamp was used to investigate vaginal-innervating DRG neurons excitability. A novel ex vivo electrophysiological recording preparation was used to examine mechanosensory function of pelvic afferents innervating the vagina. Pain was measured in vivo by recording the visceromotor pain response evoked by vaginal distension.

Results: Na_v channel mRNA was detected in retrogradely labeled vaginal neurons (from n = 40 vaginal neurons: $Na_v1.1$ = 33%, $Na_v1.2$ = 54%, $Na_v1.3$ = 21%, $Na_v1.4$ = 13%, $Na_v1.5$ = 26%, $Na_v1.6$ = 59%, $Na_v1.7$ = 100%, $Na_v1.8$ = 97%, $Na_v1.9$ = 72%). Inhibition of tetrodotoxin-sensitive Na_v channels significantly reduced: i) excitability of vaginal-innervating DRG neurons ($P < 0.0005$, n = 45 neurons), ii) mechanosensitivity of pelvic vaginal afferents ($P < 0.05$, n = 8 afferents), and iii) pain evoked by vaginal distension ($P < 0.01$, N = 6 mice). Conversely, activation of all Na_v channels using veratridine significantly increased mechanosensitivity of pelvic vaginal afferents ($P < 0.05$, n = 7 afferents) and induce allodynia and hyperalgesia evoked by vaginal distension ($P < 0.05$, N = 9 mice) in vivo.

Conclusions: Mechanosensory function of pelvic primary afferents innervating the vagina can be modulated by targeting Na_v channels. Peripheral inhibition of tetrodotoxin-sensitive Na_v channels could be an efficacious approach to prevent evoked vaginal pain in vivo. These results create a platform for subsequent mechanistic studies into IBS-, endometriosis- and vulvodynia- induced chronic visceral pain.

114 | Associations between dairy consumption and constipation in a population-based sample of adults in Australia: A cross-sectional study

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Background: Despite reported associations between dairy intake and constipation in children, the association between dairy and constipation in the general adult population remains understudied.

Methods: Data from women (n = 632) and men (n = 609) participating in the Geelong Osteoporosis Study were utilised. Information on dairy (milk, yogurt, and cheese) and constipation were self-reported. Total dairy was calculated by summing the intake of milk, yogurt, and cheese, which was expressed as serves per day. Multivariable adjusted (mobility, major depressive disorder, and irritable bowel syndrome) logistic regression models were used to estimate the odds ratio (OR) and 95% confidence interval (CI) to examine the link between categories of milk (<250 mL/d, 250-1000 mL/d, >1000 mL/d), yogurt (0 g/d, <200 g/d, ≥200 g/d), cheese (0 g/d, <40 g/d, ≥40 g/d), and total dairy (<1 serve/d, 1-2 serves/d, ≥2-4 serves/d, ≥4 serves/d), with constipation.

Results: In women, there was weak evidence that consumption of <250 mL/d of milk was associated with an increased likelihood of constipation (OR: 1.58; 95% CI: 0.61-2.06; $P = 0.055$) compared to consuming 250-1000 mL/d of milk. Consumption of 1-2 serves/d of total dairy was associated with a reduced likelihood of constipation (OR: 0.47; 95% CI: 0.26-0.86; $P = 0.014$) compared to consuming 1 serve/d of total dairy in women. Neither yogurt nor cheese consumption were associated with constipation in women. In men,

no association was observed between milk, yogurt, cheese, or total dairy consumption and constipation.

Conclusion: Moderate consumption of milk and total dairy was associated with reduced odds for constipation in women; however, there was no association between any form of dairy consumption and constipation in men. Further studies are warranted to confirm these results.

Keywords: constipation, dairy, milk, bowel symptoms

115 | Alpha 1 adrenoceptors may be promising targets for the treatment of functional bowel disorders

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Background & Aims: Functional bowel disorders (FBD) are common, symptom-based diseases that have substantial negative impacts on quality-of-life and work productivity of patients. However, the pathophysiology of FBD is not fully understood, and therefore definitive treatments have been difficult to establish yet. Stress is an important factor in the etiology of FBD, and responses to it include stimulation of the sympathetic nervous system and releases of norepinephrine (NE), which could be involved in pathogenesis of FBD. While activation of α2 or β adrenoceptors (ARs) inhibits colonic motility, the role of α1 ARs remains to be elucidated. We investigated the functional roles of α1 ARs in human colonic contractility to explore their potential as a new target for the treatment of FBD.

Methods: Isometric tension recording, intracellular recording, and Ca^{2+} imaging were performed on muscles of the human colon. Responses to α1 ARs agonists or electric field stimulation with AR antagonists and neuroleptic reagents were studied.

Results: Exogenous or endogenous NE released from sympathetic nerves inhibited colonic contractions through binding to α1A ARs or enhanced colonic contractions by acting on α1D ARs. The inhibitory responses were blocked by apamin, an antagonist of small conductance Ca^{2+}-activated K^+ (SK) channels. Phenylephrine, α1 AR agonists, or NE evoked increases in intracellular $[Ca^{2+}]$ in PDGFRα$^+$ cells and hyperpolarized SMCs, by binding to α1 ARs expressed by PDGFRα$^+$ cells.

Conclusions: Subtype specific α1 AR antagonists can selectively inhibit either excitatory or inhibitory actions of NE. Therefore α1 AR antagonists may be promising drugs for treating stress-associated symptoms of FBD.

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116 | The increase of demethylation markers in the obstruction-induced intestinal hypertrophy in mice

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Background and Aim: Dysregulated gene expression in the gastrointestinal tract can be changed in the phenotype and function through DNA methylation which is a key epigenetic modification mechanism and can lead to motility disorders. It has been known that DNA methylation is positively regulated by DNA methyltransferases and is negatively regulated by the demethylation enzymes which belong to Ten-Eleven Translocation (TET) family. This study aimed to investigate the changes of epigenetic markers including DNA methyltransferase and demethylation enzymes in the GI tracts.

Method: Partial obstruction hypertrophic model was constructed by insertion of the plastic ring into just above cecum in C57BL/6 mice. Seven to 10 days after the insertion of the ring, partial intestinal obstruction can be recognized by the abdominal bulging. After removing ileocecal area and confirming gross intestinal hypertrophy, immunofluorescence and Western blotting were performed for detecting the changes of phenotypes in GI tract using DNA methyltransferases and demethylation enzymes in the hypertrophied region.

Results: The changes in the expression in the DNA methyltransferases were not apparent. However, the expression of TET1, TET2, and TET3 was significantly increased in the smooth muscle and mucosa of hypertrophic region.

Conclusion: These results suggest that besides DNA methyltransferase, the demethylation enzymes could also have an essential role in the development and progress of gastrointestinal hypertrophy and can be a therapeutic target for phenotypic changes in human GI disease.

117 | Effects of intragastric vs. intraduodenal administration of the bitter compound, quinine, on fasting insulin and glucose concentrations and antropyloroduodenal pressures, in healthy men

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Background/Objective: Bitter compounds may modulate the release of gluco-regulatory hormones and gastrointestinal motor function (gastric emptying), which are important in the regulation of blood glucose. The distribution of bitter receptor subtypes varies along the gastrointestinal tract (e.g. a greater number in the duodenum than stomach). Accordingly, intraduodenal (ID) administration may affect hormone secretion and gut motility more than intragastric (IG) administration. We compared the effects of IG vs. ID administration of quinine hydrochloride (QHCl) on fasting plasma insulin and glucose as well as antropyloroduodenal motility, in healthy men.

Methods: Eight healthy lean men (age: 25 ± 3 years) received, on 2 separate occasions, 600 mg QHCl either IG or ID, in randomised, double-blind fashion. Plasma insulin (radioimmunoassay) and glucose (glucose oxidase technique) concentrations and antropyloroduodenal motility (high-resolution manometry) were measured at baseline and for 2 hours in response to quinine.

Results: ID QHCl was associated with an earlier (time to maximum [min]; ID: 33 ± 4, IG: 71 ± 5, $P < 0.05$) and greater (peak concentration [mU/L]; ID: 13.6 ± 2.2, IG: 9.0 ± 1.8, $P < 0.05$) rise in plasma insulin, and an earlier decrease (time to minimum [min]; ID: 55 ± 6, IG: 86 ± 6, $P < 0.05$) in fasting glucose, with no significant difference in the magnitude of glucose lowering (Δ baseline [mmol/L]; ID: −0.9 ± 0.2, IG: −1.2 ± 0.1). ID QHCl markedly stimulated pyloric pressures compared with IG QHCl during the first 60 minutes (number; ID: 33 ± 11, IG: 15 ± 7, $P < 0.05$), with no differences in the motility index of antral or duodenal pressures.

Conclusions: There are substantial differences between IG and ID QHCl in the time course and/or magnitude of the changes in plasma insulin and glucose and pyloric stimulation, consistent with the concept that localised stimulation of small intestinal bitter receptors elicit optimal effects.

118 | The relationships between the results of contemporary tests of anorectal structure and sensorimotor function and the severity of faecal incontinence

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Background: Diagnostic investigations for faecal incontinence (FI) assess the structure and sensorimotor function of the anorectum. Investigations can include anorectal manometry, rectal/anal canal sensory testing, pudendal nerve terminal motor latencies (PNTML), and endoanal sonography. The severity of FI and results of diagnostic anorectal investigations are often discordant and symptom resolution following treatment remains <40%. High-resolution anorectal manometry (HRAM) and three-dimensional endoanal ultrasound (3D-US) have been introduced into clinical practice in the last decade. This study aims to determine whether the strength of relationships between investigation results and symptom severity increases with the use of these contemporary tools.

Methods: Patients presenting for investigation of FI were assessed using the St Mark's FI severity score, HRAM, rectal/anal canal sensory testing, PNTML, and 3D-US.

Results: 253 patients were included. There were significant relationships between the St Mark's score and HRAM (resting pressure $r_s = -0.23$, 95% CI = (−0.34, −0.11), $P < 0.01$; squeeze pressure $r_s = -0.27$, 95% CI = (−0.38, −0.15), $P < 0.01$) and 3D-US (EAS defect $U = 3811.50$, $r = -0.15$, $P = 0.02$; IAS thickness $r_s = 0.20$, 95% CI = (0.07, 0.32), $P < 0.01$; anterior EAS length $r_s = -0.25$, 95% CI = (−0.37, −0.12), $P < 0.01$; posterior EAS length $r_s = -0.18$, 95% CI = (−0.31, −0.06), $P < 0.01$). The relationship between St Mark's score and HRAM had a greater effect size in the subgroup with urge-predominant symptoms (resting pressure: $r_s = -0.36$, 95% CI = (−0.59, −0.23), $P < 0.01$, maximal squeeze pressure: $r_s = -0.41$, 95% CI = (−0.59, −0.22), $P < 0.01$). Overall, the variance in the St Mark's score accounted for by anorectal investigations was 19% ($R^2 = 0.28$, $P < 0.01$, adjusted $R^2 = 0.19$).

Conclusions: FI severity is not a strong predictor of anorectal dysfunction. Contemporary anorectal investigations relate similarly with severity of symptoms when compared with conventional anorectal investigations. These findings reflect the multifactorial, heterogenous pathophysiology of FI, the limitations of anorectal investigations and symptom scoring, and factors contributing to symptoms which are extrinsic to the anorectum.

119 | Sedative drug effects for reduction of pediatric intussusception

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Background/Objective: Intussusception is common emergency condition in children. Hydrostatic or pneumatic reduction is considered the first-choice management strategy in cases lacking indications for surgical intervention. Generally, sedatives are not used in children undergoing interventional radiology procedures. Surgical reduction is associated with long hospitalization durations and high costs, unlike non-surgical reduction. To avoid surgery, reduction procedures are repeated despite initial treatment failure. However, in cases involving repeated failures, children should be referred for surgery.
Methods: To ensure good response to reduction, we planned hydrostatic reduction under sedation during the third reduction attempt. Sedative reduction was performed with the administration of ketamine, midazolam, and atropine. All patients with contraindications against hydrostatic reduction underwent laparoscopic reduction without hydrostatic reduction.
Results: Over 3 years, sedative reduction was performed in 43 patients, and in 28 (65.1%), the treatment was successful. Among the 15 patients in whom the procedure failed, 14 underwent laparoscopic reduction without intestinal resection.
Conclusion: Sedative drugs can reduce the intestinal contraction, patient's irritability, and poor cooperation. During the second or third hydrostatic reduction attempt, successful reduction may be ensured with the sedative reduction procedure with intravenous ketamine, midazolam, and atropine; this procedure may further reduce surgery rates in pediatric intussusception.

121 | Assessment of gut dysfunction in a mouse model of stroke

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Stroke is a debilitating disease caused by a restriction of blood flow to the brain, and it contributes to over 10% of all deaths annually. Despite early mortality being primarily attributed to the extent of brain infarction, stroke is associated with various non-neurological medical complications. Bacterial pneumonia is a common cause of death in patients with stroke. In addition, bowel dysfunction, such as constipation and incontinence, occurs in over half of all patients with stroke. Emerging research suggests that the onset of infection after stroke arises as a consequence of gastrointestinal dysfunction; however, the mechanisms involved remain unclear. This study aims to examine the effect of stroke on the neurons of the enteric nervous system and intestinal immune compositions. Using the well-established mouse model of stroke called the middle cerebral artery occlusion (MCAO), the gut of C57BL6/J mice that either underwent sham or stroke surgery was assessed. At 24 hours after stroke onset, we observed significantly reduced gut transit; however, there were no significant changes between the number or size of peristaltic contractions in vivo between the sham-operated and post-stroke group. Visualisation of the myenteric plexus through fluorescent staining revealed selective changes in neuronal populations. Furthermore, we assessed the immune population in the intestine via flow cytometry and revealed that stroke promotes an increase of immune cells in the ileum, more specifically macrophages in both the mucosal and myenteric plexus layer. Depletion of macrophages showed moderate rescue of altered gut transit after stroke suggesting stroke-induced gut dysmotility is accompanied by neural and immune changes. Addressing the mechanisms that drive gut dysfunction after stroke could aid in the identification of novel therapeutic targets to reduce gut-derived infection in patients with stroke and improve their outcome.

122 | Coordination of spontaneous electrical and mechanical activity in the isolated non-pregnant mouse uterus

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Background: Precisely coordinated contractions of the non-pregnant uterus are critical for the success of many reproductive functions. The mechanisms underlying such activity, however, are incompletely understood. We therefore sought to further characterise spontaneous electrical and mechanical activity in the mouse uterus during the oestrus (post-ovulatory) phase of the oestrous cycle.
Methods: Contractions of isolated, intact uteri from nulliparous female C57BL/6 mice were video captured to generate spatiotemporal maps, and myoelectrical activity simultaneously recorded using suction electrodes attached along the uterine horn (n = 5).
Results: Contractions typically initiated from the oviduct-end of the uterus and propagated toward the uterine body at a frequency of ~1-2 cpm. This activity appeared to occur independently in adjacent uterine horns, with only ~12% events happening concurrently. Within each contractile event, bursts of electrical activity corresponded to individual phasic contractions of both the circular and longitudinal muscle. Such electrical bursts consisted of major and minor (superimposed) peaks generated at two different frequencies: ~5 and ~25 Hz, respectively. All recorded activity persisted in the presence of the sodium channel blocker, TTX (0.6 μmol/L), and

was abolished by the L-type calcium channel blocker, nifedipine (1 μmol/L); supporting a myogenic origin. Additional calcium imaging experiments (n = 2) revealed that during contractile events, calcium waves occurred simultaneously in both muscle layers. However, spontaneous myometrial calcium waves between contractile events were not detected.

Conclusions: This is the first description of propagating bursts of electrical activity along the uterine horn. The intrinsic pacemaker mechanisms underlying such rhythmicity are critically dependent upon L-type calcium channels.

123 | Properties of sympathetic neurons that respond to inflammation and innervate gut associated lymphoid tissue in the small intestine of the rat

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Background/Objectives: Inflammatory bowel disease is associated with changes in sympathetic innervation of the intestine and may be treatable by stimulation of sympathetic pathways. Many questions remain, however, regarding how sympathetic nerves respond to inflammation and regulate immune cells within the gut. For instance, peritoneal inflammation activates sympathetic nerves that inhibit motility but intraluminal inflammation is generally prokinetic. Reports on the extent and location of sympathetic nerves to the intestinal GALT are contradictory. We therefore investigated the responses of intestinal sympathetic nerves to inflammation and the targets of sympathetic nerves within the GALT.

Methods: Extracellular recordings were made from small mesenteric nerves close to the intestine during acute inflammation in anaesthetized rats. Neurons innervating ileal Peyer's patches were identified using a combination of retrograde tracing, immunohistochemistry and 3-D confocal microscopy.

Results: Acute intraluminal inflammation of the ileum with TNBS activated a population of efferent axons in small nerves close to the wall of the intestine, in part reflexly via activation of cholinergic intestinofugal neurons. Reflexly activated efferent axons had properties consistent with non-vascular neurons and neither gut blood flow nor motility were inhibited by the inflammation. Retrograde tracing demonstrated a small population of prevertebral sympathetic, non-NPY neurons that specifically innervated the interfollicular region of ileal Peyer's patches. The submucosa between the follicles contained 2 distinct layers of enteric ganglia. Sub-groups of the abundant nerve terminals present within the Peyer's patch contained various enteric peptides but were only rarely immunoreactive for tyrosine hydroxylase. Consistent with tracing studies, these rare fibres did not contain NPY.

Conclusions: Our data suggest that a specific population of non-vascular, non-motility controlling prevertebral sympathetic ganglion neurons are activated by intraluminal inflammation and control the function of ileal Peyer's patches, both directly, and indirectly via connections with submucosal neurons.

124 | Comparative profiling of the gaseous signaling molecules NO, H_2S, HNO and the polysulfide Na2S3 on the spontaneous phasic contractions of the rat colonic muscle strips

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Background/Objectives: Endogenous gasotransmitters might have a direct impact on colonic contractility. Nitric oxide is produced by nNOS and released by enteric inhibitory motorneurons. During inflammation, several reactive oxygen and nitrogen species produced by inducible nitric oxide synthase and oxidant-generating enzymes might produce nitroxyl (HNO). Several enzymatic routes such as CSE and CBS endogenously produce H_2S. It has been postulated that polysulfides (Kimura *et al*., FASEB J. 2013 Jun;27(6):2451-7) might be the final product of these enzymatic route. Accordingly, the aim of this study is to compare the effect of these potential gasotransmitters on rat colonic motility.

Methods: Organ bath and microelectrode recordings were performed on rat proximal colon muscles strips using the polysulfide Na_2S_3, and sodium nitroprusside (NaNP), sodium hydrogen sulfide (NaHS), Angeli's salt as NO, H_2S, HNO donor, respectively. The neurotoxin TTX (1 μmol/L) was used to block neuronal activity, suppressing endogenous neural inhibitory tone.

Results: All four signaling molecules caused a concentration-dependent inhibition of spontaneous phasic contractions (SPC) observed as a reduction in the amplitude, the frequency and the area under the curve (AUC) of such contractions. Each drug however presented a specific profile in its actions. The rank of potency was NaNP > Angeli's salt > Na_2S_3 > NaHS. Only the responses of NaNP and Angeli's salt were reversed by 10 μmol/L ODQ, blocker of sGC. Except for Na_2S_3, which increased the basal tone at higher concentrations, none of the drugs substantially changed the basal tone.

Angeli's salt hyperpolarized the smooth muscle and its effect was reduced both by ODQ (10 μmol/L) and apamin (1 μmol/L, blocker of SKca). The effects of H_2S and Na_2S_3 were insensitive to ODQ.

Conclusions: All four molecules, NO, H_2S, HNO and the polysulfide Na_2S_3 reduce the basal contractile activity of the colon by activation of the sGC/cGMP pathway (NO, HNO) or not (H_2S, Na_2S_3). Overexpression of both pathways in inflammatory diseases might have synergistic effects on motility.

125 | Nitroxyl (HNO)-induced relaxation of the intestinal smooth muscle: Mechanisms of action

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Background/Objectives: Pro-secretory properties of nitroxyl (HNO) were demonstrated in the rat colonic epithelium (Pouokam *et al.* 2013, https://doi.org/10.1016/j.ejphar.2013.05.031). Whether HNO presents additional effects as potential signaling molecule in the regulation of gastrointestinal tract functions is not known. Therefore, we investigated the properties of this molecule in the regulation of gastrointestinal contractility considering potential interactions with known gaseous signaling molecules like nitric oxide (NO) and hydrogen sulfide (H_2S).

Methods: Organ bath, Ca^{2+} imaging and microelectrode recordings were performed on rat intestinal samples, using Angeli's salt as HNO donor.

Results: Angeli's salt caused a concentration-dependent relaxation of longitudinal or circular muscle strips of the ileum and the proximal colon. This relaxation was strongly inhibited by the Rho-kinase inhibitor Y-27632 (10 μmol/L), by the reducing agent DTT or by the inhibitor of soluble guanylate cyclase (sGC) ODQ (10 μmol/L) alone or in combination with the inhibitors of the endogenous synthesis of H_2S β-cyano-L-alanine (5 mmol/L) and amino-oxyacetate (5 mmol/L). Preventing endogenous synthesis of NO by the NO synthase inhibitor L-NAME (200 μmol/L) did not affect the relaxation induced by HNO. HNO induced an increase of cytosolic Ca^{2+} concentration in colonic myocytes. It also caused myocyte membrane hyperpolarization. ODQ (10 μmol/L) and Apamin (1 μmol/L), a selective inhibitor of small conductance Ca^{2+}-activated K^+ channels (SK), strongly antagonized this effect.

Conclusions: HNO induces a relaxation of the gastrointestinal tract musculature by (1) hyperpolarizing myocytes via activation of the sGC/cGMP pathway, (2) increasing cytosolic Ca^{2+} for activation of SK_{Ca} contributing to hyperpolarization, (3) inhibiting the RhoK and activating MLCP when tissues are pre-contracted.

126 | Evaluation of endoscopic and clinicopathologic features of eosinophilic gastritis in Korea: A preliminary study

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Recently, an endoscopic activity assessment and scoring system for eosinophilic gastritis (EG) has been proposed in Western countries. However, this scoring system has not validated in Asian patients. The aim of this study was to investigate the endoscopic and clinicopathologic characteristics of EG in Korea. We retrospectively reviewed 223 990 gastric biopsies performed during upper endoscopies between January 2005 and August 2019 at a tertiary care center. We diagnosed EG based on ≥30 eosinophils/high-power field (HPF), upper gastrointestinal symptoms, and evaluated peak eosinophil count (PEC), and the Eosinophilic Gastritis Endoscopic Reference System (EG-REFS) included features of erosion/ulceration, granularity, raised lesions, erythema, friability, fold thickness and pyloric stenosis. We diagnosed EG in 52 patients (34 men and 18 women, median age: 63.5 years; interquartile range [IQR] 34.25-62.5 years) who presented with symptoms, including epigastric pain (32.7%), nausea/vomiting (21.2%), and lower abdominal pain (17.3%). Peripheral eosinophilia was present in 27 patients (51.9%). A history of allergy was reported in 11 patients (21.2%), including asthma (15.4%), allergic rhinitis (5.8%) and atopic dermatitis (3.8%). The median PEC was 80.5 (IQR 32.75 to 80.5) per HPF. The median total score of EG-REFS scores was 4.0 (IQR 3.0-6.0) but ranged from 0 to 12 out of a total of 46. The total score of EG-REFS was correlated with PEC (rho = 0.312, P = 0.024, Figure 1).

The EG-REFS scoring system was well correlated histologic severity of Korean EG patients. A high suspicion of EG is warranted when patients have unexplained gastrointestinal symptoms and abnormal endoscopic findings.

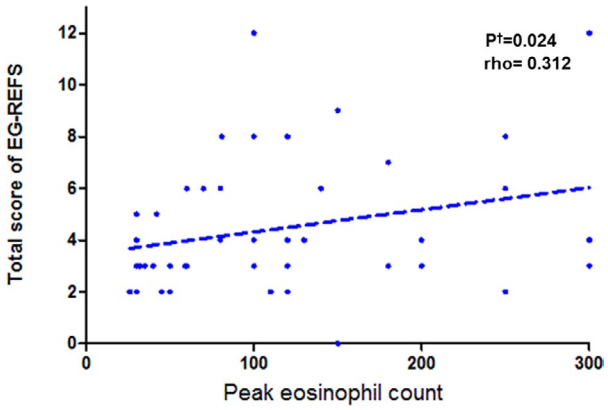

Figure 1. Scatter plot comparing total score of EG-REFS and peak eosinophil count

127 | Differential effects of the gut microbiota on the pharmacokinetics of antipsychotic drugs

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Background/Objectives: The role of the gut microbiome in drug bioavailability has recently come under scrutiny. It remains poorly understood whether the gut microbiome influences the pharmacokinetic parameters of psychotropic drugs. In this study, we aimed to investigate the pharmacokinetics of olanzapine and risperidone in rats whose microbiota had been depleted with an antibiotic cocktail or following probiotic supplementation.

Methods: Following two-week pre-treatment with either vehicle, probiotics (VSL#3) or antibiotics (ampicillin 1 g/L, vancomycin 500 mg/L and imipenem 250 mg/L), a single dose of OLZ (20 mg/kg) or RISP (15 mg/kg) was administered to Sprague Dawley rats via oral gavage (n = 7/group). Whole blood samples were collected via tail-bleeds over an 8 hours time-period and liver and intestinal tissue samples were harvested.

Results: The bioavailability of olanzapine, but not risperidone, was significantly increased in rats pre-treated with antibiotics. There was no direct effect of the microbiota depletion on the expression of CYP450 enzymes involved in antipsychotic metabolism, as assessed by RT-qPCR and Western blotting. Antibiotic-induced microbiota depletion, however, significantly downregulated the expression of UGT1A3 in the duodenum. Moreover, antibiotics significantly altered the metabolic activity of the gut microbiota. Among the bacterial genera detected by 16S sequencing, the relative abundance of Alistipes significantly correlated with the AUC in olanzapine-rats, suggesting that this bacterium might play a role in the pharmacokinetic alterations observed for olanzapine.

Conclusions: These results suggest that the gut microbiome is an important variable determining the bioavailability of orally administered olanzapine but not risperidone. Additional research is warranted to understand the implications and the role of the gut microbiota in the pharmacokinetics of olanzapine in humans, both in terms of the therapeutic and adverse effects of this antipsychotic.

128 | A longitudinal study of fodmap intake on the metabolome and pain signalling in IBS patients

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Background: The low FODMAP (fermentable oligo-, di-, monosaccharides and polyols) diet reduces symptoms of irritable bowel syndrome (IBS). Previous work suggests that FODMAP-microbiota interactions change the metabolome possibly modulating pain signaling in the gut. The aim of this randomized cross-over study was to further characterize the effect of FODMAPs on symptoms, the metabolome and pain signaling.

Methods: In this longitudinal IBS (Rome IV) study, patients underwent 4 assessments: two non-intervention periods, followed by a low- and high-FODMAP diet given in random order for 3-weeks each. Patients were advised that either diet may have therapeutic potential. At the end of each period, patients completed a 7-day food diary, the IBS Severity Scoring System, and provided stool and urine. Quantification of 117 urine metabolites was performed using LC-MS. The effects of fecal supernatants from 4 IBS-D patients were assessed during in vitro extracellular recordings of the lumbar splanchnic nerves of the distal colon in mice. Basal firing and mechanical sensitivity to ramp distension (0-60 mm Hg) was assessed before and after fecal supernatants were applied intraluminally. Analysis was conducted by two-way ANOVA and multivariate analysis.

Results: Twenty-five patients (4 male) completed the study (16 IBS-D, 3 IBS-C, 6 IBS-M). Symptoms were significantly reduced with the low FODMAP diet ($P < 0.01$), which correlated with urinary metabolites at baseline (PC36:0AE: $r = 0.7$, PC36:0AA: $r = 0.7$, C14:2OH: $r = 0.7$, n = 10). Fecal supernatants at baseline significantly increased the basal firing frequency of afferent nerves ($P < 0.05$), an effect which was lost when fecal supernatants from the low FODMAP diet were applied (P = NS).

Conclusions: Lowering FODMAP intake improved symptoms which correlated with the urinary metabolome at baseline. Furthermore, it reduced pain signaling induced by fecal supernatants suggesting mediators secreted by the microbiome may underlie these changes. Further studies are needed to identify specific active metabolites driving this response.

129 | Gut immune system activation in chronic idiopathic demyelinating polyneuropathy

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Background/Objectives: There is a growing recognition of the role of gut dysbiosis, gut inflammation and altered intestinal permeability in the pathogenesis of autoimmune diseases. Chronic inflammatory demyelinating polyneuropathy (CIDP) is a complex autoimmune disease caused by dysregulated response to unrecognized antigens. Some association between CIDP and Crohn's disease has been reported, but the exact pathophysiological links of these diseases are not well understood. The aim of the study was to evaluate fecal calprotectin as a biomarker of gut inflammation in CIDP patients without inflammatory bowel disease.

Methods: Fifteen patients with CIDP (mean age 64, range 52-70 years) and 15 healthy controls (mean age 59, range 46-75 years) were included in the study. The CIDP diagnosis was based on the American Academy of Neurology Task Force criteria. The prevalence of gastrointestinal symptoms was assessed based on a questionnaire. The quantitative evaluation of fecal calprotectin level was performed by the ELISA test.

Results: The duration of CIDP in the studied group ranged from 1 to 27 years. The fecal calprotectin level (μg/g) expressed as median along with the lower and upper quartiles [25Q-75Q] was significantly higher in CIDP patients compared to the controls: 26.6 [17.5-109.0] vs. 15.6 [7.1-24.1], $P < 0.01$. Applying the cut-off value of 50 μg/g, abnormal fecal calprotectin level was found in 33% of all CIDP patients and in none of the control subjects. The most frequent gastrointestinal symptoms reported by CIDP patients included constipation (33%), feeling of incomplete evacuation (33%), bloating (27%), alternating bowel movement pattern (27%), abdominal pain (7%) and diarrhea (7%).

Conclusions: In one-third of CIDP patients the signs of gut immune system activation have been observed. This finding may be associated with CIDP pathogenesis and induction of autoimmune response as well as with gastrointestinal symptoms frequently observed in CIDP patients. Elucidating the link of gut inflammation in CIDP may support immunomodulatory therapeutic approach including the gut microbiota modification.

130 | Cannabis & the Gut: Is supplementing with phytocannabinoids a worthy treatment?

Ben Cowin
Action Spine & Sports Medicine

Cannabis has been thrust into the forefront of the athletic world of late with the ban on CBD being lifted by WADA, CBD being taken off of the FDA's regulatory list, and the multitude of countries who have now legalized cannabis usage for medicinal purposes. As it continues to grow in popularity, so do the myths and 'wives tales' of what cannabis potentially can do. We want to take a look at the literature and tell exactly what science says about the plant, phytocannabinoids, and the therapeutic potential it may have for your patients.

Learning Objectives:

1. To know the difference between CBD & THC
2. To understand the basic function of the endocannabinoid system (ECS)
3. To understand the clinical implications of supplementing with certain phytocannabinoids

Three Key Terms: Tetrahydrocannabinol (THC), Cannabidiol (CBD), Therapeutic

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132 | A diagnostic algorithm discriminating non-celiac gluten sensitivity from irritable bowel syndrome

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Background: Non-celiac gluten sensitivity (NCGS) is characterized by intestinal and extra-intestinal symptoms related to the ingestion of gluten-containing foods, in the absence of celiac disease (CD) and wheat allergy. The pathophysiology of NCGS is unclear and no biomarkers are available. Distinguishing NCGS from IBS-D is of greater value in clinical practice, where the clinician is confronted with the dilemma of placing a patient on a gluten-free diet. The aim of the present work was to develop a diagnostic algorithm to discriminate NCGS and IBS.

Methods: Eighty-six patients with NCGS, 59 patients with diarrhoea-predominant irritable bowel syndrome (IBS-D), 15 patients with CD and 25 asymptomatic controls (ACs) were enrolled in a multicenter study. Zonulin serum levels were assessed by immunoenzymatic assay. Clinical and symptomatic data were recorded. Logistic regression analysis was used to develop a diagnostic algorithm to differentiate NCGS and IBS-D.

Results: Compared to IBS-D, the NCGS patients had significantly increased levels of zonulin ($P < 0.001$). The diagnostic accuracy of zonulin levels in distinguishing NCGS from IBS-D was 81%, with a sensitivity of 71% and a specificity of 83%. After exclusion of CD, a diagnostic algorithm combining zonulin levels, demographic and clinical data, improved the diagnostic accuracy (89%).

Conclusions: Zonulin serum levels combined with demographic and clinical data differentiate NCGS from IBS-D with high accuracy.

133 | Bloating and abdominal distension were associated with more frequent gastroesophageal reflux (GER) after dinner

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Bloating and abdominal distension (B/D) were commonly developed in the afternoon and getting worse in the evening as the fermentable carbohydrate in breakfast, lunch and dinner reaching to the colon. GER symptoms also commonly occur after meals. Whether there is an association between B/D and GER is not known.

Methods: Consecutive patients with typical GER symptoms who underwent esophageal manometry and 24-hour impedance pH monitoring during off therapy were retrospectively analyzed. Patients who had moderate to severe bloating with abdominal distension, [B/D(+)] were 1:1 gender, BMI and reflux symptoms severity matched with patients without B/D, [B/D(−)]. Patients with major esophageal dysmotility were excluded. The 2-hour post prandial (breakfast, lunch, dinner) reflux parameters were compared between groups.

Results: Nineteen patients (17 Female, age 47.7 ± 9.7) of 132 patients (14.4%) with typical GER symptoms had B/D(+). Among B/D(+) group, the postprandial reflux frequency increased from breakfast to dinner [4 (2-6) vs. 6 (3-9) vs. 8 (4-12) times/2-hour)] and reflux frequency during post-dinner was significantly higher than post-breakfast period ($P = 0.03$). In contrast, post-prandial reflux frequency in B/D(−) group was similar between meal (5 times/2-hour after breakfast, lunch, and dinner, $P > 0.05$). Additionally, post-dinner reflux frequency in B/D(+) group significantly higher than B/D(−) group ($P = 0.04$). The post-breakfast and lunch reflux frequency and other reflux parameters including %acid exposure time, acid clearance time, bolus clearance time, proximal extension and nocturnal reflux number were not significantly different between B/D(+) and B/D(−) group, $P > 0.05$ (Table 1).

	GERD without B/D (N=19)	GERD with B/D (N=19)	p-value
Age (years)	52.4±11.4	47.7±9.7	0.18
Gender, Male:Female	2:17	2:17	1
Body mass index (BMI), kg/m^2	23.7±4.7	22.3±3.9	0.47
Hiatal hernia, n(%)	4(21%)	5(26.3%)	0.7
Global symptom severity (0-10)	6.42±2.2	7.16±1.9	0.28
Reflux parameters			
% acid exposure time	0.8(0.2-3.8)	0.4(0.1-1.3)	0.25
Total reflux number (times/24 hours)	34(19-43)	34(19-55)	0.87
Acid clearance time (seconds)	15.2(4.4-42.0)	8.5(1.4-31.3)	0.12
Reflux frequency (times/2-hour)			
- Post-breakfast	5(2-7)	4(2-6)	0.59
- Post-lunch	5(4-8)	6(3-9)	0.43
- **Post –dinner**	**5(2-8)**	**8(4-12)**	**0.04**
Bolus clearance time (seconds)			
- Post-breakfast	10.2(7.0-13.3)	10.3(2.1-13.3)	0.47
- Post-lunch	10.6(4.5-13.3)	9.2(5.6-11.4)	0.50
- Post-dinner	7.9(5.5-10.6)	11.5(6.2-14.7)	0.10
Proximal extension (cm from LES)			
- Post-breakfast	9(6.5-12.3)	9(7-17)	0.14
- Post-lunch	9(9-17)	9(7-16)	0.95
- Post-dinner	9(9-15)	9(7-13.2)	0.93
Supine reflux number at night (times)	3(1-8)	3(0-6)	0.74
Supine duration at night (hours)	8.36(7.5-9.2)	7.57(7-9)	0.14

-Data expresses as mean±SD or median (interquartile range)

Conclusions: GERD patients who B/D(+) had increase reflux frequency from breakfast to dinner and had significantly higher post-dinner refluxes frequency compared to patients with B/D(−). This study suggests bloating and abdominal distension symptoms are significantly associated with increase GER frequency after dinner. This study provides insight into the interaction between GERs and bloating/distention symptoms.

134 | Biofeedback therapy improved upper gastrointestinal symptoms in patients with dyssynergic defecation (DD)

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Constipation is commonly overlapped with other functional GI disorders. We aim to evaluate the effect of biofeedback therapy for constipation on GI symptoms other than constipation symptoms.

Methods: Patients diagnosed DD by Rome IV criteria were enrolled for manometry-based biofeedback therapy every 1-2 weeks (maximum of 4 sessions). Global symptoms score (GSC; 0-100), degree of satisfaction with defecation, upper GI and constipation symptoms, were evaluated at 1 week before treatment and then at every biofeedback session. Participants who rated moderately satisfied and very satisfied with defecation at the end of treatment were defined as responders. Prevalence of each GI symptom was compared between biofeedback non-responders vs. responders. GI medication except laxatives was not adjusted during biofeedback training.

Results: Seventy-two patients (50 female, age 54 ± 15 years) were enrolled. At baseline, bloating (81.9%), post-prandial fullness (59.7%), epigastric pain/discomfort (51.4%), early satiation (33.3%), regurgitation (30.6%), nausea/vomiting (23.6%), and poor appetite (23.6%) were reported with GSC of 70.7 ± 20.9. After finish the biofeedback training, 11 (15.3%) patients were dissatisfied, 9 (12.5%) were slightly, 33 (45.8%) were moderately, and 19 (26.4%) were very satisfied with defecation. Fifty-two patients (72.2%) were responders. Baseline prevalence of GI symptoms except poor appetite and early satiation was not significantly different between responders and non-responders ($P > 0.05$). After biofeedback therapy, responders had significant decrease of the prevalence of bloating (baseline vs. post-treatment; 42 (80.8%) vs. 28 (53.8%), $P < 0.005$, but not in non-responders (17 (85%) vs. 15 (75%), $P > 0.05$)). Prevalence of epigastric pain/discomfort significantly decreased from baseline in both responders and non-responders [baseline vs. post-treatment; responders: 23 (44.2%) vs. 11 (21.2%), $P < 0.01$, non-responders: 14 (70%) vs. 6 (30%), $P < 0.01$]. Non-responders had more prevalence of poor appetite and early satiation than responders both at baseline and end of treatment. Other upper GI symptoms were not significantly different between responders and non-responders. For lower GI symptom, there was no difference in lower abdominal pain between baseline and end of treatment in both non-responders and responders, but it was higher at the end of treatment in non-responders ($P < 0.05$) (Table 1).

Conclusion: Bloating and upper GI symptoms were prevalent in patients with DD. Biofeedback responders had significant improvement of bloating and epigastric pain/discomfort without adjusting PPI/prokinetic dosage. GI symptoms other than constipation especially bloating, epigastrium pain/discomfort might be associated with defecation disorder and obtain benefit from biofeedback therapy.

Table 1: The prevalence of upper GI symptoms comparing between baseline vs. end of treatment in biofeedback and non-responders vs. responders

Symptoms prevalence	Biofeedback outcome						Baseline symptoms	End of treatment symptoms
	Non-responders (n=20)			Responders (n=52)				
	Baseline	End of treatment	Baseline vs. end of treatment	Baseline	End of treatment	Baseline vs. end of treatment	non-responders vs. responders	non-responders vs. responders
			p-value			*p-value*	*p-value*	*p-value*
Bloating, n(%)	17 (85)	15 (75)	0.625	42 (80.8)	28 (53.8)	0.001	1.000	0.117
Postprandial fullness, n(%)	15 (75)	11 (55)	0.125	28 (53.8)	19 (36.5)	0.108	0.117	0.188
Epigastric pain/discomfort, n(%)	14 (70)	6 (30)	0.008	23 (44.2)	11 (21.2)	0.008	0.067	0.537
Early satiation, n(%)	13 (65)	11 (55)	0.727	11 (21.2)	10 (19.2)	1.000	0.001	0.008
Regurgitation, n(%)	7 (35)	4 (20)	0.453	15 (28.8)	8 (15.4)	0.118	0.776	0.727
Nausea/vomiting, n(%)	8 (40)	5 (25)	0.453	9 (17.3)	8(15.4)	1.000	0.063	0.494
Poor appetite, n(%)	9 (45)	7 (35)	0.687	8 (15.4)	7 (13.5)	1.000	0.013	0.051
Lower abdominal pain n(%)	12 (60)	13 (65)	1.000	23 (44.2)	19 (36.5)	0.481	0.296	0.037

135 | On the nature of high amplitude propagating pressure waves in the human colon and the best techniques to induce them during high resolution manometry

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Background/Objectives: High Amplitude Propagating Pressure Waves (HAPWs) are universally assessed to identify colonic dysmotility, yet our understanding of normal HAPWs is rudimentary and a consensus on quantitative control values is lacking. We aimed to characterize normal HAPWs, explore the best method to induce them and provide quantitative analysis.

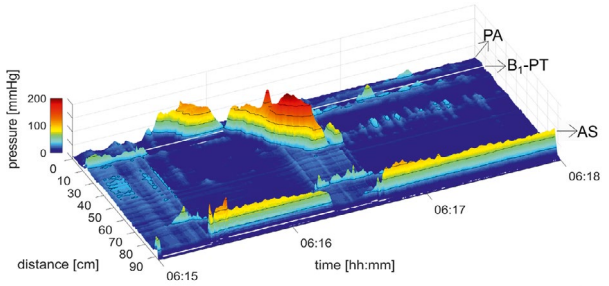
Methods: Nineteen healthy subjects (age: 20-54) underwent HRCM using an 84-channel water-perfused catheter. Interventions included: proximal balloon distention, distal balloon distention, rectal balloon distention, meal, prucalopride, and rectal bisacodyl, following a baseline period.

Results: 295 HAPWs were identified. 11% started in the proximal colon and propagated to descending, sigmoid, or rectum. 8% were proximally contained. 32% were proximally originating and transformed to simultaneous pressure waves (SPWs). 49% originated in the transverse/descending colon. Amplitudes ranged between 50 and 250 mm Hg, and velocities were between 0.2 and 2.2 cm/s.

Average control values for amplitude and occurrence are not sufficient to understand normal HAPWs; to that end we invented Symbol Maps which schematically show all HAPWs in all volunteers. To obtain a quantitative measure of HAPWs we developed the HAPW Index; based on the esophageal distal contractile interval. Rectal bisacodyl and proximal balloon distention induced most HAPWs and from all categories. An amplitude of 50 mm Hg and an HAPW index of 200 mm Hg.m.s were determined to be the cut-off values for differentiation between HAPWs and Low-amplitude propagating

pressure waves (LAPWs). A critical feature of normal HAPWs is the accompanying anal sphincter relaxation which amounts to 66% of baseline values, a much higher relaxation compared to the RAIR; 100% relaxation can occur, indicating autonomous relaxation of the external anal sphincter.

Conclusions: Several HAPW configurations need to be considered as normal, associated with autonomous internal and external anal sphincter relaxations. Symbol maps and the HAPW-Index provide a significant advance in understanding normal HAPW activity and will be useful tools in identification of ineffective HAPWs in constipated patients.



HAPW originating in the proximal colon transforms into simultaneous pressure wave (SPW) about the descending colon and is associated with complete anal sphincter relaxation. It is evoked by rectal administration of bisacodyl. White line represents 10-cm balloon (PA-proximal ascending; PT-proximal transverse; AS-anal sphincter).

136 | The role of the sphincter of O'Beirne in normal colon motor function and constipation

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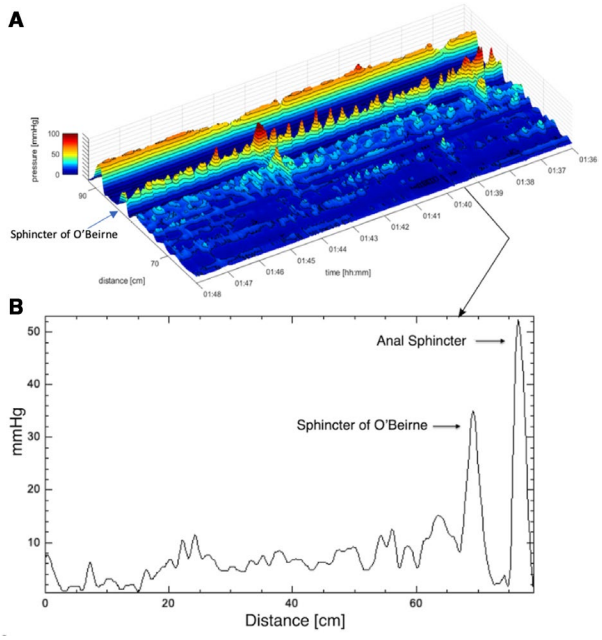
Background/Objectives: Gastroenterologists have ignored or emphasized the importance of the rectosigmoid junction in continence or constipation on and off for 200 years. Here we characterize it as the sphincter of O'Beirne in 18 healthy subjects and show its relevance to constipation in a patient case study.

As Method we employed High-Resolution Colonic Manometry (HRCM) using an 84-channel water-perfused catheter.

Results: The rectosigmoid junction shows as an intermittent pressure band of 26.2 ± 7.2 mm Hg, or intermittent phasic contractile activity at a dominant frequency of 3 cpm and an amplitude of 28.6 ± 8.6 mm Hg, 10-17 cm above the anal verge. When a motor pattern such as the high-amplitude propagating pressure wave (HAPW) or the simultaneous pressure wave (SPW) descends upon the junction, it often relaxes in concert with relaxation of the anal sphincters. This indicates that the pressure decreases as part of a neurally driven program. The junction is also a site where motor patterns end or where they start; we observed cyclic motor patterns emerging from the junction, retrogradely propagating. In a patient with severe longstanding constipation, suspected of having an inert colon, HRCM showed normal colonic activity but a persistent presence of the junction that did not relax when HAPWs or SPWs descended upon it. Paradoxical contraction of the junction was also seen in response to HAPWs, that can be considered as autonomous dyssynergia. In this patient, the high-pressure zone identified by manometry was consistent with a tight sphincter encountered on colonoscopy.

Conclusions: We propose that the rectosigmoid junction is a functional sphincter that can be referred to as the sphincter of O'Beirne and that it is part of the mechanism to maintain continence by keeping content away from the rectum. O'Beirne described the sphincter in 1834. In the patient, its excessive presence, and poor ability to relax in concert with propagating motor patterns, is likely the primary pathophysiology of her severe constipation.



A prominent and persistent anal sphincter pressure and persistent activity of the sphincter of O'Beirne in a patient with severe chronic constipation assessed by High Resolution Colonic Manometry using 84 sensors throughout the colon.

137 | Associations between colonic motor patterns and autonomic nervous system activity assessed by high-resolution manometry and concurrent heart rate variability

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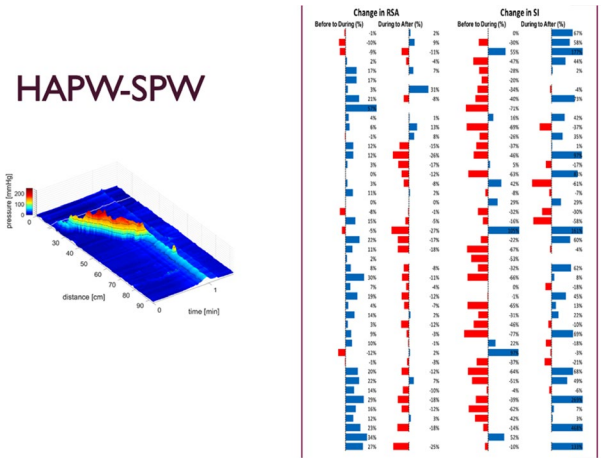
Background/Objectives: Abnormal colonic motility may be associated with dysfunction of the autonomic nervous system. Our aim was to evaluate if associations between colonic motor patterns

and autonomic neural activity could be demonstrated by assessing changes in heart rate variability in healthy volunteers.

Methods: Colonic motor patterns were assessed in 11 healthy volunteers by High-Resolution Colonic Manometry using an 84-channel water-perfused catheter. Motor patterns were evoked by balloon distention, a meal and luminal bisacodyl. The electrocardiogram and cardiac impedance were assessed during colonic manometry.

Results: As measures of parasympathetic reactivity we used respiratory sinus arrhythmia (RSA) and root mean square of successive differences of beat-to-beat intervals (RMSSD). As measures of sympathetic reactivity, we used the Baevsky's Stress Index (SI) and pre-ejection period (PEP). We show here that Motor Complexes (more than one propulsive motor pattern occurring in rapid succession) are significantly associated with increased RSA ($P < 0.0001$) and RMSSD ($P < 0.001$) (increased parasympathetic activity), and increased PEP ($P < 0.0001$) and decreased SI ($P < 0.05$) (both indicating decreased sympathetic activity). Proximal high amplitude propagating pressure waves (HAPWs) that are followed by a simultaneous pressure wave (SPW) are significantly associated with increased RSA ($P < 0.0001$) and RMSSD ($P < 0.001$) and decreased SI ($P < 0.0001$). Isolated HAPWs (without SPWs) evoke significantly increased activity in the parasympathetic system alone ($P < 0.05$). Isolated pan-colonic SPWs, are associated with decreased SI ($P < 0.05$) hence decreased sympathetic activity. In all measures, the ANS activity returns to baseline values within 2 minutes after the motor pattern subsided. The autonomic nervous system (ANS) changes during all four types of motor patterns studied were correlated with ANS changes in response to posture change from supine to standing, suggesting that the latter might predict the strength of motor activity.

Conclusions: Colonic motor patterns are associated with activity in the autonomic nervous system that is reflected in cardiac autonomic measures. These measures may serve as proxies for autonomic neural dysfunction in patients with colonic dysmotility.



Associations between the motor pattern HAPW-SPW (45 occurrences) and measures of parasympathetic reactivity (RSA) and sympathetic reactivity (SI) observed in 11 healthy volunteers.

139 | Human and bacterial-derived amyloids differentially affect cell cycle regulation in primary myenteric networks

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**Shared authorship*

Background/Objectives: A mounting body of evidence suggests that the enteric nervous system might be a vulnerable node in neurodegenerative pathologies that are associated with the transmission of amyloid-like proteins. It is, however, not known to what extent enteric neurons change their gene expression profile in response to amyloid exposure and whether they respond differently to host-derived or bacterial amyloids.

Methods: We isolated myenteric neuronal networks of the colon of WT Black6 mice and exposed them to medium containing oligomers of pro-aggregant human $A\beta_{1\text{-}42}$, peptides with a scrambled sequence ($A\beta_{scr}$), or bacterial amyloid aggregates (Curli). After 24 hours of incubation, RNA sequencing was performed using a QuantSeq 3' mRNA Library Prep kit and an Illumina NextSeq 500 sequencer. A data analysis workflow combining Rsubread and DESeq2 was used to identify differentially expressed genes, and dysregulated pathways were scrutinized using open-source bioinformatics tools such as GeneOntology, EnrichR, DAVID, GSEA, SPIA and Pathview.

Results: We found that exposure of myenteric neurons to $A\beta_{1\text{-}42}$ induced specific transcriptomic alterations that were not observed after incubation with $A\beta_{scr}$. These changes mainly pertained to inhibition of the cell cycle (57 genes including mdm2, PCNA, Cdk's and Orc6) and dysregulation of genes implicated in DNA repair (31 genes). Interestingly, exposure to Curli (as compared to control medium) induced a transcriptomic signature that pointed to activation rather than inhibition of the cell cycle (15 genes).

Conclusions: We have shown that enteric neurons respond differently to host-derived and bacterial amyloids, but that both amyloids may induce gene expression changes that adversely affect cell physiology. We are currently investigating whether these pathways also become dysregulated/activated in vivo, and what the long-term consequences are in the context of pathology development.

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140 | Why do antegrade cecostomy enemas fail?

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Introduction: Children with medically resistant constipation often end up with an appendicostomy with antegrade colonic enemas (ACE). Despite this, a subset of children for unclear reasons, continue to have defecatory problems.

Aim: To study demographics, colonic caliber and manometric tracings in a cohort who have been on ACE for constipation and continue to have defecatory problems.

Methods: Medical records of children on ACE and followed-up in our collaborative colorectal clinic over 7 years were reviewed. Contrast enema and CM tracings were examined in all subjects and findings compared among the cohort who had a normal vs. abnormal CM and duration of ACE use <30 months vs. >30 months. Wilcoxon test and Fisher's test were used to examine group differences.

Results: A total of 17 children over a 7 years period met the inclusion criteria.

Colonic Manometry: Normal (n = 10) vs. Abnormal (n = 7):

There were no significant differences in the two groups with respect to gender, BMI, age at ACE placement and duration since ACE, volume or composition of flush, age at CM and caliber change in colon. Subjects with an abnormal CM had a significantly decreased total number of HAPCs (P = 0.01), Bisacodyl-induced HAPCs (P = 0.01) and amplitude of HAPCs in the right colon (P = 0.01).

Nine of the 10 cases with a normal CM had a contrast enema and four (44%) had right colonic dilation.

Duration of ACE: >30 months (n = 10) vs. <30 months (n = 7):

In subjects that used ACE for >30 months, there was significant decrease in the total number of HAPCs (P = 0.06) and amplitude of HAPCs in the right colon (P = 0.05) and all of these subjects had a dilated right colon (P = 0.0001). Within this group, there was also a significant decrease in the amplitude of HAPCs in the right colon vs. the left colon, and was lower in both right and left colon when compared to those who used the ACE for <30 months.

Summary: With chronic use of ACE, the right colon dilates in a subset of subjects, resulting in unfavorable changes in both neuromuscular-integrity and mechanical properties.

141 | Diet and the microbiota in irritable bowel syndrome: Baseline associations and the impact of dietary intervention

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Background/Objective: Diet is a major modulator of the gastrointestinal microbiota and is an important therapy in irritable bowel syndrome (IBS). We aimed to identify microbiota-diet interactions in adults with IBS consuming habitual diet and to assess the impact of two interventions on the microbiota.

Methods: Data were analysed from 95 individuals with irritable bowel syndrome (IBS) participating in a large 4-week 2 × 2 factorial design randomised controlled trial investigating the impact of the low FODMAP diet and co-administration of a probiotic supplement. Diet was assessed at four hierarchical levels using 7-day food record data and metataxonomic data from 16S rRNA sequencing was used to measure the microbiota. All data were collected at baseline and at 4 weeks post intervention.

Results: Numerous diet-microbiota associations evident at each diet hierarchy at baseline were lost after correction for multiple testing, but were more evident at nutrient level rather than diet pattern level. There were also no associations in hypothesis-driven correlation analysis of microbiota with nutrients and food components previously established to modulate the microbiota (fibre, non-starch polysaccharides, fructans, galacto-oligosaccharides). Diet and probiotic interventions had clear impact on microbiota composition. The low FODMAP diet led to significant changes in the relative abundance of major saccharolytic genera compared with controls, including a higher *Bacteroides* (34.1% (15.7%) vs. 23.3% (15.2%), q = 0.01) and lower *Bifidobacterium* abundance (0.9% (1.0%) vs. 2.1%, (2.5%) q = 0.029). Compared with placebo, probiotic supplementation led to higher *Lactobacillus* (0.08% (0.1%) vs. 0.03% (0.2%), q < 0.001) and *Streptococcus* abundance (2.0% (2.2%) vs. 0.6% (1.2%), q = 0.001). The probiotic treatment appeared to buffer the low FODMAP microbiota effects on *Bifidobacterium* in a four-group analysis. Baseline microbiota could not predict clinical response to either intervention.

Conclusions: Although diet intervention modifies the gut microbiota, cross-sectional bivariate correlation analysis is not well suited to elucidate nutrient, food, food group or diet pattern interactions with individual gut bacteria in IBS. This may be as a result of altered microbiota and/or dietary intake in IBS, or a relative dietary homogeneity across individuals with IBS compared with healthy individuals.

142 | The CLIMB trial: Determining the effect of red meat on the human gut microbiome

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Background: There is an ongoing debate on how the consumption of red meat impacts human health. Studies have suggested red meat consumption increases levels of trimethylamine-N-oxide (TMAO) partially via the microbiome, thus increasing cardiovascular disease risk, while plant protein may have a protective effect against GI disorders such as inflammatory bowel disease (IBD). The aim of this study was to determine the impact of the habitual consumption of different types of red meat (pasture-fed Wagyu, grain-finished Angus) and soy alternatives on the composition and functional gene relative abundances of the human gut microbiome.

Methods: Fifty overweight and dyslipidaemic, male subjects (age 35-55) were single blindly randomised to 1 of 3 diets (1) New Zealand pasture-fed Wagyu Beef, (2) Grain finished Angus-mixed breed beef, or (3) Soy-based meat alternative for 8 weeks (170 g/3× week). Participants were instructed to avoid all other red meat and fatty fish. Faecal samples were collected pre and post intervention and the microbial communities were determined using shotgun metagenomic sequencing (Illumina). Sequencing data were analysed using the Biobakery pipeline, diamond, and MEGAN to determine taxonomic composition and total gene content.

Results: Taxonomic analysis of the dietary interventions showed no difference in microbial diversity or community structure between the 3 groups. Statistical analyses also indicated there were no differences in abundance of individual microbes between treatment groups. Gene compositional analyses using the KEGG and humann2 (UniRef90) databases did not identify any significant differences between the 3 intervention groups.

Conclusions: These study results suggest that consumption of red meat at levels recommended by Australian Dietary Guidelines does not substantially alter the microbial taxonomic composition or functional potential of the human gut microbiome. This contrasts with previous work suggesting consumption of animal proteins alters the human gut microbiome.

143 | Characterising human gastric-derived interstitial cells of Cajal

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Background/Objective: Interstitial Cells of Cajal (ICC) are the pacemaker cells within the smooth muscle of the gastrointestinal (GI) tract that are involved in regulating peristalsis. Two ICC markers have been well-documented: the receptor tyrosine kinase, KIT proto-oncogene; and the calcium-activated chloride channel, Anoctamin 1 (ANO1). However, KIT is not exclusive to ICC; other hematopoietic cell types such as macrophages and mast cells express high levels of KIT. Loss or dysfunction of ICC has been implicated in several GI motility disorders, including gastroparesis. Despite this, the molecular mechanisms of ICC in normal and diseased human GI biology are yet to be clearly defined. Accordingly, this study aims to perform molecular characterisation of ICC purified from human gastric tissue.

Methods: Excess human gastric tissues were collected from patients undergoing sleeve gastrectomy. ICC from gastric muscles were analysed by immunofluorescence and fluorescence-activated cell-sorting (FACS). Cell populations enriched via FACS were assessed using semi-quantitative PCR and primers for a number of cell-associated marker genes (*KIT*, *ANO1*, *CD68* and *CPA3*).

Results: Immunofluorescence revealed KIT^+ and $ANO1^+$ cells morphologically-similar to published images of human ICC. FACS performed using antibodies against KIT, CD45 and CD11b revealed a population of candidate ICC (i.e., $KIT^+CD45^-CD11b^-$ cells) representing 1.88 ± 0.65% of the total live cell count. PCR analysis of the FACS-purified candidate ICC showed a 12-fold enrichment in *ANO1* expression levels ($P < 0.0001$), and 10- and 4-fold reduction of the hematopoietic markers, *CD68* and *CPA3*, respectively.

Conclusions: We have optimised a FACS-based approach to isolate highly-enriched candidate human ICC with increased *ANO1* gene expression from gastric tissue. This approach will form the foundation for future pioneering transcriptomic and proteomic studies aimed at defining the molecular characteristics of human ICC. Such knowledge will provide fundamental biological insights into the functional roles of human ICC within GI functioning.

144 | Feeling down? A systematic review of the gut microbiota in anxiety/depression and irritable bowel syndrome

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Background: Anxiety/depression and irritable bowel syndrome (IBS) are highly prevalent and burdensome conditions, whose co-occurrence is estimated between 44% and 84%. Shared gut microbiota alterations have been identified in these separate disorders relative to controls; however, studies have not adequately considered their comorbidity. This review set out to identify case-control studies comparing the gut microbiota in anxiety/depression, IBS, and both conditions comorbidly relative to each other and to controls, as well as gut microbiota investigations including measures of both IBS and anxiety/depression.

Methods: Five databases were systematically searched using comprehensive search terms (OVID Medline, Embase, PsycINFO, and PubMed), following PRISMA guidelines.

Results: Systematic review identified 16 studies (10 human, 6 animal). Most studies investigated the gut microbiota and anxiety/depression symptoms in IBS cohorts. Participants with IBS and high anxiety/depression symptoms had lower alpha diversity compared to controls and IBS-only cohorts. Machine learning and beta diversity distinguished between IBS participants with and without anxiety/depression by their gut microbiota. Comorbid IBS and anxiety/depression also had higher abundance of Proteobacteria, *Bacteroides*, *Prevotella*/Prevotellaceae, and lower Lachnospiraceae relative to controls.

Conclusions: Well-designed case-control and longitudinal studies are required to disentangle whether the gut microbiota is predicted as a continuum of gastrointestinal and anxiety/depression symptom severity, or whether reported dysbiosis is unique to IBS and anxiety/depression comorbidity. These findings may inform the development of targeted treatment through the gut microbiota for those with both anxiety/depression and IBS.

145 | Irritable bowel syndrome and gastrointestinal symptoms are characterised by alterations in the faecal microbiome

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Background: Irritable Bowel Syndrome (IBS) is a functional gastrointestinal (GI) disorder featuring chronic or recurrent abdominal discomfort. Although the GI microbiome has been implicated in IBS, the exact role of the microbiome is uncertain. To improve our understanding of the links between the microbiome and IBS, we analysed faecal samples from a case-control study.

Methods: Faecal samples from 167 individuals were analysed by shotgun sequencing; 95 controls, 22 constipation-predominant IBS (IBS-C), and 50 diarrhoea-predominant IBS (IBS-D). Taxonomic classifications were assigned by aligning sequences against the NCBI non-redundant database using DIAMOND. GI symptoms were assessed using the Patient Reported Outcomes Measurement Information System (PROMIS) tools for GI symptoms.

Results: The GI microbiome in IBS was characterised by differences in a range of Firmicutes belonging to the *Lachnospiraceae* family, including *Blautia*, *Ruminococcus* and *Lachnoclostridium* ($P < 0.05$). Other differentiating taxa included *Bilophila* (higher in IBS-D) and *Methanobrevibacter* (lower in IBS-D) ($P < 0.05$). *Blautia*, *Bilophila*, and *Methanobrevibacter* are prominent hydrogen users, suggesting IBS is characterised by altered hydrogen metabolism in the GI tract. The major source of hydrogen in the GI tract is from microbial carbohydrate fermentation. At the phylum level, GI symptoms such as bloating and constipation were correlated with Firmicutes relative abundance, and negatively correlated with Bacteroidetes (canonical correlation > |0.5|). At the genus level, *Blautia* and an unclassified group of *Lachnospiraceae* were correlated with diarrhoea and faecal incontinence (canonical correlation > 0.5).

Conclusion: Our results show that IBS is associated with an altered faecal microbiome composition and these changes may involve functional differences in carbohydrate and hydrogen metabolism.

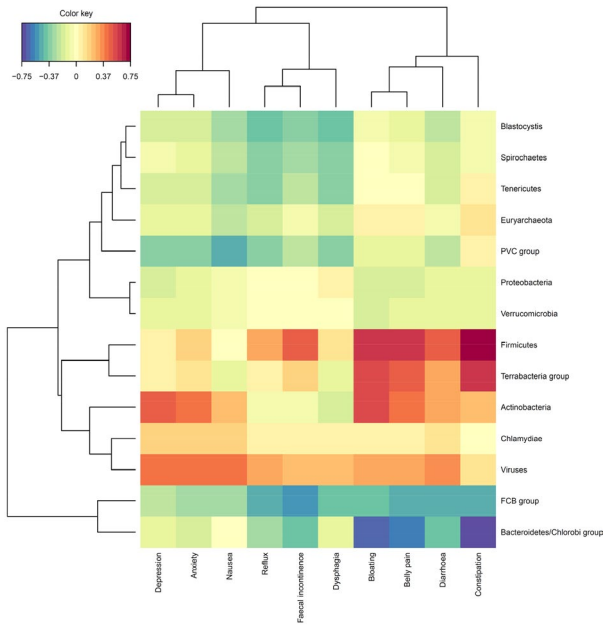

Figure 1 Canonical correlation heatmap showing associations between GI symptoms and microbiome composition at the phylum level

146 | Gut hormone secretion, gastric emptying and glucose absorption after frequently sampled oral glucose and liquid mixed meal tests in young men

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Background & Objectives: Entero-pancreatic hormone secretion has been reported during the pre-absorptive cephalic and gastric meal phases, but never with a temporal resolution allowing close scrutiny and correlation with gastric emptying and glucose-absorption.

We hypothesized that the release of insulin and insulinotropic gut peptides would coincide with glucose absorption after ingestion of liquid test meals.

Methods: Ten healthy (fasting) young men ingested (t = 0-2 minutes) on separate days liquid test meals (a 75 g glucose drink, OG) and a liquid mixed meal, LMM). Blood was collected at time −30, −20, −18, −16, −14, −12, −10, −8, −6, −4, −2, 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30 minutes for measurements of plasma glucose, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), gastrin, cholecystokinin (CCK), glucagon, pancreatic polypeptide (PP) & 3-ortho-methyl-glucose (3-OMG), and serum insulin, C-peptide and acetaminophen.

Results: Glucose and hormone concentrations were stable at baseline. The individual timing of substrate absorption and hormonal responses were highly variable. Mean acetaminophen was higher 8 minutes after OG (P < 0.001) and LMM (P < 0.05). Mean 3-OMG was higher 8 minutes after LMM (P < 0.0001), 10 minutes after OG (P = 0.04). Mean PP was higher 4 minutes after LMM (P < 0.03), but did not significantly increase after OG.

Mean gastrin was higher 6 minutes after LMM (P < 0.003) and OG (P < 0.003); mean CCK was higher than baseline 6 minutes after LMM (P = 0.0001).

Mean GIP was increased after 8 minutes (OG, P < 0.05) and LMM, P < 0.03); glucose, 8 minutes after OG (P < 0.001) and 12 minutes after LMM (P < 0.02); GLP-1, 12 minutes after OG (P < 0.01), 10 minutes after LMM, (P < 0.01). Glucagon was attenuated 30 minutes after OG (P < 0.02), but not after LMM. Mean insulin increased 12 minutes after LMM (P = 0.02) and OG (P = 0.002); C-peptide increased 12 minutes after OG (P = 0.002) and LMM (P = 0.04).

Conclusion: Gastric emptying, glucose absorption and gut hormone releases occur within minutes. Individual postprandial responses varied, which should be considered when interpreting mean responses. Within 5 minutes after liquid meal ingestion, gastric emptying, glucose absorption and gut hormone release are underway.

147 | Faecal incontinence: Identification and classification of phenotypes using contemporary, objective clinico-physiological parameters

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Background/Objectives: Faecal incontinence (FI) is a chronic and debilitating disorder. Symptomatically, FI can be categorised as either passive, urge or post-defecation. However, physiological testing allows identification of more homogenous populations[1]. Indeed, formal phenotyping of defecatory disorders has recently been achieved using hi-resolution anorectal manometry (HRAM)[2]. Therefore, the aim of this study was to identify and phenotype patients with FI using objective, clinico-physiological measures of anorectal structure and function using HRAM.

Methods: Consecutive FI patients undergoing physiological assessment of anorectal function at a tertiary referral specialist pelvic floor clinic were included. All patients underwent symptom severity assessment using the modified (Vaizey) Incontinence Score and physiological assessment using 3D HRAM, 3D endoanal ultrasonography and pudendal nerve terminal motor latency (PNTML). Nine parameters of anorectal function and structure were selected a priori to characterise phenotypes: Vaizey score,

functional anal canal length, average incremental squeeze pressure, average resting pressure, rectoanal inhibitory reflex relaxation, maximum tolerable volume, bilateral PNTMLs and the Starck score (measure of anal structural integrity on ultrasound). Results were stratified according to the three major patterns in disorders of anal tone and contractility described by the London classification[3]. Phenotypes were characterised using the statistical technique of 'K-means clustering', which identifies a number of centroids, and then allocates every data point to the nearest cluster, thus grouping observations together because of similarities.

Results: 79 patients (58 females) were included. K-means clustering of the selected anorectal parameters identified six distinct clusters. Based on the mean values of selected parameters of each cluster, six clearly identifiable patient phenotypes were characterised: (i) complete structural and functional anal impairment, (ii) neuropathic sphincter weakness, (iii) anal hypotension with normal anal contractility, (iv) anal normotension with hypocontractility, (v) severe hypocontractility and neuropathy and (vi) 'idiopathic' phenotype (Table 1). Rectoanal inhibitory reflex relaxation and maximum tolerated volume during rectal distension did not contribute to clustering.

Conclusion: K-means clustering analysis of nine objective clinical and physiological measures of anorectal structure and function has characterised 6 distinct phenotypes of FI. Classification of patients into these phenotypes may facilitate delivery of tailored and more precise treatments in the era of personalised medicine.

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148 | Validity of 3D high resolution anorectal manometry in the assessment of anal sphincter defects

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Introduction: For preservation of continence, the anal sphincter complex needs to be structurally intact and functionally strong. Traditionally, endoanal ultrasound (EAUS) has been the gold standard for the assessment of anal sphincter integrity. The recent advent of 3D high resolution anorectal manometry (HRAM) allows potential for diagnosis of anal sphincter defects in addition to its historical use for assessment of anorectal function only. Therefore, the aim of this study was to assess agreement in the identification of sphincter defects on 3D HRAM compared to 3D EAUS.

Methods: Consecutive patients undergoing assessment of anorectal function, including 3D HRAM and 3D EAUS, at a tertiary referral specialist pelvic floor clinic were included. 3D HRAM and 3D EAUS were analysed blindly by two independent assessors. 3D HRAM was used to record pressures within the anal canal during rest and squeeze, reflecting the function of the internal and external sphincters, respectively. Sphincter defects were defined as areas of at least 50% reduction in pressure, 30° or more in length and at least half of the time (Figure 1). Subsequently, the presence, localisation and size of defects as diagnosed on 3D HRAM were compared to 3D EAUS using the Bland Altman method of agreement.

Results: 98 patients (68 F; median age: 58; range: 15-84 years) were included. The positive and negative predictive values of 3D HRAM for the diagnosis of internal sphincter defects were 31.7% and 51.4%, respectively. By contrast, the positive predictive value for the diagnosis of external sphincter defects via 3D HRAM was 67.6% and the negative predictive value was 12.5%. When present on both 3D HRAM and 3D EAUS, the agreement for defect location was suboptimal for both internal and external sphincters. The Bland Altman method of agreement showed suboptimal agreement for the size of external sphincter defects. Insufficient data were available for internal anal sphincter defect size to assess agreement.

Conclusion: The detection and characterisation of anal sphincter defects on 3D HRAM was poor and cannot replace 3D EAUS in the detection of anal sphincter defects, which remains the gold standard. Nonetheless, 3D HRAM is effective for physiological evaluation of anorectal function.

Figure 1. 3D HRAM showing (a) criteria met for a right lateral IAS defect: the centre of the "defect" had a pressure of 7 mm Hg compared to the resting pressure of 68 mm Hg (~90% decrease); size of 75°, extending 1/3 of the length of IAS; and (b) criteria met for a left lateral EAS defect: the centre of the defect has a pressure of 16 mm Hg compared to the resting pressure of 132 mm Hg (~88% decrease), size of 90°, extending 1/4 of the length of the EAS

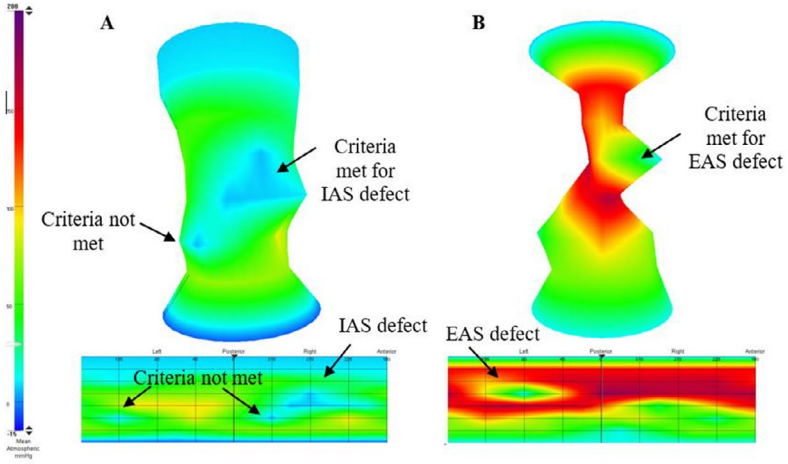

Figure 1: 3D HRAM showing a) criteria met for a right lateral IAS defect: the centre of the "defect" had a pressure of 7 mmHg compared to the resting pressure of 68 mmHg (~90% decrease); size of 75°, extending 1/3 of the length of IAS; and b) criteria met for a left lateral EAS defect: the centre of the defect has a pressure of 16 mmHg compared to the resting pressure of 132 mmHg (~88% decrease),size of 90°, extending 1/4 of the length of the EAS

151 | Effects of deoxycholic acid on the human colon contractility

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Background: Bile acid malabsorption accounts for approximately 30% of cases of diarrhea-predominant irritable bowel syndrome.
Methods: This study focused on investigating the effects of bile acid on the human colon muscle contraction, and elucidating its mechanism of action. Sigmoid colon longitudinal muscle strips were obtained from subjects who underwent colectomy for colon cancer. Isometric force measurements were calculated in response to electrical field stimulation (EFS, 0.3 ms in trains of 10 Hz for 20 seconds, 150 V). Peak and AUC during and after EFS, were measured in a basal state, and after sequential addition of deoxycholic acid (DCA, 1 mmol/L), atropine (1 μmol/L), MRS2500 (1 μmol/L), and N-nitro-L-arginine.
Results: Peak contraction and AUC during EFS significantly decreased after addition of DCA, decreased further after addition of atropine, and persisted after sequential addition of MRS2500 and L-NNA. Peak contraction and AUC off EFS significantly decreased after addition of DCA, and persisted after sequential addition of atropine, MRS2500, and L-NNA. Pretreatment with L-NNA decreased the inhibitory effect of DCA on peak contraction and AUC during EFS.
Conclusions: DCA inhibits the contraction of longitudinal muscle in the human sigmoid colon. Nitrergic pathway partly involves the mechanism of action.
Keywords: deoxycholic acid; colon; motility

152 | Risk of severe ventricular arrhythmia in domperidone users: A population-based, self-controlled case series analysis

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Background: There has been controversy over the cardiovascular safety of domperidone, attributable to the lack of a well-designed study as well as inconsistent results. This study aimed to examine the risk of severe domperidone-induced ventricular arrhythmia (VA), compared to mosapride, itopride, or non-use of all three prokinetics, in the general population.
Methods: We conducted a population-based, self-controlled case series analysis. Enrolled subjects were individuals who were diagnosed with severe VA and were prescribed domperidone, mosapride, or itopride from 2003 to 2013 in the National Health Insurance Service-National Sample Cohort. The incidence rate ratio for severe VA was measured during exposure to prokinetics and compared with unexposed periods and itopride (no-proarrhythmic effect)-exposure periods, as control.
Results: A total of 2817 subjects were included. Domperidone, mosapride, or itopride use was associated with increased risk of severe VA, compared with non-use (adjusted incidence rate ratios (IRR) of 1.342 (95% CI 1.096-1.642), 1.350 (95% CI 1.105-1.650), and 1.486 (95% CI 1.196-1.845), respectively). The risk of severe domperidone-induced VA was lower, compared to that of itopride (adjusted IRR of 0.548 (95% CI 0.345-0.870)). Of the subjects who had been prescribed all three prokinetics, domperidone-exposure was associated

with a lower risk of severe VA, compared to itopride-exposure (crude IRR, 0.571; 0.358-0.912). Mosapride-exposure did not show IRR difference for severe VA, compared to itopride-exposure.

Conclusions: Domperidone, mosapride, or itopride use is associated with an increased risk of severe VA. However, the magnitude of association was modest and domperidone use does not increase further the risk, compared with other prokinetics.

Keywords: Domperidone, Ventricular arrhythmia, Prokinetics, Self-controlled case series

153 | Effects of intragastric administration of L-tryptophan on gastric emptying, glucoregulatory hormones and plasma glucose in people with type 2 diabetes

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Background/Objective: The rate of gastric emptying and the glucoregulatory hormones, insulin and glucagon, play key roles in the regulation of postprandial blood glucose. Intragastric administration of L-tryptophan ('TRP'), at a dose of 3 g, slows gastric emptying in both lean and obese individuals, associated with a reduced blood glucose response to a mixed-nutrient drink. Whether TRP has the capacity to slow gastric emptying, affect glucoregulatory hormones and reduce the postprandial blood glucose response to a carbohydrate-containing, mixed-nutrient drink in patients with type-2 diabetes (T2D) is currently unknown.

Methods: 12 men with T2D (age 63 ± 2 years; HbA1c: 6.7 ± 0.1%) received, on 3 separate occasions, 1.5 g TRP, 3 g TRP or control, intragastrically, 30 minutes before a mixed-nutrient drink (500 kcal, 74 g carbohydrates), in randomised, double-blind fashion. Plasma glucose (n = 12), C-peptide and glucagon (n = 9) concentrations were measured at baseline, for 30 minutes in response to TRP alone, and for 2 hours after the drink. Gastric emptying of the drink (n = 12, expressed as % meal retention) was calculated from antral areas, which were measured for 2 hours using 2D ultrasound).

Results: TRP alone did not affect plasma glucose, C-peptide or glucagon. In response to the drink, there was no overall effect on plasma glucose, and maximum glucose concentration did not differ between treatments (mmol/L; control: 5.7 ± 0.5, TRP-1.5: 5.8 ± 0.5; TRP-3: 5.8 ± 0.4). However, the early glucose response (t = 0-45 minutes) was less after TRP-3 compared with TRP-1.5 and control (AUC, mmol/L*min; control: 96 ± 10, TRP-1.5: 92 ± 12, TRP-3: 50 ± 16, $P < 0.05$). C-peptide and glucagon increased on each day, however, did not differ between treatments. Gastric emptying of the drink did not differ between treatments. There was no correlation between the early postprandial plasma glucose response and early gastric emptying.

Conclusions: In people with T2D (in contrast to healthy or obese people), intragastric administration of TRP does not slow gastric emptying or affect the glycaemic response to a carbohydrate-containing drink.

154 | Goat milk increases gastric emptying compared with cow milk in a rat model

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Goat milk is often reported to be easy to digest and its curd formation differs from that of cow milk, but its effect on gastric emptying (GE), or gastrointestinal (GI) transit has not been reported. We hypothesised that goat milk would increase GE and GI transit and compared its effect to that of cow milk on these two parameters in a rat model using high resolution X-ray imaging. Concentrations of short chain fatty acids (SCFA) were also determined because some influence GI transit. Male rats (n = 12 per group) were fed a non-dairy diet supplemented with one of three drinks: goat milk, cow milk, or a dairy-free (water) control for 2 weeks. Transit of metallic beads was assessed by X-ray, and bead location was rated in vivo over 15 hours. This was used to compare GE, and transit through the small and large intestine. Caecal content was collected post-mortem for SCFA analysis. GE increased in animals treated with goat milk compared with animals consuming cow milk or water. By 4 hours, beads in goat milk treated animals had moved further through the small intestine than the cow milk treated animals. Both milks slowed transit through the large intestine compared with the control. Goat milk altered the short chain fatty acid (SCFA) profile in a manner that differed from controls, suggesting altered carbohydrate fermentation and lower levels of amino acid fermentation. There was no significant difference between the SCFA profile of cow milk and control animals. Our study demonstrated increased GE of the solid content (beads) in animals supplemented with goat milk. However, we did not detect a significant difference in transit in the lower GI tract. The faster GE for goat milk treated animals compared with cow milk correlates with the comparatively lower curd density reported previously for goat milk.

155 | Effects of intragastric administration of the branched-chain amino acids, leucine, isoleucine and valine, on gastric emptying, plasma C-peptide and glucose concentrations in healthy, lean men

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Background/Objective: Whey protein slows gastric emptying, stimulates insulin and lowers postprandial blood glucose in health and type-2 diabetes. Whey is rich in the branched-chain amino acids, leucine, isoleucine and valine, which may, at least in part, mediate

the effects of whey. In healthy men, 10 g of leucine or isoleucine, administered intragastrically, modestly lowered the blood glucose response to a mixed-nutrient drink containing predominantly fructose-based carbohydrates. The effects of valine, and the comparative effects of these amino acids, on gastric emptying, glucoregulatory hormones and blood glucose are currently unknown, and were investigated in this study using a mixed-nutrient drink containing glucose-based carbohydrates.

Methods: 12 healthy lean men (age: 26 ± 3 years), participated in a double-blind, randomised study, in which they received, on 4 separate occasions, either 10 g leucine, isoleucine or valine, or control, intragastrically 30 minutes before a mixed-nutrient drink (500 kcal; 74 g carbohydrates). Plasma glucose and C-peptide (n = 6) were measured at baseline, in response to the amino acids, and for 120 minutes following the drink. Gastric emptying of the drink was measured using ^{13}C-acetate breath-test.

Results: Amino acids alone did not affect plasma glucose or C-peptide. In response to the drink, there was a treatment effect on plasma glucose (peak concentration [mmol/L]; control: 6.8 ± 0.2; leucine: 6.3 ± 0.2; isoleucine: 6.1 ± 0.1; valine: 6.9 ± 0.3); isoleucine lowered glucose compared with control, and leucine and isoleucine compared with valine (all $P < 0.05$). There were treatment effects on C-peptide ($AUC_{0\text{-}120min}$ [pmol/L*min]; control: 169 478 ± 34 369; leucine: 199 249 ± 42 379; isoleucine: 168 215 ± 38 139; valine: 170 999 ± 38 552; $P < 0.05$), and on gastric emptying ($AUC_{0\text{-}120min}$ [%recovery $^{13}CO_2$/hour]; control: 2553 ± 130; leucine: 2148 ± 127; isoleucine: 2322 ± 117; valine: 2214 ± 105; $P < 0.05$), although differences between treatments were not statistically significant.

Conclusions: In healthy lean men, intragastric administration of leucine and isoleucine, but not valine, lowers postprandial blood glucose, apparently independently of stimulation of C-peptide or slowing of gastric emptying.

156 | Reflux esophagitis patients showed a more rapid worsening of clinical parameters associated with life style disease

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Background/Objectives: Although risk factors of reflux esophagitis (RE) have been investigated in numerous cross-sectional studies, little is known about predictive factors associated with future onset of RE. We investigated time courses of clinical parameters before RE onset by a longitudinal case-control study using long-term health checkup records.

Methods: We used health checkup records between April 2004 and March 2014 at nine institutions in Japan. The time courses of the clinical parameters of RE subjects were compared with those of non-RE subjects by the restricted maximum likelihood method or multivariate logistic analysis. We also implemented cross-sectional comparisons for each of the 5 years prior to RE onset.

Results: Initial data were obtained from 230 056 individuals, and 2066 RE subjects and 4132 non-RE subjects were finally included in the analysis. The time courses of body mass index, fasting blood sugar, serum triglyceride, glutamate oxaloacetate transaminase, glutamic pyruvic transaminase, γ-glutamyl transpeptidase, percentages with acid reflux symptoms and feeling of fullness, and percentage with hiatal hernia in the RE group were significantly worse than in the non-RE group.

Conclusions: The RE group displayed a more rapid worsening of the clinical parameters associated with lifestyle diseases, including obesity, diabetes, hyperlipidemia, and fatty liver compared with the non-RE group. These results suggest that RE is a lifestyle disease and thus lifestyle guidance to at-risk person may help to prevent RE onset.

157 | A novel method for quantifying periodicity and time delay in dynamic neural networks using unstable sub-action potential threshold depolarisations

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Background/Objectives: In the central and peripheral nervous systems of vertebrates and invertebrates, the coordinated firing of large populations of nerve cell bodies in neural networks is well described. In many electrophysiological studies, recordings are often made from multiple recordings sites. These multisite recordings can detect the propagation of action potentials, for example, around the cerebral cortex. In these cases, the waveform of the action potentials is usually relatively stable. However, problems can arise when trying to correlate the degree of synchronization and time lag between two or more independent recording sites, when their waveform is unstable (e.g. with fluctuations in amplitude or duration). This occurs when recording electrical events that are below threshold for action potential generation, such as synaptic potentials, or calcium transients. This is compounded by the fact that the extent and directionality of propagation of electrical signals through neural networks can be highly dynamic. Recently, we used calcium imaging to record the activity from hundreds of enteric neurons simultaneously (Spencer NJ *et al*. 2019; J. Neurosci).

Methods: A problem arose as to how to spatially correlate and analyse the degree of synchronization between two recording sites, when calcium transients were of inconsistent waveform.

Results: We report the development of a novel analytical approach to cross correlate signals from two independent recordings sites

whose waveform varies over time, including the frequency and time delay between the recording sites.

Conclusions: The approach described allows determination of directionality of the spread of excitation along a neural network based on measurements of the time delay between recording sites. This new method could also be used for the analysis of low frequency rhythmic events in other biological systems, where sub-action potential threshold recordings show dynamic variability.

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158 | Effects of whey protein on energy intake, gastric emptying and glycaemia in obese, older and younger men

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Background: A strategy to reduce energy intake in obese, young adults is to increase dietary protein. It is not clear if this works in obese, older individuals; we have reported that a 30 g whey protein drink suppresses energy intake less in older than young non-obese men. The whey protein slowed gastric emptying, more in the older than younger men. The aim of this study was to determine the effect of a whey-protein drink on energy intake, gastric emptying and glycaemia in obese, older and younger men.

Methods: In randomised, double-blind order, 10 younger (age: 27 ± 2 years; body mass index: 36 ± 2 kg/m²) and 10 older (72 ± 1 years; 33 ± 1 kg/m²) obese men ingested a 30 g whey protein (120 kcal) and control (2 kcal) drink on separate study days. Gastric emptying, as measured by antral area (ultrasonography), blood glucose concentrations (t = 0-180 minutes), and ad libitum energy intake (t = 180-210 minutes) were determined following overnight fasting. Data were analysed using repeated measure mixed-effect model.

Results: Energy intake was not altered by the whey-protein drink ingestion, when compared to control, in either the older (decrease 2 ± 8%) or younger (decrease 3 ± 8%) obese men ($P > 0.05$). There was a trend for energy consumption to be lower in the older than younger men on both study days ($P = 0.16$). Whey protein ingestion slowed gastric emptying, compared to control, to a similar degree in both age groups (gastric emptying half time: older and younger combined protein vs. control: 40 ± 13 vs. 20 ± 1 minutes, $P < 0.001$). Fasting and postprandial blood glucose concentrations were higher in the older than younger obese men (protein and control day combined old vs. young; fasting: 6.2 ± 0.1 vs. 4.9 ± 0.6 mmol/L and postprandial (area under the curve 1110 ± 30 vs. 992 ± 20 mmol/L/180 minutes, $P < 0.05$). There was no effect of whey-protein ingestion on glycaemia in either age group.

Conclusions: Our data suggest that obesity may blunt/abolish the age-related effect of whey protein on suppression of energy intake and slowing of gastric emptying.

160 | The role of gut microbiota and gastrointestinal dysfunction in a mouse model of Huntington's disease

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterized by progressive motor dysfunction associated with cognitive and psychiatric symptoms. The R6/1 transgenic mouse model of HD recapitulates the progressive onset of clinical symptoms and pathology. The emerging field of the microbiota-gut-brain axis has resulted in new hypotheses where microbiota could represent a link between genetic and environmental factors. Indeed, by profiling the fecal microbiome of R6/1 mice at 12 weeks of age (i.e. prior to overt motor onset), we have recently provided the first evidence that gut dysbiosis occurs in HD. We observed gut dysbiosis in the HD mice, including a change in the ratio of the major bacterial phyla, *Bacteroidetes* and *Firmicutes*. Our central hypothesis is that the gut microbiota and gut physiology are important features of the HD pathophysiology and targeting gut microbiota before the onset of symptoms provides an important new modulation target as a possibility of treatment. In order to test this hypothesis, we have modulated the gut microbiota directly through treatment with antibiotics followed by fecal microbiota transplantation from wild-type mice to HD mice. We have also used exercise and environmental enrichment interventions to evaluate experience dependent modulation of the gut microbiota and HD pathogenesis. Our preliminary results indicate that we have well-established models for the evaluation of the impact of the gut microbiota on HD mice and promising results that may inform future treatments for HD. This is the first study to investigate the direct and indirect effects of gut microbiota modulation in HD and extend our understanding of gastrointestinal dysfunction and novel therapeutic targets to combat this devastating disease.

162 | A high fat diet alters endocannabinoid and ghrelin mediated regulation of orexigenic receptor expression in nodose ganglia

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Background: Anandamide (AEA) has biphasic effects on gastric vagal afferent (GVAs) sensitivity to stretch. At low concentrations, AEA

decreases GVA sensitivity in a cannabinoid type 1 (CB1) receptor, TRPV1 and ghrelin-dependent manner. At high concentrations, AEA increases GVA sensitivity in a TRPV1 and CB1-dependent manner. In high fat diet (HFD)-induced obesity AEA only has an inhibitory effect. This may be due to altered levels of gastric ghrelin and AEA and subsequent changes in expression of their receptors. The current study aimed to determine the effect of ghrelin and AEA on the expression of CB1, ghrelin receptor (GHSR), TRPV1, and fatty acid amide hydrolase (FAAH; responsible for AEA breakdown) in vagal afferent cell bodies.

Methods: Eight-week-old male C57BL/6 mice were fed a standard laboratory diet (SLD) or HFD for 12 weeks. Nodose ganglia were removed and vagal afferent cell bodies cultured for 14 hours in the absence and presence of ghrelin (3 nmol/L) or methAEA (mAEA, stable analogue of AEA; 100 nmol/L). Quantitative RT-PCR was performed to determine expression of CB1, GHSR, TRPV1, and FAAH.

Results: Cells from SLD-mice: mAEA increased TRPV1 and FAAH, and decreased CB1 and GHSR mRNA expression. Ghrelin decreased expression of TRPV1, CB1, and GHSR mRNA.

Cells from HFD-mice: mAEA increased CB1, GHSR and FAAH but had no effect on TRPV1 expression. Ghrelin decreased TRPV1 and increased CB1 and GHSR mRNA expression.

Conclusions: AEA and ghrelin modulate receptors and breakdown enzymes involved in the mAEA-GVA satiety signalling pathway. This modulation was disrupted in HFD-induced obesity and may contribute to the changes in vagal afferent signalling observed in obesity.

163 | Pharyngeal contractile integrals as diagnostic markers for swallowing dysfunction: A concurrent videofluoroscopic and pressure-flow analysis study

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Background & Aims: High-resolution pharyngeal manometry (HRPM) can reliably measure pharyngeal motor function through the derivation of contractile integrals (CI). Being based on expert opinion and limited published data, these metrics require formal evaluation to determine their clinical relevance. We assessed the diagnostic value of whole and regional pharyngeal CIs in relation to signs of pharyngeal swallowing safety (aspiration) and efficacy (residue), as defined on simultaneous radiological imaging.

Method: We performed a retrospective study of 72 adult patients with oropharyngeal dysphagia who underwent simultaneous HRPM and videofluoroscopic swallowing studies (VFSS) at a tertiary university hospital and who demonstrated objective evidence of global or regional pharyngeal weakness. A total of 263 swallows were analyzed. Pharyngeal pressure and impedance recordings from each patient were extracted and analyzed using pressure-flow analysis (PFA) on Swallow Gateway™ to obtain metrics for pharyngeal contractility, UES function, and hypopharyngeal intrabolus distension pressure were. VFSS video clips were scored for airway invasion, pharyngeal residue, and associated physiologic impairments using the Penetration Aspiration Scale (PAS), Normalized Residue Ratio Scale (NRRS), and Modified Barium Swallow Impairment Profile (MBSImP) scales, respectively. Logistic regression analysis was applied to assess the relationship between pharyngeal CIs and radiologic outcomes.

Results: Distinct signs of pharyngeal impairment were observed to occur with specific regional CI abnormalities, but not with the whole pharyngeal CI (PhCI). Aspiration was related to decreased velopharyngeal CI (VCI < 9 mm Hg.cm.s; OR 3.27 [95% CI 1.5-7.4]). Significant vallecular residue was associated with decreased mesopharyngeal CI (MCI < 45 mm Hg.cm.s; OR 2.83 [95% CI 1.6-4.9]), while pyriform sinus residue was associated with decreased velopharyngeal CI (OR 3.75 [95% CI 2.0-7.5]) and increased UES relaxation pressure (UES-IRP; OR 1.76 [95% CI 1.3-2.2]). Low hypopharyngeal mean peak pressure (HypoPeakP < 88 mm Hg) was a strong predictor of aspiration and residue. Pharyngeal CIs appeared to be influenced by age and robust to changes in bolus consistency.

Conclusion: Regional pharyngeal contractile integrals add important diagnostic information to the current pharyngeal HRPM technology. By combining these with other HRPM or VFSS metrics, a comprehensive characterization of the biomechanics of inefficient or unsafe pharyngeal bolus clearance can be made.

164 | Relationship between gender roles and psychological comorbidities in patients with irritable bowel syndrome

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Background/Aim: Irritable bowel syndrome (IBS) is a disease that typically shows gender differences. In addition, psychiatric comorbidity plays an important role in the pathogenesis of IBS, and depression and anxiety are frequently accompanied by IBS. We aimed to measure the properties of gender role and investigate their relation to psychiatric factors in patients with IBS.

Methods: Patients diagnosed with IBS by Rome III were enrolled at multiple sites in Korea. Participants completed the short form of the Korean Sex Role Inventory (KSRI-SF) for assessing masculinity and femininity, stress questionnaire, hospital anxiety depression scale (HADS), and SF-36 questionnaire.

Results: A total of 102 patients with IBS (M:F = 35:67 mean age 42.6 ± 16.7) were recruited, 55 subjects who showed normal findings on colonoscopy were controls (M:F = 20:35 mean age 42.4 ± 11.1). IBS patients had more psychological stress than the control group. (9.69 ± 8.23 vs. 4.56 ± 8.31, $P < 0.0001$) Anxiety and depression scores were higher in IBS patients than in the control group (16.12 ± 7.17 vs. 10.22 ± 5.74, $P < 0.0001$). IBS patients with anxiety and depression showed a significantly lower masculinity score and total gender score in comparison with IBS patients without anxiety and depression, respectively. In patients with IBS, there was no definite difference in score of HADS and KSRI-SF between males and females. There was no difference between IBS and controls regarding masculinity score and femininity score. However, masculinity score was significantly lower in patients with IBS with anxiety or depression than in patients without anxiety or depression and in controls. Quality of life is poor in IBS patients as a result of SF 36. A low score of the physical component score was related to IBS symptoms.

Conclusion: IBS patients were more depressed and anxious, more stressed, and had lower quality of life than the normal control. Lower masculinity rather than sex is associated with psychological comorbidities.

Kew Words: Irritable Bowel Syndrome; Anxiety; Depression; Stress, Psychological; Masculinity

165 | The outcome of fecal incontinence treatment in patients with congenital anorectal malformation can be predicted by anorectal manometry

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Objectives: Patients with congenital anorectal malformations (CARM) are known to suffer from fecal incontinence. We aim to investigate whether patients with CARM have the anal-external sphincter continence reflex (AESCR) and the puborectal continence reflex and whether the presence of these reflexes improves the outcome of therapy.

Methods: We included 40 patients with CARM, who suffered from fecal incontinence and who underwent anorectal manometry between 2010 and 2019. The balloon retention test was performed to examine the presence of the fecal continence reflexes and the defecometry test to test whether they use the pelvic floor muscles adequately. Furthermore, we analyzed the fecal continence outcomes; complete fecal continence, improvement or no improvement, after physical therapy. Improvement was defined as a decrease in frequency of bowel management or when patients with bowel management became continent with bowel management.

Results: Out of 40 patients, the AESCR was present in 32 patients and the puborectal continence reflex in 39. All 9 patients who were either treated conservatively, or underwent anoplasty, anterior sagittal anorectoplasty or bucket-handle resection possessed both reflexes. Of the 31 patients who underwent other types of surgery, 8 patients lacked the AESCR and 1 lacked the puborectal continence reflex.

Of all patients, 25 patients underwent pelvic physical therapy. Out of 20 patients who had both continence reflexes, 6 became completely continent and 9 showed improvement. Out of the 4 patients without AESCR, but with the puborectal continence reflex 2 showed improvement but none became completely continent.

Conclusions: Patients with all types of anorectal malformations can have both fecal continence reflexes. Presence of both fecal continence reflexes in fecal incontinent patients predicts good clinical outcome after pelvic physical therapy.

166 | Characterising the gut microbiome in anxiety and depression – A systematic review

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Background/Objectives: Growing evidence supports the role of the gut microbiome in anxiety and depression, which are highly prevalent, comorbid affective disorders whose aetiology presently remains unclear. Whilst clinical studies in anxiety are sparse, an integrated investigation of the possible shared, underlying microbial features of both debilitating disorders may inform future diagnosis and treatment avenues. Here we present the first systematic review of the human gut microbiota in anxiety, combined with an essential update of recently published clinical studies of gut microbiota in depression.

Methods: A systematic search was conducted using the MEDLINE (Ovid), Embase Classic+Embase, PsycINFO, and PubMed databases to identify human case-control studies of gut microbiota in clinical anxiety (n = 2) and depression (n = 16), and cross-sectional associational studies with anxiety/depression symptom measures in humans (n = 6).

Results: Compared to existing reviews, 24 eligible studies provide evidence that alpha and beta diversity findings are inconsistent between anxiety and depression cohorts relative to controls. However, several taxa were consistently identified, including a lower abundance of *Faecalibacterium* and *Prevotellaceae*, and a higher abundance of Actinobacteria in major depression disorder cohorts relative to controls. Generalized anxiety disorder cohorts had a decreased abundance of Firmicutes, Ruminococcaceae, Prevotellaceae, Faecalibacterium, Subdoligranulum, *Dialister*, and *Coprococcus;* and an increased abundance of *Bacteroides*, *Escherichia/Shigella* and Enterobacteriaceae/Enterobacteriales relative to controls. These findings indicate that both disorders may be characterised by an increase in pro-inflammatory species, and a decrease in short-chain fatty acid producing bacteria.

Conclusions: Whilst diversity findings across studies are largely inconsistent, several taxa and their mechanisms of action are highlighted as they may relate to anxiety and depression aetiology. A comprehensive evaluation of confounding factors is presented, which may potentially contribute to the lack of consensus in taxonomical findings of the extant literature. Future research should carefully assess the confounding effects of comorbid gastrointestinal syndromes and diet, and assess functional variation of differential microorganisms.

167 | Placebo response rate in patients with chronic idiopathic constipation: A systematic review and meta-analysis

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Background: It is essential to know the placebo response rate in patients with chronic idiopathic constipation when designing a treatment clinical trial. However, no one has reported its specific value in a systematic review and meta-analysis.

Objective: The purpose of this study is to calculate the pooled placebo response rate and discuss relative factors.

Method: PubMed, Cochrane Library, Embase were electronically searched for randomized controlled trials (RCTs) on treatment in constipated patients compared with placebo or sham stimulation. Data extraction and assessment of risk of bias were performed independently by two reviewers. The pooled placebo response rate and its 95% confidence interval (95% CI) were calculated and all the statistical analyses were performed using R 3.6.0. Our protocol has registered in PROSPERO with registration number: CRD42019121287.

Result: There are 87 studies included with 14 374 constipated patients allocated to placebo arm in total. The pooled placebo response rate is 28.31% (95% CI: 25.54-31.07%). The placebo response rate is higher in studies with single center than that in multicenter (34.36% vs. 25.83%, $P < 0.05$). Patients respond less to placebo when the treatment is given once a day than twice or three times a day (23.75% vs. 33.44% or 48.51%, $P < 0.05$). Treatment efficacy assessed using global improvement can significantly elevate the placebo response rate (31.72% vs. 21.05% for improvement in CSBM, $P < 0.001$), especially reported by patients subjectively (33.98% vs. 25.81% for objective assessment, $P < 0.05$). Patients diagnosed by Rome I present a dramatically high response to placebo than that in the rest three criteria: Rome II, Rome III, diagnose based on clinical symptom (60.80% vs. 28.71%, 27.62% or 25.94%, $P < 0.001$). However, this consequence may not reliable because of inadequate simple size in Rome I group. There is no statistical difference in placebo response rate among patients diagnosed with FC, IBS-C and CIC.

Conclusion: Patients with constipation have a significant response level to placebo. This response rate differs within the subgroups associated to study design parts, as described above, suggesting more cautious design in further RCTs.

Keywords: chronic idiopathic constipation, placebo response rate, systematic review and meta-analysis

169 | The RECLAIM study: Symptoms, bowel movements, psychological comorbidity and the balloon expulsion test confirm differences between IBS-C, FC and HV as defined by Rome IV criteria

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Background: Approximately 50% of patients with constipation are not satisfied by current medical management. Whether this is because medications are directed to treatment of symptoms and not underlying pathophysiological mechanisms is unclear. The RECLAIM study uses MRI and high-resolution manometry to define colonic responses in individuals with different subtypes of constipation and examines their different response to prokinetic and antispasmodic treatment in a randomised clinical trial.

Aim: Analysis of baseline data for recruited trial subjects to assess clinical differences between subgroups.

Methods: Participants across two sites have been classified as healthy volunteers (HV), constipation-predominant IBS (IBS-C) and functional constipation (FC) based on ROME IV criteria. Before undergoing MRI and manometry, baseline data acquired included: Cleveland Clinic Constipation Score (CCCS), PAC-SYM, Hospital Anxiety Depression Scale (HADS) score, 2-week off-treatment bowel habit diary reporting number of weekly spontaneous bowel movements (SBM) and complete SBM (CSBM). Patients also underwent a balloon expulsion test (BET: test normal if expulsion within 2 minutes).

Results: Baseline data from 87 enrolled participants ([age 36 ± 13; BMI 25.5 ± 5]: 41 HVs [36F], 28 IBS-C [27F] and 18 FC [17F]) were analysed. Age and BMI were similar between groups.

Both patient groups have significantly higher constipation and depression scores than HV; IBS-C also had higher anxiety scores (Table 1). IBS-C present a similar number of SBM to health but a lower number of bowel movements they considered complete. FC have lower numbers of both SBM and CSBM and have impaired evacuation.

Conclusion: As expected, both constipated groups scored higher in the CCCS and PAC-SYM questionnaire and had higher anxiety and depression scores. Sensation of completeness of evacuation is selectively impaired in IBS-C but how this relates to colonic motility remains to be defined.

170 | Quantifying abnormal colonic function in constipation using MRI

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Background: RECLAIM is a multicentre study examining patients with functional constipation (FC) and IBS with constipation (IBS-C), along with healthy volunteers (HV), to correlate MRI findings with those from colonic manometry, and clinical response.

Aim: MRI assessment of the colonic response to an osmotic laxative in patients with constipation and health.

Methods: Participants meeting ROME IV criteria (confirmed by bowel habit diary) were recruited at 2 sites. A fasting baseline scan was performed using a 3T Philips Ingenia scanner. Participants then consumed MoviPrep™ and had two further scans at 60/120 minutes. MRI measures included: colonic volume, transit time (using the weighted-average position score 0-7 of transit markers taken the previous day; higher scores = slower transit) and a metric of mixing of colonic contents (% coefficient of variance in MRI data tagged to give sensitivity to movement, averaged over ascending colon region of interest). Image analysis was performed blind to participant condition. Baseline and maximum value reached were used to allow for different oral-caecal transit and mid-study defaecation. Time from MoviPrep™ to first bowel movement (TBM) was recorded using a cut off at 150 minutes (average time spent at centre).

Results: To date, 66 participants have completed MRI (31 HVs, age and gender matched to 23 IBS-C and 12 FC: results outlined in Table 1). After MoviPrep™, largest volumes and increase from baseline was seen in the FC group compared to IBS-C and HV. Transit scores and TBM were variable but showed slower transit for the patient groups compared to HV. Whilst mixing decreased in patients compared to HV this was not significant.

Conclusion: The MoviPrep™ challenge, as previously reported, demonstrates larger colons and slower transit in patients with constipation compared to health and could now be used to quantify abnormal function in clinical practice.

171 | The enteropancreatic nerve activity modulates insulin and glucagon secretion

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Background: Enteric neurons of the proximal duodenum project out of the gut to pancreas through enteropancreatic nerves. Our study found that the activity of enteropancreatic nerves was altered with duodenum motility suggesting gut might play a role in modulating insulin/glucagon secretion through enteropancreatic nerves. In this study, we investigated the possible role of enteropancreatic nerve activity in insulin and glucagon secretion.
Methods: Isolated perfused pancreases from rats were used. The enteropancreatic nerves were tested with repetitive electrical nerve stimulation at low-frequency (2 Hz) or high-frequency (16 Hz). Insulin and glucagon releases were detected by enzyme-linked immunosorbent assay at different glucose (2.7, 5.6, 16.7 mmol/L) condition.
Results: With 2.7 mmol/L glucose, low-frequency enteropancreatic nerve stimulation reduced insulin secretion significantly by 46.7% ($P < 0.05$) and reduced glucagon secretion by 8.0% ($P < 0.05$). The inhibition on insulin secretion was not altered by 100 μmol/L hexamethonium (HEX). High-frequency enteropancreatic nerve stimulation had no effect on insulin or glucagon secretion at 2.7 mmol/L glucose. With 5.6 mmol/L glucose, enteropancreatic nerve stimulation had no significant effect on insulin secretion at low-frequency but increased insulin secretion significantly by 57.4% ($P < 0.05$) at high-frequency. HEX totally removed the high-frequency nerve stimulation-induced increase of insulin secretion and further suppressed insulin secretion by 36.5% ($P < 0.05$, compared to the control). With 5.6 mmol/L glucose, enteropancreatic nerve stimulation had no significant effect on glucagon secretion. With 16.7 mmol/L glucose, low-frequency nerve stimulation had no significant effect on insulin secretion. High- frequency nerve stimulation trended to increase insulin secretion and trended to decrease glucagon secretion.
Conclusions: Our results suggested that enteropancreatic nerves contribute to insulin and glucagon secretion modulation and the modulation is glucose-level dependent. (Supported by National Natural Science Foundation of China, Grant No. 81670492).

172 | TRPV4 modulates motility in the mouse colon

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Background: Activation of the stretch-sensitive ion channel, TRPV4, has been proposed to both stimulate and inhibit colonic motility by distinct mechanisms. TRPV4 is functionally expressed by colonic resident macrophages and glia but not neurons and smooth muscle. This is inconsistent with the proposed mechanisms for TRPV4 in motility. TRPV4-dependent effects on motility have been studied using muscle strips and in vivo transit assays. However, complex motility patterns have not been examined. We assessed the role of TRPV4 in motility of the isolated mouse colon.
Methods: *Colonic motor complexes* (CMCs) generated in intact colons were analysed from spatiotemporal maps. The *occurrence of CMC initiation* (number of transition locations from non-contracting to contracting state) and *the rate of contraction* (difference in colon diameter (at the start − end of the contraction)/contraction 'on/off' duration) were compared.
Results: Colon dilation increased CMC occurrence and the rate of contraction in all conditions. Bath addition of TRPV4 activator GSK1016790A or inhibitor HC067047 did not evoke an immediate response (n = 8 and 5, respectively) but altered CMCs properties. At diameters 2.7-3.3 mm, GSK1016790A increased CMC occurrence by 1.2-2.1 times when compared to control. HC067047 decreased CMC occurrence compared to control when the diameter of the colon was between 2.1 and 3.9 mm. The rate of contraction compared to control did not change with GSK1016790A but was reduced by HC067047. At diameters between 2.1 and 4.0 mm, HC067047 decreased the ratio of the rate of contraction to a proportion of 0.82-0.61 times that in control. Although CMCs were unaffected in tissues from mice where macrophages were acutely depleted, GSK1016790A did not change the occurrence of CMC initiation.
Conclusion: We demonstrate that TRPV4 modulates but does not mediate mouse colonic motility and suggest a role for resident macrophages. Our work challenges currently proposed mechanisms by which TRPV4 and resident macrophages' influence colonic motility and highlights the importance of studying an intact system, devoid of central influence.

173 | Changes of duodenal electromyography in type 2 diabetes rats and rats with RYGB surgery

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Backgrounds: Roux-en-Y gastric bypass (RYGB) surgery exerts powerful antidiabetic effect for type II diabetes (T2DM) but its mechanism underlying remains unclear. Duodenum has been suggested playing an important role in the bariatric surgery-induced diabetes remission with its strategic position in the upper digestive tract and influence on both endocrine and exocrine activity of pancreas. In this study, we evaluated the changes of duodenal electromyography (EMG) induced with T2DM and T2DM with RYGB.
Methods: Normal rats in control (NC) group and T2DM rats induced with streptozotocin in DM group were receiving operation with electrode implantation on duodenum, and T2DM rats in DM-RYGB

group were receiving RYGB surgery and electrode implantation. Duodenal EMGs were recorded 2 weeks after surgery.

Results: All rats in DM group maintained diabetic state throughout experiment time and the rats in DM-RYGB groups regained normal glycemic control 2 weeks after surgery. All three groups had comparable length of MMCs. However, the durations of phases I and III were significant ($P < 0.05$) shorter in DM group and phase III was significant longer in DM-RYGB group, compared with NC group. In DM group, the bursts per minute (BPM) of phase II was significant higher ($P < 0.05$) than NC group at proximal site but not at distal site, but was significant lower ($P < 0.05$) than DM-RYGB group at both sites of duodenum. The propagation velocity of phase III in duodenum was significant ($P < 0.05$) higher in DM group compared with NC group and DM-RYGB group. The BPM in 120-minute fed period was increased significantly ($P < 0.05$) in DM group compared with NC group and further increased ($P < 0.05$) in DM-RYGB group. The time for phase III recovery in DM group was significant ($P < 0.05$) longer than NC group and DM-RYGB group.

Conclusion: It was the first time to investigate the duodenal EMG changes with T2DM and after RYGB surgery and the mechanism behind these changes might play an important role in the diabetes remission with RYGB surgery. (Supported by Natural Science Foundation of China, Grant No. 81270900, 81670492).

174 | Association with gastroesophageal reflux disease and noncardiac chest pain and/or extraesophageal symptoms: A symptomatic, endoscopic, and proton pump inhibitor trial study

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Background: Gastroesophageal reflux disease (GERD) is known to be the most common cause of NCCP being in up to 60% of patients with NCCP. Also, a variety of extraesophageal symptoms have been attributed to GERD, including asthma, coughing, hoarseness, throat clearing, and globus. Unlike classic manifestations of GERD such as heartburn and acid regurgitation, NCCP or extraesophageal symptoms may be difficult to accurately diagnose and properly treat. Therefore, we examined the symptomatic, endoscopic and pH metric associations between GERD and NCCP, and GERD and extraesophageal symptoms. Also we analyzed the response to a proton-pump inhibitor (PPI) using 1- or 2-week clinical trial in patients with NCCP or extraesophageal symptoms.

Methods: Sixty-three patients with at least twice per week NCCP and 108 patients with extraesophageal symptoms at least twice per week were enrolled. The NCCP and extraesophageal symptoms were assessed using the 1-week symptom score and the typical GERD symptoms of heartburn and acid regurgitation. Transnasal-esophagogastroduodenoscopy (TN-EGD) and ambulatory 24-hour esophageal pH monitoring were performed to diagnose GERD (+) or GERD (−). Then, patients were treated with 30 mg of lansoprazole twice a day for 14 days. The PPI test was considered positive if a symptom score improved ≥50% compared with baseline. In patients with NCCP or extraesophageal symptoms, documentation of reflux esophagitis on TN-EGD and/or pathologic acid exposure on ambulatory 24-hour esophageal pH monitoring was used to determine the positivity of GERD.

Results: In NCCP patients, 31 were diagnosed with GERD (+) and 32 were diagnosed with GERD (−). Also in patients with extraesophageal symptoms, 22 were diagnosed with GERD (+) and 86 were diagnosed with GERD (−). There was no significant difference for a positive ratio between GERD (+) NCCP group (56%) and GERD (−) NCCP group (56%) in the 1-week PPI test, however in the 2-week PPI test, GERD (+) NCCP had a higher positive ratio (80%) than GERD (−) NCCP (27%) ($P < 0.05$). On the other hand, in patients with extraesophageal symptoms, both the 1-week PPI test and 2-week PPI test showed no significant difference between the GERD (+) group and the GERD (−) group.

Conclusions: In NCCP patients, the empirical trial of PPI was diagnostic for patients with frequent GERD-related NCCP, and its optimal duration was determined to be at least 2 weeks, not 1 week. However, in patients with extraesophageal symptoms, while these symptoms may be associated with GERD, a 1 or 2-week PPI trial is not long enough to be a diagnostic tool.

175 | Defecation-associated disorders following resection surgery for (recto)sigmoid infiltration by endometriosis: Towards better long-term outcomes

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Background/Objectives: Patients with deep infiltrating endometriosis (DIE) are known to experience defecation related problems, such as constipation and fecal incontinence (FI). (Recto)sigmoid resection performed for DIE has been reported to improve the defecation associated outcomes. The purpose of this study is to investigate the prevalence of fecal problems and the pattern of symptoms in women after surgical treatment for deep infiltrating endometriosis in comparison with healthy women.

Methods: This cross-sectional study was designed as a single tertiary-level academic center. We included 130 female adult patients who had undergone (recto)sigmoid resection for deep infiltrating endometriosis between January 2005 and December 2015.

Patients were randomly age-matched to two disease-free controls from the general Dutch population. We measured the prevalence of constipation, FI, Irritable Bowel Syndrome (IBS) and the Low Anterior Resection Syndrome Score (LARS).

Results: The prevalence of constipation, FI, and IBS in the patients was significantly higher than in the controls (50.8% vs. 26.2%, $P < 0.001$, 15.4% vs. 5.4%, $P = 0.001$, and 14.6% vs. 5.4%, $P = 0.002$, respectively). The prevalence of constipation and FI was lower in the patients who had undergone surgery longer than 24 months ago in comparison with those who had undergone surgery less than 24 months ago (46.7% vs. 69.9% and 15.0% vs. 17.4%), which is still significantly higher in comparison with the control group. Finally, the LARS score was significantly higher in the patients than in the controls.

Conclusion: Following surgery for deep infiltrating endometriosis, patients do still experience constipation, FI, and IBS more frequently than healthy controls. Even after surgery for DIE, patients do not get close to outcomes comparable of the general Dutch population.

176 | Defecation disorders and their association with quality of life among women after (recto)sigmoid resection due to deep infiltrating endometriosis

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Background/Objectives: Deep infiltrating endometriosis (DIE) negatively affects women's QoL and bowel functions. To improve condition of the patients, (recto)sigmoid resection is often performed. The QoL of the patients however still tends to be poorer after the treatment than in a control group. We aimed to explore the relationship between the quality of life (QoL) and fecal problems in women after (recto)sigmoid resection due to deep infiltrating endometriosis (DIE).

Methods: In this cross-sectional study we prospectively collected data of 124 adult female patients treated with a (recto)sigmoid resection at the University Medical Centre Groningen between January 2005 and December 2015. All patients completed the validated Groningen Defecation and Fecal Continence questionnaire (DeFec) and the Endometriosis Health Profile (EHP-30) core questionnaire. Constipation, Fecal Incontinence (FI) and Irritable Bowel Syndrome (IBS) were defined according to the Rome IV criteria. Patients with comorbidities, which are known to influence defecation and fecal continence, were excluded from analysis.

Results: Patients had the lowest mean score in case of the domain "Control and powerlessness" (33.7, SD ± 42.8) and the highest in case of the domain "Emotions" (42.8, SD ± 16.3). When looking at patients with exclusively one fecal disorder, we found that solely constipated patients had significantly higher scores than non-constipated patients in the core domains of EHP-30 ($P < 0.003$). Patients who suffered from exclusively IBS had significantly higher scores in three core domains: "Pain" ($P = 0.018$), "Emotions" ($P = 0.006$) and "Self-image" ($P = 0.035$), when compared to patients without (exclusively) IBS. FI was not associated with increased scores in any of the EHP-30-core domains. Multiple regression analysis confirmed that constipation and IBS significantly contributed to the prediction of "Pain", "Emotions" and "Self-Image".

Conclusions: Constipation and IBS are the major factors which negatively contribute to decreased QoL among women who had undergone surgery for DIE. It is however still unclear whether the disease, the surgery or both contribute directly to the fecal disorders among women who were treated with rectosigmoid resection due to DIE.

177 | A noninvasive gastric function test for the assessment of major pathophysiology of functional dyspepsia

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Background and Aims: Functional dyspepsia is characterized by impaired gastric accommodation, visceral hypersensitivity and impaired gastric motility. The aim of this study was to develop a simple one test to assess these pathophysiologies of FD.

Methods: A simple noninvasive test is proposed as follows: simultaneous recordings of 4-channel electrogastrogram (EGG) for 30 minutes before and after a nutrient drink (0.6 Cal/mL, fat: 19%, protein: 18% and carbo: 63%) test in which the subject was asked to drink at a speed of 60 mL/min until completely full. Symptoms during the 30 minutes after the drink were recorded. The study was performed in 98 FD patients (48 ± 1.4 years) and 14 healthy controls (53 ± 2.2 years) from 2 hospitals in China.

Results: (1) The FD patients showed a significant lower percentage of normal slow waves in all 4 channels of the EGG recordings (typically 10% lower, $P < 0.05$, vs. controls), suggesting impaired gastric motility. (2) The maximum intake of nutrient drink was substantially less in FD patients (637 mL vs. 1091 mL, $P < 0.05$ vs. controls), suggesting a reduced accommodation. (3) The drink test induced bloating in both controls and patients; however, the recovery of bloating was slower in FD patients compared with the controls ($P < 0.05$) and pain was reported after the drink only in patients with FD, suggesting visceral hypersensitivity. (4) The combined test was not able to differentiate subtypes of FD. However, the postprandial distress syndrome patients showed significantly decreased dominant frequency and power of the gastric slow waves in both fasting and fed state ($P < 0.05$ for all). (5) The severity of bloating is negatively correlated

with the percentage of normal slow waves measured in channel 4 of the postprandial EGG ($P < 0.05$).

Conclusions: This easy and noninvasive test provides comprehensive information on pathophysiologies of FD and is capable of differentiating FD patients from healthy controls; correlations have been observed between subjective symptoms and objective physiological measurements. It may be used as a noninvasive surrogate method for assessing gastric accommodation, visceral hypersensitivity and antral motility.

179 | Microbial regulation of stress-induced alterations in the gut metabolome

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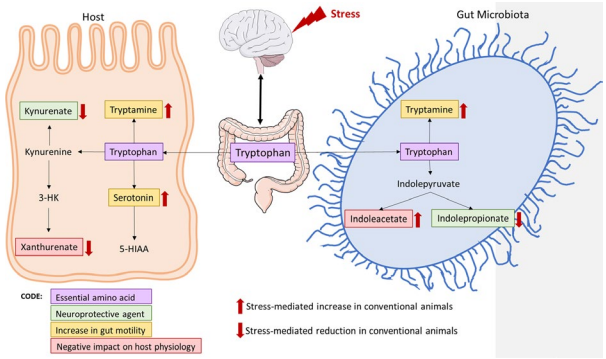
Background/Objectives: The physiological consequences of stress exposure often manifest in the gastrointestinal tract. Chronic psychological stress has implications for host physiology, behaviour and gut microbiome composition. The immediate host-microbe response following an acute stressor and the implications for gut homeostasis have been neglected.

Methods: Adult C57/BL6 male conventional (Conv), germ-free (GF), and recolonised GF (ColGF) mice were randomly allocated to unstressed or stressed groups. The latter were subjected to a single acute stressor and culled immediately or 45 minutes following stress. Caecal contents and mucosal scrapings were taken for metabolomic analysis.

Results: The levels of tryptophan in the cecum decreased after stress in all groups. In stress-exposed Conv animals, the mucosal levels of serotonin and tryptamine were increased meanwhile levels of kynurenines were reduced. The metabolic profile following stress exposure in GF animals was markedly different. Colonisation of GF animals restored the metabolic profile in cecum to Conv levels but not in the mucosa. In GF and ColGF, stress induced an increase in the mucosal levels of kynurenate and xanthurenate. An elevation of cecal tryptamine was found in Conv and ColGF animals in response to stress. Finally, there was an increase in indoleacetate and a reduction in indolepropionate levels only in Conv and ColGF following acute stress.

Conclusions: Both host and microbial tryptophan metabolic pathways were markedly altered by stress. To our knowledge, this is the first time that a direct effect of acute stress on microbial metabolism has been demonstrated, with changes in indoleacetate and indolepropionate, metabolites produced exclusively by gut microbes. Our results suggest that acute stress induces rapid changes at the host-microbe interface and that the microbiota is responsible for directing host and microbial metabolism of tryptophan along specific and physiologically relevant homeostatic pathways.



180 | Mechanophysiological analysis of anorectal function

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Background/Objectives: Defecation is a complex process that may easily get disturbed. Defecatory disorders are commonly diagnosed with anorectal manometry and the balloon expulsion test. Recently we developed a simulated electronic stool named Fecobionics that integrates several tests and simultaneously assesses pressures, orientation, bending and geometry during expulsion of the device. The aim was to use new analytical tools to calculate frictional force and tension during expulsion of Fecobionics in normal subjects.

Methods: The 12-cm-long Fecobionics contained pressure sensors at the front, rear and inside a bag, two motion processor units for measurement of orientation and bending, and impedance rings for measurement of serial cross-sectional areas/diameters. Eight presumed normal subjects (4 F/4 M) defecated Fecobionics. The bending angle of the device, the frictional force between the probe and the surrounding tissue, the membrane tension, and the probe expulsion velocity were calculated.

Results: The bending angle and pressures changed during expulsion of the device with the maximum pressure recorded at the rear. Pressure change at the front was closely associated with changes in the bending angle (Figure 1). The averaged circumferential tension, longitudinal tension and friction force change were associated with the pressure difference between the rear and the front of the device ($R^2 = 0.878 \pm 0.011$, $P < 0.005$; $R^2 = 0.885 \pm 0.01$, $P < 0.005$; $R^2 = 0.411 \pm 0.007$, $P < 0.005$; respectively). The peak tensions and friction force immediately before expulsion of the rear were significantly higher than that when the front entered into the anal canal (circumferential and longitudinal tension $P < 0.005$, and friction force $P < 0.05$).

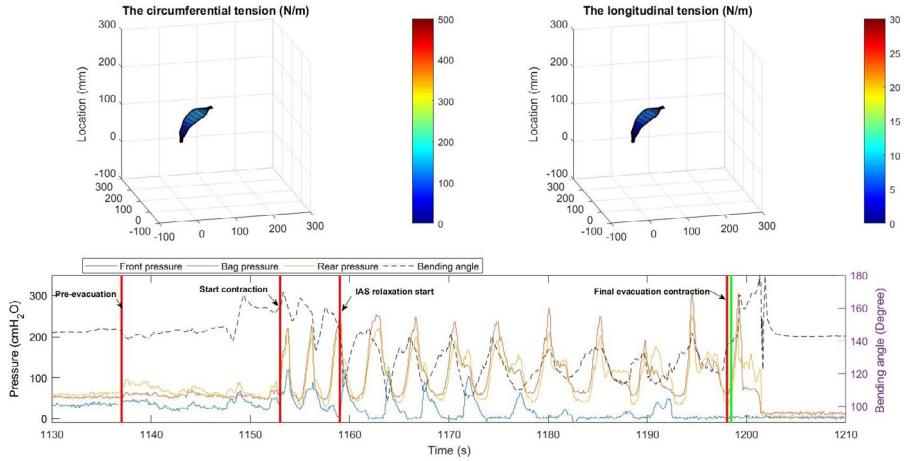

Figure 1. Top panels: The geometry and the membrane tension distributions of the Fecobionics during expulsion. Bottom panel: The recorded pressures and bending angles in the Fecobionics during expulsion.

Conclusions: Fecobionics obtained reliable data under physiological conditions. Mechanical features such as frictional force and membrane tension were assessable during Fecobionics expulsion using the developed analysis.

182 | Incongruent views of quality of life between patients and gastroenterologists: A mixed-methods approach

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Background: Functional Gastrointestinal Disorders (FGIDs) are disorders of brain-gut interaction that result from a combination of motility disturbance, visceral hypersensitivity, altered mucosal, immune function, altered gut microbiota and central nervous system processing. The objective of the present study was to examine the relationship between incongruence, which was defined as a differing perception of the illness between the patient and the physician, and type of diagnosis (functional or organic) with psychopathology.
Methods: The present study involved 1562 patients with gastrointestinal disorders attending primary care centres. Incongruence was calculated as the difference between the clinician-rated functionality (Karnofsky Performance Status Scale) and the subjectively rated quality of life (physical functioning of the SF-36 subscale). Psychopathology was measured by means of scoring the responses given on the Brief Symptom Inventory–18 (BSI-18).
Results: The results indicated that both the presence of incongruent views between the patient and the physician and a functional gastrointestinal diagnosis predict higher levels of psychopathology. Interestingly no interaction was found between the aforementioned variables suggesting that they function independently in psychopathology, thus suggesting that neither of the diagnostic groups had greater levels of incongruence. Whilst both factors contribute to psychopathology, incongruent views between the patient and the physician had a much higher explanatory power on psychopathology than the diagnosis of the patient.
Conclusions: The study has allowed us to identify a psychosocial factor (i.e. incongruence) that may be indicative of lower wellbeing and quality of life.

Keywords: Psychopathology, Incongruence, Diagnosis, Primary Care, Patient-Physician Relationship

183 | Role of enteric glial cells and ICAM-1 in myenteric plexitis

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Half of Crohn's disease (CD) patients require surgery within 20 years of diagnosis, and post-operative recurrence (POR) is frequent. Among the risk factors of POR, the presence of myenteric plexitis at the proximal resection margin has been incorporated in the European guidelines. Our objectives were to determine which cells of the enteric nervous system interact with T cells, and to identify the molecules responsible for these interactions.
In vivo: 29 patients (20 CD, 9 cancer) who underwent an ileocolonic resection were included. Full-thickness slices of the proximal resection margin were analysed by immunohistochemistry (IHC) to identify enteric glial cells (EGC, S100β) and T cells (CD3). T cells in contact with EGC were counted on each slide.
In vitro: EGC were co-cultured with activated T cells. To determine the impact of inflammatory conditions, EGC were pre-treated with

lipopolysaccharide (LPS) or IL-1β/TNFα (IT). Adhesion of T cells to EGC was analysed by immunocytochemistry (ICC). The expression of ICAM-1 was determined by qPCR, western blot and ICC.

IHC showed the presence of T cells in contact with EGC of myenteric ganglia in both CD and control patients. The number of T cells per ganglion was significantly higher in CD patients as compared to controls. In vitro, pre-treatment of EGC with LPS and IT significantly increased the number of T cells in contact with EGC as compared to the control condition. An overexpression of ICAM-1 in EGC was also observed as determined by qPCR, western blot and ICC.

Our results indicate that T cells interact with EGC in vitro and in vivo. These interactions are increased under inflammatory conditions and are associated with an upregulation of ICAM-1. Further experiments will be carried out to confirm the implication of ICAM-1 in T cells/ECG interactions.

185 | Barostat assisted sensory adaptation training for IBS and rectal hypersensitivity: Randomized controlled trial

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Background: Rectal hypersensitivity (RH) is a key pathophysiological dysfunction associated with IBS and abdominal pain, but there are ineffective therapies. Antidepressants help but their efficacy and tolerability remain challenging. In a RCT, we investigated the efficacy and safety of a novel treatment, sensory adaptation training (SAT), in IBS-C patients and compared this with escitalopram.

Methods: IBS-C patients (Rome-III) with RH (≥2/4 sensory thresholds below normal values) were randomized to receive either biweekly SAT or escitalopram 10 mg daily for 3 months. SAT was performed by placing a 10 cm highly compliant barostat balloon in the rectum. After assessment of sensory thresholds, the rectal balloon was distended in 1-2 mm Hg increments above pain thresholds until maximum tolerability or 45 mm Hg threshold. Patients were motivated to divert their attention from pain. Prospective stool diaries assessed daily pain episodes and bowel symptoms. Treatment effects and those between therapies were compared. Patients with ≥30% decrease in pain and ≥20% increase in ≥2/4 rectal sensory thresholds were considered responders.

Results: Forty-nine patients (F/M = 45/4) were enrolled, SAT = 26, Escitalopram = 23; 24/26 (92%) completed SAT, 2 were lost to follow up; 17/23 (74%) completed escitalopram, 5 withdrew from adverse events and 1 lost to follow up. Significantly greater ($P < 0.001$) number of IBS patients were both overall responders and hypersensitivity responders in SAT arm compared to escitalopram, but no differences for pain responders (Table). Sensory thresholds and number of CSBM/week significantly improved only with SAT (Table). Bowel satisfaction increased and daily pain score decreased significantly in both groups, without group differences. SAT was well tolerated.

Conclusions: SAT appears to be an effective and safe treatment for IBS-C with abdominal pain. It was more efficacious and better tolerated than escitalopram. SAT is a promising novel treatment for IBS with rectal hypersensitivity.

Acknowledgement: NIH-2R01-DK057-100-06A.

	SAT (n = 26)			Escitalopram (n = 23)			$P^{\#}$
Overall responder (Hypersensitivity + Pain) (%)	54%			4%			**<0.001**
Hypersensitivity responder (%)	69%			17%			**<0.001**
Abdominal pain responder (%)	58%			44%			0.4
	Before	**After**	*P*	**Before**	**After**	*P*	$P^{\#}$
Rectal sensory pressure thresholds (Barostat)							
First sensation (mm Hg)	14.4 ± 0.9	20.2 ± 2.1	**0.0071**	13.5 ± 0.7	15.2 ± 1.0	0.08	0.3
Desire to defecate (mm Hg)	20.7 ± 0.9	34.2 ± 2.4	**0.0001**	20.6 ± 1.0	22.8 ± 1.4	0.06	**0.006**
Urgency to defecate (mm Hg)	26.8 ± 1.5	41.6 ± 1.8	**<0.001**	27.3 ± 1.4	28.9 ± 1.5	0.09	**<0.001**
Global Bowel satisfaction score (VAS)	20.4 ± 3.9	50.0 ± 6.3	**0.0001**	22.5 ± 3.3	46.7 ± 5.5	**0.002**	0.6
Mean daily abdominal pain score (0-4)	1.7 ± 0.2	1.1 ± 0.2	**0.002**	1.6 ± 0.2	1.3 ± 0.2	**0.03**	0.8
No. of CSBMs/week ((Median (LQ, UQ))	0 (0, 0.75)	2.5 (0, 5.75)	**0.003**	1 (0, 5.5)	1 (0, 6.5)	**0.306**	**0.041**

$P^{\#}$: Sensory adaptation training (SAT) vs. Escitalopram. Data expressed as Mean ± SEM unless shown

186 | Characteristics of a subset of achalasia with normal integrated relaxation pressure

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Background/Aims: Integrated relaxation pressure (IRP) is a critical diagnostic criterion to define achalasia. However, there are some cases with typical symptoms and signs of achalasia but with normal IRP. The aim of this study was to evaluate the clinical characteristics of patients with achalasia with normal IRP and outcomes after peroral endoscopic myotomy (POEM).

Methods: Patients with achalasia were collected in whom peroral endoscopic myotomy was performed from November 2014 to April 2018 at ~ Medical Center. Achalasia with normal IRP was defined by findings compatible to achalasia in Eckardt score, endoscopy with endoscopic ultrasound, high-resolution manometry, impedance planimetry (EndoFlip®), and timed esophagogram.

Results: POEM was performed in 89 patients with achalasia; among them, 24 (27%) patients were diagnosed with achalasia with normal IRP. Patients with achalasia with normal IRP were older, had longer duration of symptom, and had a more tortuous esophagus. In EndoFlip, the distensibility index and cross-sectional area were higher in patients with normal IRP. Therapeutic outcomes showed no statistically significant differences. On correlation analysis, IRP had negative correlations with age, disease duration, and distensibility index.

Conclusions: Patients with achalasia of normal IRP value were older and had longer disease duration and higher distensibility index and cross-sectional area than patients with achalasia with abnormal relaxation of lower esophageal sphincter (LES). Therapeutic outcomes were not different between the two groups.

187 | Submucosal neurons of the porcine colon respond to different mechanical stimuli

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Background/Objectives: Due to anatomical and physiological similarities, the pig intestine represents a well-suited model for human studies. Nevertheless, data on porcine enteric neuronal functionality are scarce. Aim of the present study was to identify and characterize mechanosensitive neurons of the inner submucous plexus of the porcine colon.

Methods: Samples from the proximal and transverse colon were taken from 26 adult and 20 juvenile pigs, and the inner submucosal plexus was isolated. Enteric ganglia were stained with the voltage-sensitive dye Di-8-ANEPPS and responses were recorded by an ultra-fast CMOS camera system (DaVinci 1K). The neurons underwent two mechanical stimuli. An intraganglionic volume injection of small volume of physiological solution was used to simulate compression and a self-designed, two-armed stretching device was used to exert a bidirectional stretch on single ganglia. In order to characterize mechanosensitive neurons immunohistochemistry followed the experiments.

Results: Reaction to compression was investigated in a total number of 730 neurons of the proximal colon. 7.9% showed reproducible responses with a mean burst frequency of 13.5 and 11.4 Hz for the first and second stimulus. Responses to bidirectional stretch were investigated in 469 neurons of the same region. 8.4% of these neurons showed reproducible responses firing action potentials with 6.3 and 5.5 Hz. In the transverse colon 402 neurons underwent compression. 9.3% of them showed reproducible responses with a mean burst frequency of 10.8 and 9.9 Hz. Responses to bidirectional stretch were investigated in 488 neurons. 5.1% showed reproducible responses with a mean burst frequency of 4.2 and 3.3 Hz for the first and second stretch.

Conclusions: We identified mechanosensitive neurons in the inner submucosal plexus of the porcine colon which respond to compression and stretch. Mechanosensitive properties did not differ between the different localizations and between different age animals.

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188 | Nerve-mediated secretion is evoked by serosal and mucosal distension in the porcine distal colon

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Background/Objectives: From a physiological point of view the porcine large intestine appears a good model for the human one, however little is known about distension evoked secretory functions. The aim of this study was to investigate porcine colonic secretion in response to mucosal and serosal distension.

Methods: Distal colonic tissue preparations (n = 24) obtained from 6 pigs (~ 120 kg, both sexes) were mounted in Ussing chambers and short circuit currents (I_{sc}) were measured. Tissue distension was evoked four times (each 30 minutes apart) by applying a 60 seconds lasting 60 mm Hg pressure with a pump on the serosal and mucosal side, respectively. Amplitude of secretory response and secretion index were calculated and compared in a paired manner in control tissues and tetrodotoxin (TTX) treated tissues (each n = 12), respectively.

Results: Serosal and mucosal distension evoked an increase in I_{sc} (27.83 ± 12.0 and 24.99 ± 11.10 μAs/cm², respectively). Amplitude of the secretory response as well as secretion index evoked by serosal application were significantly reduced by pretreatment with TTX (19.49 ± 6.10 vs. 27.26 ± 8.41 μAs/cm² and 61.87 ± 20.49 vs. 82.12 ± 22.03 μA/cm^2 × min^{-1}). Reduction in maximal secretory response after TTX treatment was also recorded when pressure was applied from mucosal side (22.26 ± 9.51 vs. 29.19 ± 9.18 μAs/cm²).

Conclusion: In porcine distal colon, distension of the intestinal wall from both the serosal and mucosal sides evokes secretion as indicated by increase in I_{sc}. This secretion response is largely nerve mediated shown by the effects of TTX. Further experiments will show whether this secretion is chloride dependent as it is the case in other species.

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189 | The effect of gastrin-releasing peptide (GRP) on motility in isolated, vascularly perfused rat colon

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Background/Objectives: Gastrin-releasing peptide (GRP) induced stimulation of smooth muscle contraction and peristalsis in gastrointestinal tract. The effect of GRP on motility, however, has not been studied in the vascularly perfused rat colon. The aim of this study was to investigate the effect of GRP on the isolated rat colon and to clarify whether the action of GRP on colonic motility is mediated via the influence of cholinergic input.

Methods: Effect of GRP on colonic motility was studied in totally isolated rat colon vascularly perfused with Krebs solution containing 0.1% BSA and 3% Dextran at 1.2 mL/min via SMA. Luminal pressure was monitored via microtip catheter pressure transducers from proximal and distal colon. After control, GRP was administered at 14, 28, 140, 276 pM in a stepwise increase fashion. Motility index was calculated for the last 5 minutes of each 15 minutes period and expressed as %change over basal level.

Results: GRP increased colonic motility in rats. The stimulating effect of GRP on colon was almost completely abolished by atropine, tetrodotoxin, respectively.

The effect of GRP on colonic motility

GRP	MI of Proximal colon	MI of Distal colon
Dose (pM)	(mean ± SE %, n = 6)	(mean ± SE %, n = 6)
0	0	0
14	23.7 ± 7.0**	31.0 ± 10.3*
28	107.6 ± 25.8**	72.5 ± 10.2**
140	102.5 ± 41.8*	80.7 ± 21.7**
276	79.4 ± 28.8*	173.5 ± 79.1
28(P), 140(D) + Atropine	0 ± 0##	1.0 ± 0.6##
28(P), 140(D) + Tetrodotoxin	16.7 ± 10.2#	15.1 ± 15.1#

MI: Motility index, (P): Proximal colon, (D): Distal colon, *P < 0.05 vs. basal, **P < 0.01 vs. basal, #P < 0.05 vs. 28pM(P) or 140pM(D), #P < 0.01 vs. 28pM(P) or 140pM(D).

Conclusions: GRP increase colonic motility in rats. The stimulatory action of GRP on colon requires local cholinergic input.

190 | In vivo administration of an intraluminally acting 5-HT4 receptor agonist enhances colonic motility ex vivo

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Objective: The prokinetic action of 5-HT4 receptor agonists is well documented and is used to alleviate various forms of constipation by enhancing the release of acetylcholine from myenteric nerve terminals and/or by stimulating epithelial receptors. However, 5-HT4Rs are also expressed in many other regions of the body such as the heart and bladder, potentially complicating the use of 5-HT4R agonists. We have shown previously that the prokinetic action of 5-HT4R agonists can be mediated through luminal targets (PMID 22226658; Konen et al., FNM2018). We developed 5-HT4 receptor agonists that are minimally absorbed to prevent systemic distribution and tested their effectiveness in enhancing motility in the isolated murine colon.

Methods: Male C57BL/6 (B6) mice received either 0.1 mL of vehicle or intraluminally acting 5-HT4 agonist (10 mg/kg) via enema 30 minutes prior to being euthanized. The entire colon was immediately removed and pinned loosely in an organ bath containing aerated Krebs' solution (37°C). Wall motions and propulsion of natural contents was recorded by video and analyzed using PTCL analysis (VolumetryG9d, GWH).

Results: Isolated colons from either vehicle or drug compound treatments had similar lengths (vehicle: 52.3 ± 4.0 mm vs. agonist: 49.0 ± 3.1 mm) and distribution of natural contents (n = 5; P > 0.05). However, the degree of contractile activity, measured as the % of time that differential wall motions (> = 0.16 mm/s) were detected along the preparations, was markedly enhanced in colons treated with the agonist, particularly in mid-distal colon (205 ± 33%, n = 5, P < 0.01). Rhythmic bursts of contractile activity were more prevalent in colons treated with the agonist.

Conclusions: These results indicate that an intraluminally acting 5-HT4 receptor agonist is effective in enhancing contractile activity in isolated mouse colons, consistent with their prokinetic effect shown in vivo.

193 | Gluten increase bacterial fermentation, but do not affect gastrointestinal symptoms in patients with irritable bowel syndrome (IBS): A randomized, double-blind, placebo-controlled, cross-over study

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Background/Objectives: Although the use of gluten-free diet (GFD) is common in patients with IBS, its effects on gastrointestinal (GI) symptoms is not clear. This study aimed to investigate the effects of gluten on symptoms, visceral sensitivity and bacterial fermentation.
Methods: IBS subjects (Rome IV), who reported food-induced, but not necessarily gluten-related GI symptoms, were challenged with gluten in this randomized, double-blind, placebo-controlled, cross-over study. They had gluten-free meals, in two 14-days periods separated by a washout period of 14 days. The subjects sprinkled either gluten (14 g/d) or placebo powder over the meals during the two periods in a randomized order.
Before and after the diet periods, the subjects reported overall GI symptom severity (IBS-SSS and GSRS-IBS), and stool form (Bristol Stool Form scale), and they underwent a Lactulose Nutrient Challenge Test (*LeNeve, Am J Gastroenterol;2013*) to asses visceral sensitivity (GI symptoms) and bacterial fermentation (breath methane and hydrogen).
Results: Twenty IBS subjects [18 females, aged 25.5 (range 18-33) years] were included.
No significant differences were found in overall GI symptom severity between the gluten-free and gluten-containing periods. Exhaled methane concentration, as a measure of bacterial fermentation, was higher after the gluten-containing period ($P = 0.03$), whereas exhaled hydrogen concentration was not significantly different. The subjects reported worse overall GI comfort ($P = 0.02$), and more severe urgency ($P = 0.01$) during the Lactulose Nutrient Challenge Test after the gluten-free period compared to the gluten-containing period. There was an order effect, with more severe symptoms during the first compared with the second diet period. The key results are presented in Table 1.
Conclusion: Using a blinded dietary challenge paradigm, we were unable to demonstrate a clear short-term effect of gluten on GI symptoms in IBS patients with food-related symptoms, even though gluten increased bacterial fermentation.

194 | Dynamic full field optical coherence tomography imaging of alive myenteric plexus

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Imaging the enteric nervous system architecture and function remains a major challenge to better understand its role in gut health and diseases. Up to now, imaging of ENS in living full thickness preparations is limited by scattering properties of the tissue, limited depth of imaging and also phototoxicity of the dyes used. Therefore, new generation of 3D imaging techniques such as optical coherence tomography (OCT) or full-field OCT (FFOCT) is of interest as it allows non-invasive and staining free living tissue imaging with high spatial resolution. In the present study, we imaged full thickness colon preparation of mice with FFOCT both in static mode (S-FFOCT) and dynamic mode (D-FFOCT). FFOCT images of myenteric plexus were analyzed and compared to whole mount preparation of the same tissue stained with S100, Hu. In addition, the origin of D FFOCT signals was studied by modulating neuronal activity using TTX or changes in osmolality. S-FFOCT allowed to image the whole depth of colon from the serosa up to the base of the crypt. Mainly highly refractive tissue probably composed of highly reflective molecules such as collagen could be distinguished. In addition, at the interface of longitudinal and circular muscle, the myenteric network of ganglia could be identified. D-FFOCT imaging allowed the identification of cellular and subcellular structures that were identified as enteric neurons and probably also glial cells. Modulation of neuronal activity with TTX did not modify D-FFOCT signals in enteric neurons. However, changes in osmolarity reversibly extinguished D-FFOCT signals. This study identifies D-FFOCT as a novel imaging modality of the ENS allowing dye and dissection free in situ imaging of the ENS with a high spatial resolution. The origin of the optical signals in the ENS observed in D-FFOCT remains to be identified. These techniques might be of interest to analyze changes in neuronal/glial density and network organization associated with various pathologies.

195 | Effect of repeated stress on the spatial distribution of the host-microbiota interactome along the mice intestine

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Background/Objectives: Psychological stress is recognized as being a key environmental factor contributing in the evolution of chronic

diseases. However, its impact on the geographic composition of gut microbiota and its association with host transcriptomic responses remains largely unknown. Therefore, we aimed to analyze the changes in host-microbiota interactome between intestinal epithelial cells, the luminal as well as adherent microbiota in various gut regions induced by repeated stressors in mice models.

Method: A model of C57BL/6 mice subjected to water avoidance stress (WAS) for four consecutive days was used to evaluate the physiological effect of stress. To identify how host epithelial functions associate to microbes in adherent or luminal regions we used reductionist and associative approaches on 16S rRNA and host transcriptomic data of the various gut regions.

Results: Differential analysis between control and stressed mice revealed region specific changes in epithelial gene expression especially in the Ileum where the transcriptional response was the most impacted. Furthermore, the prefrontal cortex and the hippocampus were strongly affected in terms of number of differentially expressed genes compared to other brain regions. However, changes in microbial abundances were less numerous and only noticeable at the species level. Eventually, using associative studies, we were able to correlate bacterial signatures to host transcriptomic response in the proximal colon with specific functional changes in corticosterone levels.

Conclusion: This stratified spatial characterization is a first step to explore biological functions associated to gut microbial regions and to the gut-brain axis in C57BL/6 mice. We propose new insights on how the gut functional biogeography can be modulated by the host physiology and identify novel bacterial or host transcriptomic signature that could contribute to organ dysfunctions induced by stress setting up the basis to develop novel therapeutic strategies to prevent/treat these dysfunctions.

196 | Strict anaerobic bacteria involvement in gut-brain axis: A novel regulator of visceral pain and well-being

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Background/Objectives: The potential role of gut microbiota in visceral pain modulation arouses an emerging interest during recent years. The composition of the gut microbiota could have an influence on the neurological component, both on behavior and pain processing. This could be due to an indirect communication *per* immune response or direct host/microbiota interaction involving activation of nociceptive neurons by metabolites. Although probiotics are recommended for the treatment of visceral pain, mechanisms involved in bacteria/host interaction are not well understood. Commensal anaerobic bacteria constitute a novel target of investigation in the field of probiotics. Several representants of gut microbiota may have beneficial effects. This is the case of *Faecalibacterium prausnitzii* which exhibits anti-nociceptive properties. The aim of this study was to highlight and characterize potential interactions between other commensal anaerobic bacteria and neuronal cells.

Methods: Several commensal anaerobic bacteria were cultivated before being lysed to separate soluble intracellular bacterial content from insoluble membrane parts. Excitatory or inhibitory potential of those bacterial fractions were evaluated on primary culture of neuronal cells from Dorsal Root Ganglia. Briefly, fraction was applied on mice neuronal cells and neuronal activity was followed by calcium imaging. Then, non-activating-fractions have been tested for their inhibitory potential under algic compound stimulations.

Results: Species-specific and fraction-specific direct interactions between neurons and bacteria have been observed. Moreover, it appears that several bacterial fractions may have an inhibitory potential on neuronal activation. Such effects were observed using intracellular content of *Faecalibacterium prausnitzii* or membrane fraction of *Bifidobacterium adolescentis*.

Conclusion: Bacterial content can modulate the activity of primary culture neurons. Further studies are necessary to characterize these interactions and to evaluate the ability of some bacterial fractions to cross the intestinal barrier and rich peripheral and central neurons.

197 | Post-natal development of extrinsic and intrinsic enteric circuits of the colon and defects in Hirschsprung disease mouse models

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Normal colon function and homeostasis requires extensive coordination among diverse populations of neuronal and non-neuronal cell types. Additionally, extrinsic sensory and autonomic innervation allows for bidirectional communication between the colon and CNS. To interrogate the circuits by which colon homeostasis is maintained, we developed in vivo and ex vivo mouse colon preparations that keep all, or specific, parts of the "Extrinsic-Intrinsic ENS Connectome" intact and have recently described these circuits in healthy, adult mice. Here, we expanded these techniques to (a) characterize the post-natal development of extrinsic-intrinsic ENS circuits, and (b) determine the effects of *Ednrb* mutations, that are associated with distal colon aganglionosis, on the development of functional circuits in ganglionic colon regions.

Mice heterozygous for the *Ednrb* mutation were bred to wild-type or other heterozygous mice to generate $Ednrb^{+/+}$, $Ednrb^{+/-}$, and $Ednrb^{-/-}$ mouse pups. At post-natal day 2 (P2), AAV9-GCaMP6s virus was injected intraperitoneally, and pups were used for GCaMP experiments from P15-P25. Activity in myenteric neurons was recorded during spontaneous contractions and in response to activation of (1)

ascending, (2) descending, (3) parasympathetic, and (4) sympathetic circuits and compared across genotypes.

To date, we have successfully performed GCaMP imaging in colon preparations from 13 mouse pups (n = 7 *Ednrb*$^{+/-}$ and n = 6 wild-type littermates). Preliminary results suggest that adequate expression of GCaMP is achieved within 13 days of injection. In juvenile colon tissue, fewer myenteric neurons responded to oral stimulation compared to anal stimulation (P = 0.03). However, in proximal colon regions of *Ednrb*$^{+/-}$ mice, fewer neurons responded to anal stimulation, but this was not quite significant (P = 0.07). Similar to adult colons, sympathetic nerve stimulation inhibited activity in myenteric neurons in wild-type juvenile colons (P = 0.0092), but not in *Ednrb*$^{+/-}$ colon preparations (P = 0.6509).

Polarized intrinsic enteric circuits and extrinsic sympathetic input to the colon develop by P15 but appear altered in *Ednrb*$^{+/-}$ mice at this time point. Future experiments will include results from ganglionic proximal colon tissue from *Ednrb*$^{-/-}$ mice. With more experiments at various post-natal time points, we will have a complete understanding of the development of extrinsic-intrinsic ENS circuits and the defects caused by *Ednrb* mutations associated with Hirschsprung's Disease.

198 | Effects of fecal supernatant from autism spectrum disorder patients on intestinal permeability and enteric nervous system phenotype

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder in which dysfunctions of the microbiota-gut-brain axis are increasingly recognized. In particular, alterations in gut microbiota or their metabolites have been suggested to contribute to ASD pathology. In order to study how dysbiosis could affect gut functions and enteric nervous system (ENS) homeostasis, we used fecal supernatants (FS) prepared from ASD patients and healthy controls (HC) to study its impact on intestinal permeability and ENS phenotype.

Antibiotic-treated mice received enema of FS from ASD patients or HC. Gut permeability was measured in vivo and ex vivo in proximal colon. Gene and protein expression for glial (S100β, GFAP), neuronal (GAP43, Synapsin1) and inflammatory molecules (IL-1β, TNFα) were analyzed in colon and brain of treated mice. Finally, concentrations of several bacterial metabolites (short chain fatty acids and bile acids) were measured in FS and 16S metasequencing of the human intestinal microbiota was performed for ASD patients and HC.

No change of in vivo permeability was observed in mice treated with FS from ASD patients while ex vivo transcellular and paracellular permeability of proximal colon was decreased as compared to HC. FS from ASD patients induced a decrease of mRNA expression of inflammatory. In addition, the protein expression of Synapsin1 and GFAP were decreased in the colon of mice treated with FS from ASD patients and GFAP expression showed a trend of increase in the cortex. In ASD patient, we observed an increase in the concentration of deoxycholic acid, a bacterial metabolite. Furthermore, in these patients, we observed changes in bacterial proportion with a relative increase of *Bacteroidetes* and decrease of *Firmicutes*.

FS from ASD patients alter intestinal permeability, expression of inflammatory and ENS molecules. However, the factors in FS from ASD patients responsible for these changes remain to be identified.

199 | Maternal exposure to a mixture of prebiotics fosters colitis development in offspring mice

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Background/Objectives: An alteration of the gut microbiota is involved in the pathogenesis of inflammatory bowel diseases. Several probiotic strains and non-digestible food ingredients such as prebiotics are used as dietary supplements to improve intestinal health. One alternative to prevent or reduce colitis development could consist in modulating maternal immunity and microbiota through prebiotics. For this purpose, we studied the effects of prebiotics given to mice during gestation on the development of colitis in their offspring.

Methods: 8-10 weeks old male offsprings from mothers exposed to a prebiotics mixture galacto-oligosaccharides (GOS)/inulin during gestation or fed with a control diet, were submitted or not to 3 cycles of dextran sodium sulfate (DSS). DSS induces colitis and was administered in drinking water. At different days of the protocol, the total transit time, the fecal pellet output, the intestinal permeability and the disease activity index (stool consistency, weight loss, and gross bleeding) were measured for each mouse. At day 18, the offspring mice were sacrificed and further measurements of intestinal segments permeability, tissue remodelling, short-chain fatty acids (SCFAs) concentrations and intestinal inflammation were performed.

Results: Offspring from mothers that received GOS/inulin prebiotics presented no changes in total transit time or feces number, before and after DSS cycles. Nevertheless, when treated with DSS, offspring from mothers that received GOS/inulin prebiotics presented an increased disease activity index and tissue remodelling compared to the DSS group of offspring from mothers with control diet. Changes in butyrate and propionate concentrations and an alteration of the intestinal permeability were also observed in these mice.

Conclusions: The enrichment of pregnant mice with a GOS/inulin mixture promotes a long-term effect in their offspring which is not a protective one, but a sensitization to colitis development. The

underlying mechanisms and a characterization of the gut microbiota are ongoing, but our results are already questioning the health benefits that GOS/inulin should confer.

201 | Interstitial cells of Cajal in the lower esophageal sphincter exhibit dynamic Ca^{2+} signalling influenced by cholinergic and nitrergic innervation

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Background: The lower esophageal sphincter (LES) generates myogenic tone to prevent the reflux of gastric contents into the esophagus. Intramuscular interstitial cells of Cajal (ICC-IM) are electrically coupled to LES smooth muscle cells (SMCs), forming an electrical syncytium. In the gut, ICC-IM rely on the activation of Ca^{2+}-activated-Cl^- channels, encoded by Ano1, to exert their physiological function to modulate SMC excitability. Thus, Ca^{2+} signalling is fundamental to ICC-IM function and SMC contractility, yet nothing is known about Ca^{2+} signalling behaviours in LES ICC-IM.
Methods: We investigated LES ICC-IM Ca^{2+} signalling with *in situ* Ca^{2+} imaging, using mice expressing a genetically encoded Ca^{2+} indicator (GCaMP6f) in ICC-IM.
Results: LES ICC-IM exhibited intracellular Ca^{2+} signaling *in situ*, firing hundreds of Ca^{2+} transients min^{-1} from multiple intracellular sites. ICC-IM showed variability in spatial and temporal kinetics of Ca^{2+} transients and these events were not entrained with adjacent ICC-IM. Ca^{2+} transients were abolished by the SERCA pump inhibitor CPA (10 μmol/L) but were unaffected by the $Cav_{1.2}$ channel inhibitor nicardipine (1 μmol/L). Ca^{2+} influx played a major role in sustaining Ca^{2+} transients as they were reduced after 6 minutes incubation in Ca^{2+} free medium. Ca^{2+} transients did not undergo tonic neural inhibition as they were unaffected by tetrodotoxin (1 μmol/L) and the NO synthase inhibitor L-NNA (100 μmol/L). Electrical field stimulation (EFS) of inhibitory nerves (in the presence of 1 μmol/L Atropine; 10 Hz, 10 seconds) inhibited Ca^{2+} transients and this was blocked by L-NNA. Conversely, the muscarinic receptor agonist carbachol (10 μmol/L) or EFS (10 Hz, 10 seconds) in the presence of L-NNA and MRS 2500 (1 μmol/L) increased the firing of ICC-IM Ca^{2+} transients.
Conclusions: LES ICC-IM are active *in situ*, exhibiting spontaneous Ca^{2+} transients mediated by Ca^{2+} release from internal stores that are sustained by Ca^{2+} influx. Ca^{2+} activity of LES ICC-IM is modulated by enteric inputs.

202 | Activation of Ano1 channels in interstitial cells of Cajal contributes to tone and neural responses of mouse and monkey lower esophageal sphincter

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Background: Tone in the lower esophageal sphincter (LES) has been proposed to be due to activation of Ca^{2+}-activated-Cl^- channels (CaCC) opening $Cav_{1.2}$ channels, causing contraction of smooth muscle cells (SMC). In other regions of the gut, interstitial cells of Cajal (ICC) influence SMC by activation of CaCC encoded by Ano1, however, it is unknown if this occurs in the LES. We hypothesize that Ano1 channels in ICC may contribute to tone and neural responses in the LES.
Methods: We performed immunohistochemistry and tension recordings on LES of mouse and Cynomolgus monkey. Gene expression on populations of LES ICC and SMC, sorted by fluorescence activated cell sorting (FACS) was also assessed.
Results: Ano1 channels are highly expressed in Kit^+ (tyrosine kinase receptor, ICC marker) cells in mouse and monkey LES, as shown by immunohistochemistry. Transcripts of *Ano1*, *Kit* and *Gja1* (connexin 43) were enriched in ICC of the LES, while minimal expressions of *Myh11* (myosin heavy chain) or *Cacna1c* ($Cav_{1.2}$ channels) were detected. Conversely, SMC of the LES showed minimal expression of *Kit*, *Ano1* or *Gja1* but were highly enriched in *Myh11* and *Cacna1c*. Mouse and monkey LES strips developed spontaneous tone that was reduced by ~70% by the Ano1 channel antagonist Ani9 (1 μmol/L). The $Cav_{1.2}$ channel antagonist nifedipine (1 μmol/L) reduced mouse and monkey LES tone to a similar degree (~75%). In the presence of the muscarinic receptor antagonist atropine (1 μmol/L), electrical field stimulation (1-20 Hz) induced relaxations of mouse and monkey LES that were significantly reduced by Ani9. Excitatory contractile responses of mouse and monkey LES, induced in the presence of the nNOS synthase inhibitor L-NNA (100 μmol/L) were significantly reduced by Ani9.
Conclusions: Ano1 channels, expressed in ICC, contribute to spontaneous tone and both inhibitory / excitatory neural responses in the mouse and monkey LES.

203 | Functional lumen imaging probe in the management of pediatric esophageal motility disorders

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Functional lumen imaging probe (FLIP) has been used to evaluate pediatric eosinophilic esophagitis but its use in assessing pediatric

esophageal motility disorders has been limited. We demonstrate FLIP utility to guide management in different pediatric esophageal motility disorders.

Case 1. A 16 year-old with presumed achalasia on esophagram presented with recurrent dysphagia after two pneumatic balloon dilations. High resolution esophageal manometry (HREM) was inconsistent with achalasia, and endoscopy revealed candida esophagitis with FLIP demonstrating repetitive antegrade contractions (RACs), normal esophagogastric junction distensibility index (EGJ-DI) and diameter. She was diagnosed with ineffective esophageal motility, and pneumatic balloon dilation was not repeated. She improved with antifungals and behavioral modifications.

Case 2. A 15 year-old with chronic progressive dysphagia was diagnosed with achalasia type 1 on HREM. Although FLIP demonstrated normal values for EGJ-DI and diameter, pneumatic balloon dilation was performed with subsequent EGJ-DI and diameter increase and improvement in clinical symptoms. While it is challenging to assess normative FLIP values for pediatric EGJ-DI and diameter, post-dilation EGJ measurements can still be helpful in predicting clinical improvement in pediatric achalasia.

Case 3. A 3 year-old with dysphagia and feeding difficulties was diagnosed with cricopharyngeal achalasia on HREM and underwent cricopharyngeal myotomy. Her feeding and ability to manage salivary secretions worsened with swallow study showing aspiration from residual bolus with palatal dysfunction. Repeated HREM showed low pharyngeal and upper esophageal sphincter (UES) pressure, low LES/IRP, and poor augmentation on repeated multiple swallows. Despite low baseline UES pressure on HREM, FLIP showed low UES-DI. UES balloon dilation was performed nonetheless and FLIP showed persistently low UES-DI and unchanged diameter post dilation, suggesting development of fibrosis at myotomy site with swallowing dysfunction that probably would not respond to future UES dilations. As predicted, post dilation she continued to be symptomatic.

Case 4. A 13 year-old with chronic dysphagia and recurrent food impactions presented with a long segment stricture from mid to distal esophagus on UGI. Endoscopy with FLIP showed active eosinophilic esophagitis (EoE; 49 eos/HPF) with low esophageal body DI and diameter across the stricture. Dilation of the stricture was performed without improvement in DI but with an increase in diameter. He was started on swallowed Flovent, and esophageal dilation with FLIP was repeated. Although low DI persisted, diameter increased by 4 mm and peak eosinophil count decreased (9 eos/hpf) with improvement in clinical symptoms. Although stricture dilation in fibrostenotic EoE does not improve the DI, given the degree of fibrosis, symptoms can still improve by enlarging the stricture diameter and decreasing EoE disease activity.

204 | Enteric neural stem cell integration in ex vivo organotypic colon cultures

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Background/Objectives: Enteric neural stem cells (ENSC) have been identified as a possible treatment for enteric neuropathies. After in vivo transplantation, ENSC and their derivatives have been shown to engraft within colonic tissue, migrate and populate endogenous ganglia, and functionally integrate with the enteric nervous system (ENS). However, the mechanisms underlying the integration of transplanted ENSC in recipient tissues remain unclear. Therefore, we aimed to examine ENSC integration using an ex vivo organotypic culture system.

Methods: Donor ENSC were obtained from *Wnt1*$^{cre/+}$;*R26R*$^{YFP/YFP}$ mice allowing specific labelling, selection and fate-mapping of cells. YFP^{+} neurospheres were transplanted to C57BL6/J colonic tissue, and maintained in organotypic culture for up to 21 days. Subsequently, donor cell integration was assessed using immunolabelling and qPCR.

Results: Control (non-transplanted) colonic tissue displayed similar TuJ1 expression at day 7 (D7), D14 and D21 in organotypic culture. The density of TuJ1^{+} networks between D7 (52.5 ± 2.7 optical density units (ODU); n = 3), D14 (52.1 ± 18.2 ODU; n = 3;) and D21 (66.9 ± 11.0 ODU; n = 3;) cultures were comparable (P = 0.6286) suggesting preservation of neuronal networks within ex vivo cultured tissues. Seven days after transplantation, YFP^{+} cells were observed on the serosal aspect of transplanted colonic segments, with no evidence of tissue penetration. At D14, the majority of YFP^{+} cells were observed at the serosal aspect, with a limited degree of tissue penetration towards the endogenous ENS, at the level of the myenteric plexus. Importantly, at D21 transplanted YFP^{+} cells were observed fully integrated within the endogenous myenteric ganglia, with YFP^{+} fibres appearing to trace the endogenous ENS. Interestingly, active remodelling of the extracellular matrix (ECM) appears to occur in response to ENSC transplantation, with increased expression levels observed for *Col1a* (5.8 ± 2.4-fold increase), *Col4a* (4.0 ± 1.1-fold increase) *and MMP13* (5.5 ± 1.9-fold increase) at D21, compared to non-transplanted tissues.

Conclusions: The experiments described provide key evidence of how transplanted ENSC integrate within recipient gut, as ENSC-derived cells display dynamic integration over a 3-week period, along with significant modulation of the ECM.

205 | The biomechanics of colorectal submucosa and its relevance to visceral nociception

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Visceral pain arising from the internal organs has unique clinical, psychophysical manifestations associated with organ biomechanics; it is mechanical distention/stretch of hollow visceral organs that reliably evokes the perception of pain from the viscera. In turn, mechanotransduction of distension/stretch in distal colon and rectum (colorectum) appears to play a critical role in visceral nociception in patients with irritable bowel syndrome. We hypothesize that nociceptive afferent endings that detect tissue-injurious colorectal distension locate strategically at concentrations of mechanical stress. Here, we harvested the distal 30 mm of the colorectum from mice and separated into inner and outer composite layers from the interstitial space under the submucosa. The inner composite consists of the mucosa and submucosa while the outer composite includes the muscular layers and serosa. We then divided each composite longitudinally into three 10-mm-long segments (colonic, intermediate, and rectal) and conducted biaxial mechanical stretch tests. In addition, we quantified the principal orientation and morphology of the collagen fibers in colorectal layers by nonlinear imaging via second harmonic generation (SHG). Our biaxial stretch data reveal comparable stiffness of the inner (including the submucosa) and outer composite layers (including the serosa); inner composite is slightly stiffer in axial direction and out composite slightly stiffer in circumferential direction. In support, the SHG images reveal concentrated collagen fibers in the submucosal and serosal layers. The stiffness of the inner composite in axial direction is about twice that in circumferential direction, consistent with the orientations of collagen fibers in the submucosa approximately ±30 degrees to axial direction. Strikingly, the axial tension-stretch relations are comparable across different longitudinal regions of the colorectum that have varying wall thickness, which is likely caused by comparable submucosal thickness and similar collagen fiber morphology within the submucosa throughout the colorectum. These results collectively indicate that submucosa is the passive load-bearing structure of the colorectum. Accordingly, afferent endings in those collagen-rich regions are likely candidates of colorectal nociceptors to encode noxious distension/stretch. Outcomes of this study will guide further neurophysiological studies on sensory afferent endings in the submucosa, which likely play critical roles in visceral nociception and pain.

206 | Anti-inflammatory effect of rebamipide in combination with esomeprazole for resolution of disease symptoms in proton pump inhibitor-resistant non-erosive reflux disease patients – A pilot study

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Background/Aim: Previous studies have shown that non-erosive reflux disease (NERD) patients are less sensitive to proton pump inhibitor (PPI) treatment than patients with erosive reflux disease. This is majorly due to inflammation of the dilated intercellular space in the distal esophagus. Rebamipide plays a distinctive role in the amelioration of this inflammation and protects the mucosal barrier of the distal esophagus. The aim of the present study was to investigate if rebamipide treatment, in addition to esomeprazole therapy, improves clinical symptoms in PPI-resistant NERD patients.
Methods: Thirty-nine consecutive patients presenting with typical symptoms of PPI-resistant NERD were included in the study. They were randomly divided into two groups: group A (esomeprazole 20 mg/d along with rebamipide 300 mg/d for 4 weeks) and group B (esomeprazole 40 mg/d for 4 weeks). Symptoms were evaluated using a standardized questionnaire before and after treatment. Good response was defined as at least 50% symptom improvement.
Results: Nineteen patients were included in group A and 20 patients were included in group B. The mean ages were 42.6 ± 14.6 years in group A and 48.1 ± 14.0 years in group B (P = 0.23). There was no difference in the sex ratio between group A and B (0.58 vs. 0.55, P = 0.86). A decent clinical response was achieved in seven patients from group A, whereas nine patients from group B displayed improvement in disease symptoms (37% vs. 45%, P = 0.61).
Conclusion: Administration of rebamipide in conjunction with 20 mg esomeprazole could be an alternative to esomeprazole (40 mg) treatment alone in PPI-resistant NERD patients. The anti-inflammatory effect of rebamipide might be effective in the treatment of PPI-resistant NERD.

207 | Mosapride citrate in combination with esomeprazole is effective against gastroesophageal reflux disease by improving delayed gastric emptying – A pilot study

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Background/Aim: Previous studies have shown that patients with gastroesophageal reflux disease (GERD) have slower rates of gastric emptying than controls. However, the involvement of delayed gastric emptying (DGE) in the pathophysiology of gastro-esophageal reflux disease (GERD) remains debatable. The aim of this study was

to investigate if treatment with prokinetics in addition to proton pump inhibitor (PPI) therapy improves clinical symptoms in dyspeptic GERD patients with or without DGE.

Methods: Thirty consecutive patients refractory to PPI over a six-month period were included in the study. Gastric emptying scintigraphy was employed to determine DGE, which was designated when gastric emptying time (T1/2) was >70 minutes. Patients were divided according to the presence of dyspepsia into the dyspeptic group (n = 12) and non-dyspeptic group (n = 18). In addition to esomeprazole (40 mg), mosapride citrate was administered to patients for 4 weeks. Symptoms were evaluated using a standardized questionnaire before and after treatment.

Results: There was no statistical difference in age and sex between the dyspeptic group and the non-dyspeptic group (P = 0.92 and P = 0.23, respectively). Prevalence of esophagitis was similar between the dyspeptic and non-dyspeptic groups (58% vs. 39%, P = 0.31). DEG was detected in 75% of subjects in the dyspeptic group; while 28% subjects in the non-dyspeptic group (P = 0.01) displayed DEG. Improvement in symptoms was greater in the dyspeptic group than that in the non-dyspeptic group (75% vs. 33%, P = 0.02).

Conclusion: Gastric emptying in dyspeptic patients with GERD is significantly slower than in non-dyspeptic patients regardless of esophagitis. Combination therapy with prokinetics in addition to PPI improves typical GERD symptoms in dyspeptic patients with GERD.

208 | Effects of body position during high-resolution esophageal manometry: Systematic review of the literature

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Background/Objectives: High-resolution manometry (HRM) is considered the test of choice to evaluate esophageal motility disorders. According to the Chicago Classification 3.0, the test should be performed in supine position, but it is uncomfortable for the patient. The upright position, although more physiological and better tolerated by patients, may change the final diagnosis. We performed a systematic literature review to search whether HRM variables are influenced by body position.

Methods: A literature search was conducted according to the Preferred Reporting Items For Systematic Reviews and Meta-analyses (PRISMA) statement. Studies published in English from 2007 to 2019 that compared HRM results in different body positions were included.

Results: Sixteen studies including 1254 patients met the inclusion criteria. Five studies showed a significant increase in lower esophageal sphincter (LES) basal pressure in the supine position, while four studies did not find differences. Integrated Relaxation Pressure (IRP) was significantly higher in the supine position in 8/12 studies, and in two studies was significantly increased in the upright position. Distal Contractile Index (DCI) was higher in the supine position in eight studies. No studies demonstrated an increased contractility in the upright position, while in one study the two positions were comparable. Only six studies for a total of 574 patients reported the difference in terms of final diagnosis in the two positions. Ninety-three patients (16.2%) with normal HRM in the supine position had an Ineffective Esophageal Motility (IEM) when the test was performed in the upright position (P < 0.001).

Conclusions: Performing HRM in the upright position may be more comfortable for the patient but it may change the final diagnosis. Further studies to determine the normal manometric values in the upright position are needed. It may also be interesting to investigate the physiological effects of the semi-recumbent position and to evaluate patient reported outcomes and compliance.

209 | Abnormal reflux is uncommon in patients presenting with isolated oropharyngeal symptoms

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Introduction: Gastroesophageal reflux disease (GERD) is often implicated as a potential etiology for various oropharyngeal (OP) symptoms. Although ambulatory reflux monitoring is recommended by professional societies for the assessment of OP symptoms, it is unclear if objective measures of acid exposure in the esophagus correlate with these symptoms. The aim of this study is to determine the prevalence of abnormal 24-hour pH monitoring in patients with OP symptoms in our motility unit.

Methods: A retrospective chart review was performed on all patients referred for 24-hour pH monitoring for the evaluation of OP symptoms at the University Health Network between January 1, 2008 and June 1, 2019. Seven symptoms were examined: cough, globus, throat discomfort, voice change, dental erosion, altered taste, and sensation of phlegm in the throat. The 24-hour pH monitoring results were collected. A test was considered abnormal if off anti-secretory therapy overall acid exposure (pH < 4) in the distal esophagus was greater than 4.2% or if on anti-secretory therapy the overall acid exposure was greater than 1.2% of the total time. Descriptive statistics were performed.

Results: 384 patients were included. 167 patients (43.5%) with cough, 63 (16.4%) with globus, 86 (22.4%) with throat discomfort, 19 (5.9%) with voice changes, 13 (3.4%) with dental erosion, 17 (4.4%) with altered taste and 19 (4.9%) with sensation of phlegm in the throat. Overall, 19.5% of patients with OP symptoms had abnormal 24-hour pH monitoring. Abnormal 24-hour pH monitoring was present in 24.6% of those with cough, 15.9% with globus, 16.3% with throat discomfort, 21.1% with voice changes, 23.1% with dental

erosion, 5.9% with altered taste and 10.5% with sensation of phlegm in the throat.

Conclusion: A small proportion of patients with OP symptoms have abnormal gastroesophageal acid reflux based on objective 24-hour pH monitoring. Future studies are needed to determine factors that predict abnormal 24-hour pH monitoring in patients with OP symptoms to guide who benefits most from this testing.

210 | Longitudinal multi-omics reveals subset-specific mechanisms underlying irritable bowel syndrome

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Gut microbiome has been implicated in multiple chronic gastrointestinal (GI) disorders but it has been difficult to determine its mechanistic role in disease pathogenesis due to the apparent disconnect between animal and human studies and a lack of integrated host- microbial approach in relation to disease specific physiologic changes. To uncover such mechanisms, we integrated longitudinal multi-omics data (metagenomics, metabolomics, host transcriptome and methylome) coupling gut microbiome changes with host function in patients with irritable bowel syndrome (IBS) and healthy controls (n = 75). We found that longitudinal sampling helps overcome heterogeneity seen with cross-sectional microbiome studies. We identified IBS subtype specific and symptom related changes in gut microbiome using metagenomics and metabolomics which concurred with subtype specific changes in host physiology. These include a decrease in short chain fatty acids in constipation predominant IBS, which was independent of fiber intake and an associated decrease in secretory response of colonic biopsies to exogenous serotonin in Ussing chamber experiments. In diarrhea predominant IBS there was an increase in secretagogues such as the primary bile acid chenodeoxycholic acid and bacterial metabolite tryptamine associated with a higher baseline anionic secretion in colonic biopsies in Ussing chamber experiments. In addition using integration of multiple data layers, we identified purine metabolism as a novel host-microbial metabolic pathway which may play a role in the pathophysiology of IBS. The study shows the benefit of integrating longitudinal complementary multi-omics data to identify functional mechanisms that can serve as therapeutic targets in a comprehensive treatment strategy for chronic GI diseases.

211 | Low-FODMAP diet versus traditional dietary advice for diarrhea-predominant irritable bowel syndrome: A randomized, parallel-controlled trial with analysis of clinical and microbiological factors associated with patient outcome

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Background: To compare the efficacy of low-FODMAP diet (LFD) and traditional dietary advice (TDA) for treatment of irritable bowel syndrome with diarrhea (IBS-D) in a randomized controlled trial. A planned analysis was performed to identify clinical, metabolic, and microbial predictors of outcomes.

Methods: One hundred and eight Chinese IBS-D patients were randomized to LFD or TDA. Primary endpoint was a ≥50-point reduction in IBS Severity Scoring System at three weeks. Fecal samples before and after the intervention were assessed for changes in microbiota profiles by 16S rRNA sequencing and metabolomic profiles including short- chain fatty acids and fermentation index described by Valeur and colleagues. A logistic regression model was built to assess potential factors associated with response to diets.

Results: One-hundred patients (47 women; mean age 44 ± 13 years) completed the study, and the primary endpoint was met in 30/51 (59%) patients with LFD and 26/49 (53%) with TDA (Δ6%, 95% CI: −13% to 24%). LFD induced changes in fecal microbial composition (reduced *Bifidobacterium* (P = 0.03), increased *Bilophila* (P = 0.03)) and short chain fatty acid levels (reduced butyric (P = 0.045), increased isobutyric (P = 0.01) and isovaleric (P = 0.009) acids). Similar effects were not observed with TDA. Saccharolytic fermentation activity was associated with baseline symptom burden (P = 0.01). Higher saccharolytic fermentation activity was associated with a therapeutic response to LFD (log odds-ratio = 4.9, 95%-CI: −0.1 to 9.9), while lower activity was associated with a response to TDA (log odds-ratio = −6.2, 95% CI: −11.9 to −0.4).

Conclusions: Low FODMAP diet and traditional dietary advice both reduced symptoms in Chinese IBS-D patients; however, only LFD had a measurable impact on microbiota. At baseline, the presence of severe symptoms and microbial metabolic dysbiosis characterized by high saccharolytic capability predicted the outcomes of dietary interventions.

214 | Effects of provocative testing on phase III migrating motor complex in children with normal antroduodenal manometry

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During antroduodenal manometry (ADM), provocative testing with erythromycin/azithromycin and/or octreotide is performed to assess antral and small intestinal motility. There is a lack of data establishing normal phase III MMC patterns in fasting state and after provocative testing in children which marks a major limitation in the interpretation of ADM. The study objective was to determine phase III MMC characteristics in children with normal ADM. This retrospective study reviewed normal ADM (performed following published guidelines) of 48 children aged ≤21 years. All 48 ADM were recorded in fasting phase, 28 patients received azithromycin and octreotide while 15 patients received azithromycin only and 5 received octreotide only. The characteristics of antral contractions and phase III MMC are shown in Table 1. During fasting, there were 18 patients (37.5%) with phase III MMC originating from the antrum. Post-azithromycin, phase III MMC was observed in 12 patients (27.9%), with 66.7% originating from the antrum. Antral contraction frequency, mean and maximum amplitude, increased significantly compared to fasting ($P < 0.0001$). Post-octreotide, all patients had phase III MMC with 75.8% originating from the antrum. Phase III MMC frequency, maximum amplitude, and duration increased significantly compared to fasting ($P = 0.005$, 0.02, and <0.0001, respectively). Phase III MMC characteristics during fasting and post-octreotide did not differ between those that originated from the antrum or small intestine. However, mean and maximum amplitude of phase III MMCs that originated from the antrum were higher post-azithromycin ($P = 0.022$ and 0.059, respectively). We did not detect any changes in phase III MMC characteristics with the sequence of provocative agents and no symptom correlation (vomiting or abdominal pain) with phase III MMC was noted. This is the first study that provides normative measurements that may guide the interpretation of pediatric ADM. It also showed that azithromycin stimulated antral contractions by increasing frequency, mean and maximum amplitude of contractions compared to fasting and induced phase III MMC in a third of patients; of these, 2/3 originated from the antrum. Also, octreotide increased phase III MMC frequency, maximum amplitude, and duration compared to fasting, with the majority originating from the antrum.

215 | Gastrointestinal symptoms and irritable bowel syndrome is common in patients with Ehlers-Danlos Syndrome in a tertiary referral center

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Background: Ehlers-Danlos Syndrome (EDS) is a group of rare connective tissue disorders. Gastrointestinal (GI) symptoms are common in these patients. Specifically, disorders of gut-brain interaction appear to be more common in this patient population. The University Health Network (UHN) has the first and only multi-disciplinary clinic, including specialized gastroenterology services, for the management of EDS in Canada. The aim of this study is to describe the GI symptoms that are present in our cohort of EDS patients.
Methods: A retrospective chart review of all patients seen in the gastroenterology clinic of the GoodHope EDS clinic at UHN from November 1, 2017 to September 26, 2019 was performed. Demographic information including age, sex and EDS subtype were

Table 1. Characteristics of antral contraction and phase III MMC in fasting, post-azithromycin, and post-octreotide phase

	Antral contractions		Antral phase III MMC			Intestinal phase III MMC		
Characteristics [mean (SD)]	Fasting (N = 48)	Post-AZI (N = 43)	Fasting (N = 18)	Post-AZI (N = 8)	Post-OCT (N = 25)	Fasting (N = 48)	Post-AZI (N = 12)	Post-OCT (N = 33)
Frequency (cpm)	1.2 (0.41)	2.8 (0.64)	3.4 (0.92)	3.9 (0.38)	3.5 (0.87)	10.7 (0.41)	10.6 (0.39)	10.9 (0.43)
Mean amplitude (mm Hg)	38.4 (16.59)	79.1 (19.52)	60.6 (18.21)	87.0 (28.02)	56.8 (14.89)	25.6 (4.47)	27.1 (3.90)	26.3 (4.50)
Maximum amplitude (mm Hg)	139.0 (47.21)	213.0 (52.25)	144.0 (35.16)	188.4 (40.06)	151.1 (52.07)	48.1 (10.92)	55.6 (17.96)	53.6 (12.72)
Duration (seconds)	–	–	190.2 (77.86)	305.5 (77.68)	211.3 (119.10)	228.1 (69.70)	261.5 (66.84)	336.5 (115.97)

Abbreviation: MMC, migrating motor complex; cpm, cycles per minute; SD, standard deviation, AZI, azithromycin; OCT, octreotide.

collected. GI symptoms collected include constipation, diarrhea, fecal incontinence, nausea, vomiting, bloating, abdominal pain, early satiety, heartburn, dysphagia and regurgitation. A physician made diagnosis of irritable bowel syndrome (IBS) was recorded. Descriptive statistics were performed.

Results: 75 patients were included. 93.3% were female with mean age of 36.4 ± 12.5 years. 43 (57.3%) had EDS-hypermobile subtype, 13 (17.3%) EDS-classic, 3 (4%) EDS-vascular, 3 (4%) unknown EDS subtype and 13 (17.3%) had a hypermobile spectrum disorder. The most common GI symptoms observed were abdominal pain (85.5%), bloating (64.4%), heartburn (61.8%), constipation (57.9%), nausea (50%), diarrhea (43.4%), dysphagia (43.4%), regurgitation (34.2%), early satiety (32.9%), vomiting (30.2%), and fecal incontinence (13.1%). 50.7% (n = 38) had a physician made diagnosis of IBS. 16 (42.1%) had IBS-constipation, 12 (31.5%) had IBS-mixed, and 10 (26.3%) had IBS-diarrhea.

Conclusion: Our study demonstrates that GI symptoms are common in EDS patients. In our cohort, abdominal pain is the most common GI symptom, though other GI symptoms are also reported in high numbers. In this cohort, IBS appears more common than what is reported in the general population. Further studies are needed to better understand the pathophysiology and impact of these GI symptoms in patients with EDS.

216 | Do foods and fruits eaten during Korean big holiday seasons affect bowel preparation?

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Background/Objectives: Chuseok and Folk Day are the biggest holidays in Korea. It is thought that it will affect the bowel preparation because it eats a lot of food including fruits during the holiday. We aimed to determine the bowel preparation status of people who received colonoscopy between the day after the holiday season and another day.

Methods: Medical records of colonoscopy examinees who visited our hospital between 2012 and October 2016 were retrospectively reviewed.

Results: A total of 145 patients (age 58 ± 12, M = 76, F = 69) underwent scheduled colonoscopy immediately after the holiday, and 151 (age 57 ± 12, M = 89, F = 63) for about 2 weeks after the holiday seasons. There were 11 cases of poor bowel preparation (7.6%) immediately after the holiday and 10 cases (6.6%) after 2 weeks after the holidays, with no statistical significance (P = 0.748). Two (after holidays) and one (on another days) cases of endoscopy were difficult due to fruit seeds.

Conclusions: Although the result is obtained with a small number of samples, but the food eaten during the holiday season does not seem to have much to do with overall cleanliness. However, proper dietary education before endoscopy is necessary for accurate and safe examination.

217 | Low complication rate in the outpatient intrajejunal levodopa/carbidopa intestinal gel clinical program

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Background: Levodopa/carbidopa intestinal gel (LCIG) is delivered continuously through a percutaneous endoscopy gastro-jejunal tube (PEG-J) for the treatment of patients with advanced Parkinson's disease (PD). LCIG significantly reduces periods of increased motor symptoms without troublesome dyskinesia. Adverse events (AEs) have been attributed to PEG-J insertion and the device used for LCIG delivery, rather than to LCIG itself. Currently, data evaluating efficacy and safety of PEG-J insertion for LCIG administration in the outpatient setting are limited. The aim of this study is to describe short and long-term AEs associated with outpatient PEG-J tube insertion for LCIG administration.

Methods: A retrospective chart review was performed of all PD patients who underwent PEG-J insertion for LCIG therapy at UHN from March 2011 to October 2019. All AEs associated with PEG-J insertion were collected. Descriptive statistics were performed.

Results: A total of 58 patients were included. 37 (64%) were male, with mean age of 74 ± 6.17 years. The mean duration of PD prior to PEG-J insertion was 16.5 ± 2.0 years. 30 (51%) patients had post-procedural abdominal or site pain that improved with over the counter analgesics. 9 (16%) had possible site infection; 6 received oral antibiotics and 3 had the PEG-J replaced. 19 (33%) developed granulation tissue, with 2 requiring PEG-J exchange. 32 (55%) had their PEG-J removed or exchanged secondary to tube malfunction. No ER visits related to the PEG-J were recorded. 12 (21%) patients died for reasons unrelated to PEG-J insertion. There were no serious AEs, including perforation, bleeding, fistula formation, intra-abdominal collection formation or buried bumper syndrome.

Conclusion: This study demonstrates the absence of serious AEs associated with outpatient PEG-J insertion for LCIG administration in patients with advanced PD. This study supports that outpatient PEG-J insertion for the administration of LCIG is safe in patients with advanced PD.

218 | Bacterial-derived Curli fibrils trigger a transcriptional immune response in primary myenteric networks

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Background/Objectives: With phenocopies of pathological hallmarks in the enteric nervous system (ENS), and with the microbiome acting as a major source of amyloid-like proteins, the gut may represent an entry route to amyloid-driven neurodegeneration. However, the underlying mechanisms are poorly characterized. To better understand the cellular response to amyloid in the ENS, we performed a balanced RNAseq experiment, and specifically zoomed in on immune response pathways.

Methods: Myenteric neuronal networks, isolated from colon of WT Black6 mice, were incubated for 24 hours with bacterial amyloid aggregates (Curli), human amyloid oligomers ($A\beta_{1\text{-}42}$), or peptides with a scrambled sequence ($A\beta_{scr}$). After Illumina NextSeq 500 mRNA sequencing, differentially expressed genes and dysregulated pathways were explored using standard bioinformatic pipelines. Validation of top hits and associated genes was done via qPCR.

Results: Exposure to Curli, but not to $A\beta_{1\text{-}42}$, induced a signature of genes involved in innate immune response pathways. Among those, five genes (*Tirap*, *Ptges*, *Sod2*, *Cxcl2* and *Cxcl5*) were associated with TLR signaling. Since Curli fibrils are known to activate TLR1/2 signaling in cultured macrophages [1], we set out to explore whether such activation would also take place in healthy colonic tissue. We found that all known TLRs except for *Tlr8*, *Tlr11* and *Tlr12* were expressed and that active inflammation (induced by DSS or TNBS) significantly enhanced or induced their expression. To determine whether, next to immune cells, these TLR pathways could be traced back to neurons and/or enteroglia, we reverted to primary myenteric cultures. Administration of a pro-inflammatory cytokine cocktail (TNFα, IL1β and IFNγ) indeed induced upregulation of *Tlr2*, *Tlr3* and *Tlr7* in these cultures and also resulted in upregulation of the *Nlrp3* gene, a critical factor in inflammasome formation.

Conclusions: We have shown that exposure of myenteric networks to Curli induces a TLR-mediated immune response. Currently, we are investigating which TLR pathways are involved in this immune response and what the downstream cellular and systemic effects are.

[1] Tükel et al. (2010) Cell. Microbiol. 12(10): 1495-505.

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219 | Integrated pressure-impedance measures can confirm esophago-gastric outflow resistance as a mechanism of dysphagia symptoms

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Background: Esophagogastric junction outflow obstruction (EGJOO) is defined as elevated integrated relaxation pressure (IRP) and intact distal esophageal contractility. IRP may be prone to erroneous measurement, potentially leading to unnecessary treatment(s). Adjunct measures are needed in EGJOO. This study assesses the potential diagnostic relevance of measures of the intrabolus pressure domain in EGJOO.

Methods: Retrospective data on 24 patients (20 F; 64 ± 14 years) diagnosed with EGJOO on high-resolution impedance manometry (MMS Laborie; solid-state 36P/16Z, 3.2 mm Unisensor catheter), were compared to 10 asymptomatic age-matched controls (8 F; 66 ± 12 years). History of dysphagia and dysphagia during manometry (10 cm visual analogue swallowing score >5 symptomatic) were recorded. Ten right lateral 5 mL liquid, multiple rapid swallow (5 × 2 mL), 5 upright 5 mL standardised viscous bolus (IDDSI level 4; SBMkit, Trisco Foods Australia) and solid 2 × 2 cm boluses (IDDSI level 7) were administered. Data exported to www.swallowgateway.com for analysis. Two measures of the intrabolus pressure domain indicative of esophageal outflow resistance were evaluated (i) mid-domain intrabolus distension pressure (DP) and (ii) intrabolus ramp pressure (RP), quantifying the rate of pressure change in distal esophagus as the bolus is compressed between the peristaltic wave front and EGJ. Bolus emptying was quantified using bolus flow time (BFT). Comparisons by T-test, $P < 0.05$ significant.

Results: Patients who reported dysphagia were characterised by increased distal esophageal RP during viscous swallowing. Viscous RP was 27 ± 6 vs. 8 ± 4 mmHg/s ($P < 0.05$) in patients with and without a history of dysphagia. Viscous RP was 37 ± 10 vs. 14 ± 5 mmHg ($P < 0.05$) in patients with and without dysphagia symptoms during assessment. DP was increased in all EGJOO; but unrelated to dysphagia. Symptomatic patients demonstrated reduced peristaltic reserve function and descending inhibition during MRS ($P < 0.05$) and reduced EGJ bolus transit BFT compared to controls (2.0 ± 0.3 vs. 3.2 ± 0.4s; $P < 0.05$).

Conclusions: Integrated pressure-impedance measures that quantify efficacy of esophageal emptying mechanisms may help differentiate clinically relevant EGJOO.

220 | Synergistic interactions of cannabinoids and opioids potentiate analgesia in mouse colonic nociception

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Background/Objectives: Use of opioids for abdominal pain is associated with severe side effects, thus many are using cannabis as an alternative or an adjunct. However, little is known about potential benefits of cannabinoids for treating visceral pain including whether the combination of cannabinoids and opioids could enable a reduction in the amount of opioid required for analgesia. Thus, we aimed to investigate the effects of cannabinoid receptor type 1 (CB1) and type 2 (CB2) agonists alone or in combination with μ-opioid receptor agonist on colonic nociception.

Methods: Perforated patch clamp recordings were performed on dorsal root ganglion (DRG, L1-L3) neurons dissociated from male C57BL/6 mice. Neuronal excitability was examined by measuring rheobase following 30-minutes incubation of HU-210 (CB1 agonist), HU-308 (CB2 agonist), DAMGO (μ-opioid receptor agonist), or a combination. Extracellular afferent recordings on flat-sheet preparations of distal colons were used to evaluate afferent responses to probing (1 g von Frey hair) before and after superfusion of different agonists. Data were analyzed using one-way ANOVA with Bonferroni test.

Results: In patch clamp recordings, HU-210 (1 and 10 μM) decreased DRG excitability (i.e. increased rheobase, 75.5 ± 7.2 vs. 94.4 ± 9.4 and 105.5 ± 8.1 pA, $P < 0.05$ and $P < 0.001$, $n \geq 9$). When applied alone, HU-210 (100 nM) and DAMGO (1 nM) did not alter rheobase, whereas their combination decreased excitability (62.7 ± 6.4 vs. 123.0 ± 13.4 pA, $P < 0.001$, $n \geq 10$). HU-308 (100 nM and 1 μM) had no effect on DRG excitability, whereas 10 μM surprisingly increased excitability (76.4 ± 9.1 vs. 26.4 ± 6.9 pA, $P < 0.001$, $n \geq 10$). In colonic afferent recordings, HU-210 (1 μM) attenuated afferent response to probing (13.7 ± 2.5 vs. 6.2 ± 1.1 Hz, $P < 0.01$, $n = 10$). A lower dose of HU-210 (100 nM) or DAMGO (1 nM) alone had no effect on mechanosensitivity, but their combination significantly reduced mechanosensitivity (14.8 ± 2.3 vs. 8.1 ± 1.7 Hz, $P < 0.01$, $n = 10$). Conversely, HU-308 (1 and 10 μM) did not alter mechanosensitivity.

Conclusions: Activation of CB1 but not CB2 receptors inhibits colonic nociceptive signaling. Combination of CB1 and μ-opioid receptor agonist at sub-threshold concentrations inhibited colonic nociceptive signaling, suggesting that cannabinoids could enable opioid dose reduction.

221 | Cumulative effects of psychological alterations on gastrointestinal symptoms in irritable bowel syndrome

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Background/Objectives: Psychological factors are considered to be of importance for symptom generation in IBS. However, the cumulative effect of having several different psychological alterations on gastrointestinal (GI) symptom severity in IBS is unknown.

Methods: IBS patients (Rome IV) completed validated questionnaires assessing GI symptoms (GSRS-IBS; pain, bloating, constipation and diarrhoea) and psychological alterations, covering neuroticism (Big Five Neuroticism), anxiety (GAD-7), fatigue (MFI), pain catastrophizing (PCS), depression (PHQ-9), somatization (PHQ-12), stress (PSS), and GI-specific anxiety (VSI). A psychological alteration was defined by validated thresholds, or scoring in the uppermost tertile of the study cohort. Out of the 16 possible factors from the psychological questionnaires, the ten factors with significant associations with GI symptom severity were kept for further analysis on the association between the number of psychological alterations and GI symptom severity through one-way between-groups ANOVA with linear contrast analysis.

Results: In total, 186 patients with IBS were included (70% females, median age 32 [26-45] years, IBS-SSS 295 [217-370]). The number of psychological alterations in our patients (divided into quintiles) is seen in Figure 1. With increasing number of psychological alterations, there was a gradual increase in the overall severity of GI symptoms (GSRS-IBS: partial eta-squared (η_p^2) = 0.149, $P < 0.001$, IBS-SSS: $\eta_p^2 = 0.160$, $P < 0.001$, both with large effect sizes). Among the individual GI symptoms, this effect was strongest for pain ($\eta_p^2 = 0.105$, $P < 0.001$) and weakest for constipation ($\eta_p^2 = 0.026$, $P = 0.10$), with bloating and diarrhea being intermediate ($\eta_p^2 = 0.045$, $P = 0.03$, for both).

Conclusions: A distinct association was seen between increasing severity of GI symptoms and the number of psychological alterations in IBS. This finding highlights the importance of considering psychological factors when managing IBS patients in the clinical setting.

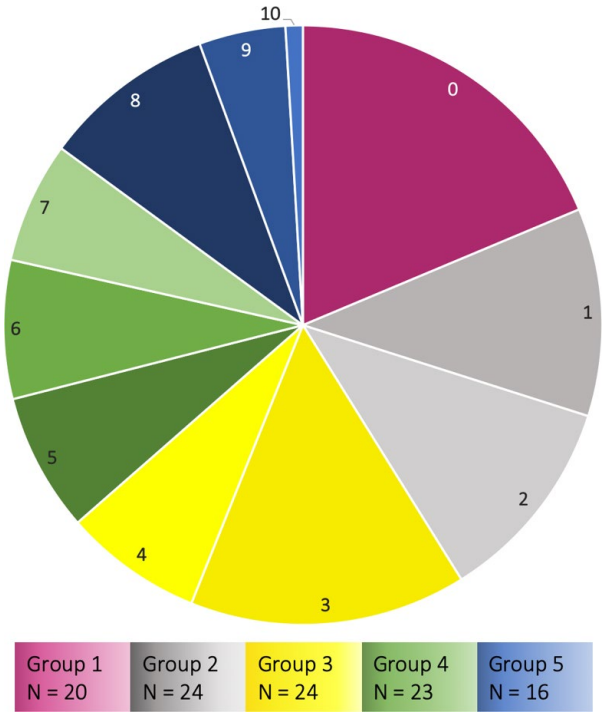

Figure 1: The number of psychological alterations and division into quintiles, in our IBS patients

222 | Olorinab, a peripherally acting, highly selective, full agonist of the cannabinoid receptor 2 (CB2), reduces visceral hypersensitivity in mice

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Background: Abdominal pain is a debilitating symptom of inflammatory bowel disease (IBD) and irritable bowel syndrome. We tested whether olorinab reduced visceral hypersensitivity in a mouse model of chronic visceral hypersensitivity (CVH). We also determined CB_2 expression in the colon and dorsal root ganglia (DRG) in CVH mice and IBD human donor DRG to understand potential sites of olorinab actions.

Methods: CVH was evaluated after resolution of dinitrobenzene sulfonic acid–induced colitis. Control or CVH mice were administered either vehicle or olorinab (3, 10, 30 mg/kg twice daily) orally on days 24-28 following induction. In vivo visceromotor response (VMR) to colorectal distension (CRD; 0-80 mm Hg) determined visceral mechanosensitivity. CB_2 mRNA expression was measured in colonic tissue (mucosa; muscle and enteric nervous system) and DRG from healthy, colitis, and CVH mice, and in healthy and IBD human donor DRG, by quantitative real-time polymerase chain reaction.

Results: Vehicle-treated CVH mice exhibited pronounced hypersensitivity compared with vehicle-treated healthy mice (N = 21/group). Olorinab-treated CVH mice (N = 12-15/group) had significantly reduced VMR to CRD ($P < 0.05$) compared with vehicle-treated CVH mice. Low but detectable levels of CB_2 mRNA were observed in all tissues studied. CB_2 was the predominant cannabinoid receptor transcript in colonic mucosa. No differences were observed in CB_2 levels in healthy versus colitis or CVH mice. There were no differences in CB_2 mRNA levels between healthy and IBD human donor DRG.

Conclusions: Olorinab reduced CVH, suggesting that CB_2 activation causes antinociceptive actions in visceral sensory pathways. Low, but detectable levels of CB_2 were observed in colonic tissue and DRG; however, no differences were observed between healthy and disease states. It is possible that subtle differences in CB_2 expression patterns may not be captured by measuring whole tissue mRNA levels. Further studies are being performed to assess localization of CB_2 on specific cell types.

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223 | Characteristics of patients referred for investigation of reflux

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Background: Patients presenting with ENT complaints are often considered to harbor atypical esophageal reflux.

Aim: To characterize patients referred for investigation of "atypical reflux" complaints and to compare them to patients referred with classic reflux complaints

Methods: Consecutive patients referred for investigation underwent a comprehensive workup including medical history, filling a symptom questionnaire, Rome 3, SCL 90 and SF 36 questionnaires. All underwent gastroscopy, esophageal HRM and pH impedance off therapy.

Results: 156 patients (90 classic reflux group-G1. 66 ENT group-G2) were included. 83 (53.2%) females, mean age 52.9 ± 16.9 years. No differences were found between the groups in demographic parameters, background diseases and psychological profile (P = NS). Patients of the 2 groups had high prevalence of functional dyspepsia, (G1-56.3% vs. G2-31.8%. $P = 0.058$) and high prevalence of IBS (G1-66.7% vs. G2-58.8%. N.S.) as expressed by the Rome-3 questionnaire. Patients with complaints of reflux used more PPIs ($P = 0.008$) and SSRIs ($P = 0.053$). Life quality was not different between the groups apart from a single parameter regarding pain ($P = 0.012$). Gastroscopy and HRM results were not different between the 2 groups apart from Higher UES pressure in G1 ($P = 0.011$). pH impedance results were not different between the 2 groups in total

acid exposure (G1-4.98% vs. G2-5.36%, NS), weakly acidic (G1-27 vs. G2-24, NS) and weakly alkaline episodes (G1-1.5 vs. G2-1.4, NS). Pathological reflux (acid exposure >6%), was similar between the 2 groups (G1-35.6% vs. G2-35.4%). Of the complaining patients during the study (104/156 = 66.6%), functional heartburn was diagnosed in G1-33.3% vs. G2-50.0%, $P = 0.094$ and hypersensitive esophagus in the rest.

Conclusions: In this cohort, apart from the major referral complaints, patients with ENT problems were not different from patients with classic heartburn in all parameters tested. Surprisingly, the majority of patients in both groups had no pathological reflux; however, the prevalence of IBS, functional dyspepsia, functional heartburn and hypersensitive esophagus was high in both groups

224 | Validation of mobile phone APP for assessment of bowel function in healthy subjects

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Background: Clinicians rely on patients' recall of bowel habit to facilitate diagnosis of bowel problems, whereas researchers use paper stool diary. Recall bias, compliance, privacy and functionality may each affect accuracy of such assessments as well as diagnosis and interpretation of results in clinical trials.

Aim: To develop and validate a mobile phone APP for constipation and symptoms and correlate this with paper form stool diary.

Methods: Healthy subjects completed either paper diary or phone APP diary for 2 weeks each, in a randomized crossover trial for 4 weeks. Each diary composed of ten previously validated questions related to constipation and evacuation difficulty. A separate questionnaire assessed functionality and user-friendliness of each stool diary. The constipation APP provides exportable weekly summary and has software for daily subject log and electronic data base for analyzing results, obviating need for manual data entry, calculation of CSBM etc, all of which should minimize errors. Correlational analyses were performed to examine reproducibility, functionality and validity of APP diary.

Results: Twenty-one healthy subjects (F/M = 13/8) participated. Stool frequency, spontaneous and complete spontaneous bowel movement (CSBM), stool consistency (Bristol Stool Form Scale), straining effort and number of gas or bloating episodes were significantly correlated between week 1 and week 2 with the mobile phone APP diary (ICC > 0.619, Table 1). Likewise, the 2-week APP diary recordings for most of the bowel symptom(s) and parameters were strongly correlated with paper dairy recordings (ICC > 0.512, Table 1). The scores for ease of use, handling, bothersomeness and overall functionality was significantly higher with APP than paper diary ($P < 0.027$, Table 1).

Conclusions: The Constipation Stool APP appears to be a reliable, valid and reproducible method of accurately documenting bowel habit whilst respecting individual's privacy. Our healthy subjects overwhelmingly (95% vs 5%) preferred the APP to paper dairy. Hence, Constipation mobile APP can be a useful tool for screening and assessment of bowel function and symptoms of difficult evacuation, for both clinical or research purposes.

225 | Patient reported symptoms of lower functional gastrointestinal disorders correlate poorly with objective faecal loading on intestinal ultrasound

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Background: Diagnosis of functional gastrointestinal disorders (FGID) is based on subjective and unreliable patient-reported symptoms. Objective biomarkers are thus urgently sought. Intestinal ultrasound can objectively and non-invasively assess luminal contents. This study assessed the utility of intestinal ultrasound in phenotyping patients with lower FGID.

Methods: Patients with lower FGID underwent an intestinal ultrasound and completed the ROME IV diagnostic questionnaire, SAGIS questionnaire and 100 mm Visual Analogue score for overall GI symptom severity. The sonographer was blinded to patient symptoms. The faecal loading score (FLS) was obtained using a modified Leech score. As previously shown, a FLS >37 is consistent with clinically significant constipation.

Results: Eighty-nine patients (19:70 m:f) were recruited. The median age was 47 (range 19-81) with mean overall GI severity scores of 68 (95% CI 59-76).

Fifty-six patients met ROME IV criteria for Irritable bowel syndrome (IBS) (IBS-M = 18, IBS-D = 18, IBS-C = 17, IBS-U = 3). Twenty-three met criteria for chronic constipation (CC), four had functional diarrhoea (FD), two faecal incontinence (FI) and two functional abdominal pain syndrome (FAP). One individual could not be defined via ROME IV.

Patients reporting constipation (either IBS/CC) had a significantly higher mean FLS score than patients describing diarrhoea (IBS-D/FD) (FLS = 42 [95% CI 33-50] vs. 26 [95% CI 14-38], $P = 0.03$). Almost half (40/88) had significant faecal loading (FLS >37) (Figure 1) with the majority describing constipation as the dominant symptom (IBS-C = 10, CC = 14). Interestingly 37% with significant faecal loading did NOT describe constipation predominance (IBS-D = 4, IBS-M = 7, IBS-U = 1 & FD = 2, FAP = 1, FI = 1); possibly indicating overflow diarrhoea.

Forty-eight patients had a FLS of ≤37 with the majority describing diarrhoea (IBS-D = 14, FD = 2, IBS-M = 11, IBS-U = 2, FAP = 1, FI = 1, no diagnosis = 1). Sixteen reported constipation (CC = 9, IBS-C = 7) despite no faecal loading on ultrasound.

Conclusions: Symptoms poorly predict severity of faecal loading. One third of patients describing diarrhoea had clinically significant faecal loading. Future research is underway to determine if symptomatic improvement correlates with change in faecal loading following laxative therapy regardless of baseline reported symptoms.

226 | A nursing / allied health led functional gastrointestinal disorders clinic is cost effective, well received by patients, highly effective and efficient and reduces medical intervention

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Introduction: Functional Gastrointestinal disorders (FGID) are highly prevalent but often poorly managed. Medical intervention is not always necessary as allied health interventions are effective and economical. Unfortunately, access is limited and patients are funnelled to high cost specialist care. We evaluated a nurse/allied health led service for patients with FGID aiming to improve access to therapies, clinical outcomes, efficiency and cost.

Methods: A composite triage questionnaire (MUST, ROME IV, SCOFF and DASS21) and blood tests (FBE, ferritin, CRP, coeliac serologies) were implemented. Low-risk patients were directed to a nurse/allied health service and high-risk patients to medical review (Figure 1). Service efficiency (2018) was compared to clinic data in 2017 (each reported over 6 months). Clinical effectiveness was determined by change in symptom severity (via 100 mm Visual Analogue Score (VAS)). Clinical responders had >20 mm improvement. Patient satisfaction was measured with 100 mm VAS.

Results: The new service was more efficient (new patients seen 2017 = 108 vs. 2018 = 297, overall patients seen 2017 = 314 vs. 2018 = 463, Total occasions of care in 2017 = 679 vs. 2018 = 1241) despite no additional medical EFT. Waiting time decreased (median days: 2017 = 345 vs. 2018 = 120, $P < 0.0001$) as did fail to attend (FTA) appointments (13.5% to 3%). The number of patients requiring medical review decreased by 36% with a corresponding cost reduction ($887 to $484 per new patient). Patients reported high satisfaction (median scores 97: IQR = 80-100) and had a 21.6% reduction in symptoms compared to 2017 patients who reported a 9% increase in symptoms over the same period. Clinical responder rates increased from 20.2% to 34.2%.

Conclusion: A nursing/allied health led FGID clinic results in significant clinic efficiencies including reduction in waiting times, reduction in FTA appointments, increase in new patients seen and an increase in overall clinic activity. This almost halved cost while being associated with excellent patient satisfaction and symptom outcomes.

229 | Megaesophagus as a complication of achalasia: Case report and narrative review of literature

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Summary: We present the case of a patient with progressive dysphagia, in whom megaesophagus was documented as complication of a long-lasting, untreated achalasia. Chagas disease was ruled out. Given the low frequency of this entity in our setting and the therapeutic implications it has for patients with achalasia, a narrative review is made in the literature about its diagnosis and management alternatives.

Key words: achalasia, dysphagia, esophageal motor disorders, high resolution manometry, megaesophagus, Chicago 3.0.

230 | Colitis induced microvascular leak triggers a neurogenic disruption in gastrointestinal motility

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Background: IBD patients suffer debilitating motility abnormalities. We investigated the hypothesis that colitis disrupts colonic motility through the inflammatory induction of a transmural colonic microvascular leak.

Methods: C57Bl/6 were fed water or dextran sodium sulfate (DSS) 3% for 10 days. Neutrophils were quantified in whole-mounts. Colonic tissue and serum cytokines were determined using a cytokine panel. Microvascular leak of FITC-dextran (70 kD) from the IB4-568λ labeled microvascular leak was imaged by confocal microscopy. The effect of colitic inflammatory mediators on cultured human intestinal endothelial cell (HIMEC) lactate dehydrogenase release was quantified. Colonic circular muscle contractions to serum in Krebs or L-nitro-arginine (100 μM) were quantified. ($P < 0.05$, N = 4-7 each). Polyethylene glycol/ammonium sulfate serial precipitations, molecular size centrifugation and HPLC were used to isolate a specific group of five serum proteins.

Results: Lamina propria demonstrated a DSS-induced dense, microvascular clustered neutrophilic infiltrate (cntrl = 162.0 ± 6.01 vs. DSS = 48.4 ± 8.05 luminosity, 100× field) and muscle whole-mounts also exhibited increased neutrophilic extravasation (24.2 ± 1.3) compared to controls (1.5 ± 0.97, cells/200×). Immune analysis of cultured

LP/ME tissue conditioned media demonstrated significant increases in VEGF, IL-10, MIP-1α & GM-CSF compared to control. And, inflamed conditioned media stimulated LDH release from HIMECs (51.1 ± 22.86% of control min/max). Serum cytokines also showed an inflammatory response. Colitis caused a profuse vascular leak of intravenous FITC-dextran. The *in vivo* intraperitoneal injection of serum (250 μL) caused an immediate delay in transit GCs compared to lactate-Ringers (5.1 ± 0.63 vs. 9.9 ± 0.53, respectively). Exposure of colonic circular muscles to serum (0.5, 1.0 and 2.5%) caused a dose-dependent inhibition of spontaneous clustered contractions (45.8 ± 9.46%, 68.6 ± 2.69%, and 78.8 ± 3.26%, respectively), which was partially blocked by LNA. Serum serial precipitations, molecular sizing and HPLC isolated the enteric leak factor (ELF) to a specific group of serum proteins >100 kD.

Conclusion: Colitis induced inflammatory mediators triggers a perfuse microvascular leak of a specific serum ELF within the colonic wall that potently activates nitrergic and NANC motor neurons to dramatically alter contractility.

231 | Prostaglandin E2 production by enteric glial cells reduces colitis development in female mice

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Enteric glial cells (EGCs) are involved in the modulation of intestinal motility, permeability and inflammation. We have shown that these regulations involve the production and release of soluble mediators, including n-3 and -6 derivatives such as prostaglandins. Prostaglandin E_2 (PGE_2) participates in the control of intestinal functions, but studies focusing on the effects of PGE_2 released specifically by EGC are still lacking. We have evaluated *in vivo* the impact of mPGES1 (PGE_2 producing enzyme) deletion induced specifically in EGC, on intestinal functions and inflammation.

mPGES1 deletion was induced in 10-20 weeks old male or female S100β$^{Cre\text{-}ERT2}$–mPGES1 $^{fl\text{-}fl}$ by a daily injection of tamoxifen (Tamo) for 5 days. Two weeks after the onset of the induction of deletion, we induced, or not, the development of colitis by adding dextran sodium sulfate (DSS 4%) in the drinking water during 4 days. The parameters measured at the end of the protocol are: the total transit time (TTT, expulsion time of carmine red given in gavage, the Disease Activity Index (DAI = presence of blood in the stool and weight loss of the animals), the paracellular permeability (sulfonic acid flux) and a histological damage score taking into account mucosal damage, goblet cell loss, muscle thickening and immune cell infiltration. An immunohistological analysis enabled to verify the expression of mPGES1 without or with inflammation (DSS) in the EGC of wild type mPGES1 mice (S and mP mice treated with Tamo = WT), and its deletion in mPGES1 Δ^{EGC} mice (S100β$^{Cre\text{-}ERT2}$–mPGES1 $^{fl\text{-}fl}$ treated with Tamo = mPΔ^{EGC}).

Results: An increase in EGC mPGES1 expression was observed after exposure to DSS in the myenteric ganglia of male and female WT mice. As expected, this induction was absent in mPΔ^{EGC} mice. TTT was unchanged after DSS or mPGES1 deletion in male, but decreased in mP^{DEGC} female mice, with or without DSS. DAI, permeability and histological damages were increased after DSS in male mice, with or without or mPGES1 deletion, but only significantly increased in mPΔ^{EGC} female mice.

Conclusion: This work represents the first *in vivo* evidence of a direct involvement of glial-derived PGE_2 in the pathophysiology of the intestine.

232 | Enteric glia utilize the cytosolic DNA sensor STING to modulate immune response

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Background/Objectives: Functional and organic bowel disorders such as inflammatory bowel disease and irritable bowel syndrome are characterized by microbial dysbiosis, immune activation, and altered enteric nerve function. Enteric glia are ideally positioned to participate in microbial and immune signaling within the enteric nervous system (ENS), but how they would fulfill this role is not clear. Related neuroglial populations utilize the cytosolic DNA sensor stimulator of interferon genes (STING) to modulate immune signaling. Thus, *we hypothesized that enteric glia express STING and enteric glial STING signaling modulates immune response in the ENS*.

Methods: We assessed glial STING expression using immunohistochemistry and glial-specific RNA sequencing in *Sox10*::$^{CreERT2+/-}$/*Rpl22*$^{+/-}$ (Ribotag) mice. STING activation in the ENS and the contribution of glial STING to immune response was determined by IFNβ ELISA in wild-type and *Sox10*::$^{CreERT2+/-}$/*Tmem173*$^{-/-}$ mice.

Results: Immunolabeling and RNA sequencing show that submucosal and myenteric glia express STING (n = 3-4 animals). Enteric glia are the primary cells expressing STING at the level of the myenteric plexus, but STING is widely expressed by glia and immune cells at the level of the submucosal plexus. Activation of STING signaling in isolated myenteric and submucosal plexus preparations produces IFNβ ($P < 0.0001$, n = 4 tissue preparations/group) and IFNβ production is greater in submucosal preparations than myenteric ($P < 0.0001$, n = 4 tissue preparations/group). Unexpectedly, the selective ablation of glial STING in *Sox10*::$^{CreERT2+/-}$/*Tmem173*$^{-/-}$ mice increases IFNβ production in response to STING agonists ($P < 0.0001$, n = 3 tissue preparations/group).

Conclusions: Our data suggest that glial STING signaling modulates IFNβ production and could impact intestinal immune responses. The potential negative feedback role of glial STING will be assessed in future work.

234 | Delivery of care for functional gastro-intestinal disorders: Randomised trial of standard gastroenterologist versus multi-disciplinary care

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Background: Functional gastrointestinal disorders (FGIDs) are common and costly to the healthcare system. Most specialist care is provided by a gastroenterologist, yet a minority of patients symptomatically improve. Although proven effective, psychological, behavioural and dietary therapies are not provided routinely. This study compared gastroenterologist-only with integrated multi-disciplinary care.

Methods: In a single-centre, pragmatic trial consecutive new referrals of patients with Rome IV criteria-defined FGIDs were randomised 1:2 to a gastroenterologist-only standard-care (SC) or multi-disciplinary (MD) clinic, MD comprising gastroenterologists, dieticians, gut-hypnotherapists, psychiatrists and behavioural ("biofeedback") physiotherapists. In the MD clinic all patients saw a gastroenterologist and were seen by allied clinicians as needed. Outcomes were assessed at clinic discharge or 9 months. Primary outcome was global symptom improvement on a 5-point Likert scale. Symptoms, specific functional disorder status, psychological state, quality-of-life, and cost were secondary outcomes. Analysis included all patients where outcomes were available at discharge (modified intention-to-treat, MITT).

Results: Of 188 randomised patients, 144 (46 SC, 98 MD) were included in the MITT analysis. 62% of MD clinic patients saw allied clinicians. 57% SC vs 84% MD clinic patients achieved global symptom improvement ($P < 0{\cdot}001$). ≥50% reduction in Gastrointestinal Symptom Severity Index occurred less often in SC than MD ($P < 0{\cdot}04$) for reflux, nausea and vomiting, constipation and diarrhoea. ≥50-point reduction in IBS Severity Scoring System occurred in 38% vs. 66% ($P = 0{\cdot}02$) of IBS patients, respectively. Hospital Anxiety and Depression Scale (HADS) was higher in SC than MD (13 vs. 10, $P = 0{\cdot}1$). Euro-QoL visual analogue was lower in SC than MD (70 vs. 75, $P < 0{\cdot}01$). Estimated incremental cost of MD care was $2909 per QALY gained.

Conclusion: Integrated multi-disciplinary clinical care is superior to gastroenterologist-only care in relation to global and specific symptoms, FGID improvement, psychological state, quality of life and cost.

235 | Effects of acute changes in fermentable fibre intake on regional colonic fermentation and transit in patients with quiescent ulcerative colitis

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Background & Objectives: Reduced carbohydrate fermentation has been described in patients with quiescent ulcerative colitis (UC). Such defects may differ across colonic regions or with acute variations in dietary fibre intake. These aspects deserve further study. Hence, we aimed to define regional colonic fermentation by direct pH-transit profiling (SmartPill) in quiescent UC patients following acute variations in fermentable fibre intake.

Methods: This is a randomized, double-blind, crossover trial. Patients with quiescent UC (Partial Mayo Score ≤1; faecal calprotectin <150 μg/g) and healthy controls were recruited. After a 7-day run-in, subjects were fed study meals high (13 g) or low (<1 g) in oligosaccharides and resistant starch over 12 hours before ingesting a SmartPill. A crossover to the other diet occurred 3 days after passage of the SmartPill. Endpoints were diet-associated differences in overall, caecal and distal colonic pH, and colonic transit time (CTT). Caecal pH was defined as the minimum pH following passage through the ileo-caecal junction whereas maximum pH was arbitrarily used as distal colonic pH.

Results: 15 UC patients (aged 24-72 years; 9 males) and 9 controls (aged 22-69 years; 4 males) were studied. A decrease in overall and distal colonic pH was observed in controls with increased fibre intake (Table 1). In UC patients, increased fibre intake reduced caecal pH but paradoxically tended to increase distal colonic pH. No differences in CTT were observed between diets in either cohort. However, subgroup analysis in the UC cohort showed highly heterogeneous responses to the high fibre diet (64% had slower CTT whilst 36% had unchanged or faster CTT). In contrast, majority (63%) of controls had no changes in CTT with the dietary interventions.

Table 1. Colonic pH and transit responses to acute changes in fermentable fiber intake

		Overall mean pH (95% CI)	Mean cecal pH (95% CI)	Mean distal colonic pH (95% CI)	Median [IQR] CTT (h)
UCn = 15	Low fiber	6.4 (6.2-6.8)	5.6 (5.3-5.7)	7.9 (7.6-8.2)	17 [9-23]
	High fiber	6.3 (6.0-6.5)	5.2 (5.0-5.4)	8.1 (7.6-8.4)	21 [16-39]
	P-value	0.20	0.001	0.09	0.13

		Overall mean pH (95% CI)	Mean cecal pH (95% CI)	Mean distal colonic pH (95% CI)	Median [IQR] CTT (h)
Healthyn = 9	Low fiber	6.9 (6.5-7.2)	5.5 (5.2-5.8)	8.2 (8.0-8.5)	16 [15-37]
	High fiber	6.3 (6.0-6.5)	5.2 (4.9-5.5)	7.7 (7.4-8.0)	18 [15-32]
	P-value[1]	0.02	0.15	0.04	0.58[2]

[1]Paired t-test or [2]Mann-Whitney test.

Conclusions: Increasing fermentable fibre intake partially increased colonic fermentative activity in quiescent UC patients compared to controls. Moreover, contrary to controls, UC patients exhibited an increase in distal pH and heterogeneous colonic transit responses after the increased fibre intake. Our findings suggest that abnormalities in regional motility and function of the colonic microbiota exist despite quiescent disease.

Conflicts of interests: Research support was received from Ferring Pharmaceuticals.

237 | Distinguishing the contributions of neuronal and mucosal serotonin to the regulation of colonic motility

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Background/Objectives: Enterochromaffin (EC) cells lining the gastrointestinal (GI) tract produce ~95% of total body serotonin (5-HT), along with serotonergic neurons of the enteric nervous system (ENS). 5-HT synthesis is limited by tryptophan hydroxylase (TPH) isoforms, TPH1 and TPH2, present in non-neuronal and neuronal 5-HT-producing cells, respectively. There is evidence that endogenous mucosal 5-HT can modulate the characteristics of colonic migrating motor complexes (CMMCs) which play a major role in propulsion of colonic contents. The role of neuronal 5-HT in gut motility remains a matter of debate. To determine the respective contribution of neuronal 5-HT in GI-motility and transit, we increased endogenous 5-HT availability at synaptic terminals using fluoxetine, in the presence and absence of mucosal 5-HT synthesis.

Methods: CMMCs were measured using a series of force transducers attached longitudinally to the muscle layer of mouse colon preparations. Firstly, we assessed the effect of 1 μM fluoxetine on CMMCs using flat-sheet colon preparations from C57/Bl6 mice in the presence and absence of the mucosal layer containing EC cells. We then repeated this using intact colon using a novel tamoxifen-inducible *Tph1*$^{CreERT2/+}$;*Rosa26*$^{DTA/+}$ mouse model for EC cell depletion, in which only mucosal EC cells had been conditionally removed.

Key Results: CMMC baseline frequency and amplitude was reduced in both mucosa-free and EC cell-depleted preparations, demonstrating the relative contribution of EC cell-derived 5-HT to colonic motility. Fluoxetine reduced CMMC frequency in the presence of mucosal 5-HT, but not in mucosa-free and EC cell-depleted preparations lacking EC cell-derived 5-HT (n = 4).

Conclusions: The increased availability of 5-HT at synaptic terminals of the ENS reduced the frequency of CMMCs in mouse colon preparations, but only in the presence of mucosal EC cells. This demonstrates that EC cell-derived 5-HT can modulate the characteristics of colonic contractions.

238 | Altered gastrointestinal motility in a mouse model of cigarette smoking induced chronic obstructive pulmonary disease

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Cigarette smoking (CS) is a major cause of Chronic Obstructive Pulmonary Disease (COPD) and gastrointestinal (GI) dysfunction reduces quality of life for COPD patients. However, the underlying mechanisms and precise effects of CS on gut contractility are not fully characterised. We therefore examined for changes in colonic motility, enteric neuronal numbers and mucus thickness in a mouse model of COPD. Male BALB/c mice (7 weeks old) were exposed to room air (Sham) or CS (9 cigarettes/day, 5 d/wk) for 2 and 6 months. Video imaging was used to construct high resolution spatiotemporal maps of colonic motor patterns *ex vivo*. The colon was cannulated in an organ bath containing physiological saline at 37°C and colonic motility was recorded over 1 hour. Wholemount immunofluorescence for the pan-neuronal marker (Hu) and nitric oxide synthase (NOS) was performed to quantify myenteric neurons. Mucus thickness was investigated using Alcian blue staining in colonic cross sections. CS reduced body weights and increased the colon length to body weight ratio. Mice exposed to CS for 2 months had more colonic migrating motor complexes (CMMCs; CS: 13.2 ± 0.6; Sham: 7.8 ± 0.6 CMMCs/15 minutes, $P < 0.0001$), reduced resting colonic diameter (CS: 3.6 ± 0.1 mm; Sham: 4.1 ± 0.2 mm, $P = 0.01$) and faster transit (CS: 3.2 ± 0.2 mm/s; Sham: 2.3 ± 0.2 mm/s, $P = 0.0024$). A 10-day cessation after 2 months CS reversed the CS-induced increase in CMMC frequency, however had no effect on reduced colonic diameter ($P = 0.816$). Exposure to CS for 2 months had no effect on enteric neuron numbers, whereas 6 months CS exposure reduced NOS

neuron numbers (CS: 35 ± 2.5%; Sham: 42 ± 0.8%, P = 0.039). As previously reported, mucus thickness was reduced in CS mice (CS: 4.8 ± 0.3 μm, n = 8; Sham: 18.6 ± 0.9 μm, n = 7, P < 0.0001). This is the first report of increased colonic motility in CS-induced COPD measured by *ex vivo* video imaging. Prolonged exposure to CS reduced the number of NOS-immunoreactive enteric neurons. Further research is necessary to identify the exact causes of dysmotility.
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239 | Association between large volume trans-anal colon irrigation and anorectal physiology

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Large volume trans-anal colon irrigation (TAI) is a common alternative method using for constipation. Our study aims to find the difference of patient characteristics and anorectal physiology between chronic constipation patients with and without regular TAI.
Methods: 242 consecutive patients from 2017 to 2019 who had chronic refractory constipation were interviewed regarding GI symptoms, their treatment on constipation, and underwent anorectal physiologic studies including high resolution anorectal manometry, balloon expulsion test, and colonic transit time (CTT). A regular use of TAI was defined by at least 1 irrigation per week. A 1:2 age and gender-matched analysis was performed between patients with and without regular TAI.
Results: 30 patients (23 females, age 54.5 ± 14.8 years; 12.4%) reported regular TAI with median frequency of 8 (4-12) sessions/month, median duration of 12 (0.5-36) months, and volume of 1 (1-1.5) liters/time. The global symptom scores and the most bothersome symptoms reported were not significantly different between TAI and non-TAI groups. Patients in TAI group was diagnosed as functional constipation (FC) 12 (40%) and irritable bowel syndrome (IBS) 13 (43.3%) which were similar to non-TAI group [FC, 20 (33.3%), and IBS, 28 (46.7%), both P > 0.05]. Prevalence of delayed CTT and dyssynergic defecation were also similar between both groups. However, patients who use TAI had significantly higher rectal sensory threshold for intolerable urgency, and a trend of higher desire to defecate threshold compared to non-TAI group [TAI vs. non-TAI; S2: 60 (40-80) vs 40 (30-60), P = 0.07 and S3: 150 (100->150) vs 120 (80-150), P = 0.049]. Patients with regular TAI had significantly more prevalence of rectal hyposensitivity compared to non-TAI group [14 (46.7%) vs 14 (23.3%), P = 0.03].
Conclusions: Chronic constipation patients with regular TAI using had significantly higher prevalence of rectal hyposensitivity and higher threshold for intolerable urgency whereas the symptoms profiles, anorectal function, and colonic transit were similar. Prospective studies regarding the causal relationship were needed.

240 | Serum Pepsinogen and Gastrin; Reliable Markers to Predict Small Intestinal Bacterial Overgrowth?

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Background: Serum pepsinogens are useful index of gastric atrophy, or the status of gastric acidity. And it could reflect the presence of small intestinal bacterial overgrowth (SIBO). This study aimed to investigate the association between pepsinogen profiles and breath hydrogen – methane gas.
Methods: This prospective study evaluated 62 patients who had functional gastrointestinal symptoms. All subjects underwent glucose breath test, endoscopy for acute gastric injury or status *Helicobacter pylori* by rapid urease test, and laboratory tests including serum gastrin, pepsinogen I, II, and gastrin.
Results: The prevalence of SIBO was 17.7% (11/62). Using cut-off values of pepsinogen I/II ratio 3.5, or gastrin (35.4 pg/mL), higher total hydrogen concentration during GBT (P < 0.01), the higher prevalence of acute gastric injury (P < 0.05), and the lower positivity to *Helicobacter pylori* (P < 0.01) were significantly shown in groups with low pepsinogen ratio, or with high gastrin than those with high ratio, or with low gastrin, respectively. A tendency of proportional correlation between total hydrogen concentration and PG I/II ratio, but hydrogen and gastrin were inverse proportion to each other. Whereas, multivariate analysis showed that old age was only independent factor to predict SIBO [(Odds ratio (95% confidence interval), 1.07 (1.01-1.14), P = 0.03].
Conclusion: Old age was the significant risk factor for presence of SIBO in patients with FGID. Although pepsinogen and gastrin profiles appear to be not independently associated with the prevalence of SIBO, further studies were needed to consider both intestinal motility function as well as gastric acidity with hydrogen producing SIBO.
Key words: gastrin, glucose breath test, pepsinogen, small intestinal bacterial overgrowth.

241 | Endoscopy assisted catheter insertion can increase high resolution esophageal manometry completeness

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Background/Objectives: Esophageal high resolution manometry(HRM) is widely used for esophageal motility evaluation in clinical practice. According to Chicago's criteria version 3.0, 10 consecutive wet swallowing of 5 mL water in the supine position is needed for evaluation. However, about 21% of HRM studies are imperfect (S. Roman et al. CGH 2001;9(12):1050-1055). In patients with large hiatal hernia and achalasia, catheter occasionally can't transverse esophageal-gastric junction. Using endoscopy to guide and assist high resolution manometry may help HRM catheter insertion. In this study, we evaluate the efficacy of endoscopy assisted catheter insertion in performing HRM exams.

Methods: We reviewed the data from a prospectively recorded functional esophageal disease database in endoscopy center of Taipei Veterans General Hospital. Records of consecutive patients who received HRM exams from June, 2017 to October, 2019 are analyzed. HRM was performed with 32 circumferential pressure channels with 16 impedance channels solid-state catheter (Medical Measurement Systems, Netherlands) and analyzed according to Chicago classification of esophageal motility disorders version 3.0. For patients failed manual catheter insertion or intolerance to catheter insertion, endoscopy guided probe insertion was performed. Snare assisted HRM catheter insertion was used in difficult cases.

Results: Total 192 patients received 207 times of HRM exams in the study period. The patient's average age was 53.91 years old. 183 HRM exams (88.4%) can be performed conventionally. 20 patients received 23 times of HRM catheter insertion with endoscopic assistance, and all of them completed HRM exams successfully. 18 patients (90%) were diagnosed with achalasia, while 1 patient had large diverticulum and 1 had large hiatal hernia. Only 1 patient didn't complete HRM exam due to rhinitis inducing nasal obstruction. With endoscopic assistance, the completeness of HRM exams increased from 88.4% to 99.5%. No clinical significant adverse events or HRM probe damage were noted in the study period.

Conclusions: In patients with difficult HRM catheter insertion, endoscopy assisted catheter insertion is highly effective. Endoscopy assisted catheter insertion can increase high resolution esophageal manometry completeness without side effects.

242 | Altered caecal neuroimmune interactions in the Neuroligin-3 R451C mouse model of autism

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Background/Objectives: Gastrointestinal (GI) dysfunction is common in individuals with autism and many rare autism-associated mutations impact synaptic cell-adhesion pathways including the well-studied neuroligin-3 R451C missense mutation. Patients expressing this mutation also experience gastrointestinal dysfunction. Changes in the host nervous system can alter microbial profiles, and microbes produce neuro-active molecules that modulate the central and enteric nervous systems. The caecum is involved in generating immune responses and is thought to act as a repository of intestinal microorganisms, however little is known about caecal neuroimmune and microbial interactions.

Methods: Using immunofluorescence in wholemount preparations, we quantified the total number of myenteric and submucosal neurons (stained for the pan-neuronal marker Hu). Using 3D image analysis we also analysed the density and morphology of Iba1-expressing immune cells in the caecal patch, a component of the gut-associated lymphoid tissue of wild-type and $NL3^{R451C}$ mice. Caecal microbial community composition was assessed using Illumina deep sequencing.

Results: $NL3^{R451C}$ mice have significantly reduced caecal weight compared to wild-type (0.65 ± 0.02 g in WT, n = 38; 0.54 ± 0.01 g in $NL3^{R451C}$, n = 36; $P = 0.0001$). $NL3^{R451C}$ mice had more caecal myenteric neurons per ganglion (WT: 11 ± 1, NL3: 15 ± 1 neurons; n = 5 in each group; $P = 0.005$; 10 ganglia/sample). The total number of submucosal neurons/ganglion was also increased in $NL3^{R451C}$ mice (WT: 5 ± 1 and $NL3^{R451C}$: 6 ± 1 neurons; $P = 0.04$). Iba1-expressing caecal patch immune cells in $NL3^{R451C}$ mice had an increased density (WT: 24 ± 1 cells/100 μm^2; $NL3^{R451C}$: 29 ± 1 cells/100 μm^2, n = 5; $P = 0.032$) and were smaller (946.9 ± 0.4 μm^3 and 559.7 ± 0.4 μm^3; WT and $NL3^{R451C}$, respectively; $P = 0.004$). In addition, caecal microbial community structures in $NL3^{R451C}$ mice were distinctly different compared to wild-types.

Conclusions: These results suggest that the $NL3^{R451C}$ mutation alters the bi-directional communication between caecal neurons and the microbial community.

***Equal contribution.** :

245 | Antibiotic-induced depletion of gut microbiota: Impact on radiation-induced oral mucositis in rats

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Objective: Gut microbiota plays a major role in the development and modulation of immune and inflammatory responses. Recently, it has been found that gut microbiota is involved in the pathogenesis of chemotherapy and immunotherapy-induced gastrointestinal mucositis through the modulation of inflammatory responses. The aim of this study was to determine whether gut microbiota has an impact on the development and severity of radiation-induced oral mucositis (OM) in a rat model.

Methods: 24 male Sprague Dawley rats were divided into two groups; antibiotic treatment/radiation (ABxRx, n = 12) and radiation only group (Rx, n = 12). To deplete gut microbiota, ABxRx rats were provided with an antibiotic cocktail for three weeks in drinking water. All rats were exposed to single-dose radiation of 20 Gy to the snout. The rats were culled at two time points, day 9 and day 15 post-radiation and tongue collected for the analysis of OM severity.

Results: OM developed from day 5 and peaked on day 9. OM severity score was similar in both groups (3.8 ± 0.3 ABxRx group vs 4.5 ± 0.3 Rx group, *P = ns*). However, there was a significant difference in the duration of severe OM (grade 4-5) between groups (ABxRx 2 ± 0.3 vs Rx 3.5 ± 0.5 days, *P = 0.0408*). Moreover, the area of ulcered mucosa was smaller in ABxRx (18.9%) compared to Rx (33.3%) (*P = 0.02*). In addition, there was a reduction in body weight in both groups between day 8 and day 11, however, ABxRx recovered faster and gained more weight after day 11 (ABxRx 7% vs Rx 2% weight gain, *P = 0.046*).

Conclusion: Antibiotic-induced depletion of gut microbiota significantly reduced severe OM duration and ulcer-like area in the tongue. Future analysis will determine if gut microbiota eradication also reduces oral histopathological injury and inflammatory markers, which would provide evidence for gut microbiota targeting to reduce OM in clinical settings.

246 | Understanding the role of the milk fat globule membrane in microbial fermentation in the small and large intestine in early life

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Background: Milk fat globule membranes (MFGMs) in breast milk are thought to influence early neurodevelopment. Most infant formulas do not contain MFGMs, but contain vegetable lipids, which differ in size and composition to MFGMs. Clinical and animal studies have shown that consuming formula containing bovine MFGMs affects the large intestinal microbiota, improves gut development and cognitive scores. MFGMs from ruminants differ to those from humans, which may lead to different effects on gut microbial fermentation and physiological outcomes. This study aimed to determine the effects of bovine, goat and sheep MFGM on (1) ileal and caecal microbial populations and (2) production of fermentation end-products, after *in vitro* fermentation of pooled luminal contents sourced from piglets fed formula.

Methods: The laboratory enriched bovine, goat, and sheep MFGM fraction and a commercial bovine phospholipid concentrate (PC; Tatua, New Zealand) were digested *in vitro* (90 minutes pepsin at pH 5, and 120 minutes pancreatin at pH 6.5) using infant conditions (6 months). The MFGM remnants were then fermented *in vitro* under anaerobic conditions by ileal and caecal inocula collected from formula-fed piglets. Microbial composition and organic acids were determined.

Results: Except for PC, 8%-14% of the remnants from bovine, caprine and ovine MFGMs were fermented by the ileal microbiota prior to caecal fermentation. PC and caprine MFGM had the largest effect on the ileal microbiota reducing alpha diversity. Caprine MFGM increased and PC reduced the ileal ratio of Firmicutes:Proteobacteria. Bovine and ovine MFGM produced higher amounts of acetic, butyric and caproic acids in the ileal digesta and reduced the proportions of ileal Proteobacteria. PC was fermented (19%) by the caecal microbiota while bovine, caprine and ovine MFGM had a limited fermentability, but all affected the microbial composition of the caecum.

Conclusions: Species-specific differences and enrichment method may be crucial to determining the effects of MFGM components on the small and large intestinal microbiota and their fermentation end-product.

247 | Activation of CRF2 receptor increases gastric vagal afferent mechanosensitivity

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Background: Gastric vagal afferent (GVA) sensing of food related mechanical stimuli is a crucial mechanism in the control of feeding behavior and gastric function. Stress is an important factor contributing to eating disorder and gastric diseases. Chronic stress has been shown to increase the mechanosensitivity of GVAs and reduce food intake and body weight in mice. The role of stress hormones in modulating the increase in GVA mechanosensitivity is unknown. This study aimed to determine the effect of the stress hormones

corticosterone and corticotrophin releasing factor (CRF) family on GVA mechanosensitivity.

Methods: The expression of receptors for corticosterone and CRF family in GVA cell bodies was determined in 8 week old male C57BL/6 mice using qRT-PCR combined with laser capture microdissection. The mechanosensitivity of GVAs was determined in the absence and presence of stress hormones using an *in vitro* single fiber recording preparation.

Results: NR3C1 and CRHR2 (mRNA isoforms of glucocorticoid receptor and CRF2 receptor respectively) were expressed in GVA neurons. The glucocorticoid receptor agonist, corticosterone (10-100 μM), had no effect on the mechanosensitivity of either tension or mucosal GVAs. The CRF2 specific analogue, UCN3 (0.3-3 μM), significantly increased the mechanosensitivity of both tension and mucosal GVAs, an effect prevented by the CRF2 receptor antagonist astressin 2B (500 nM).

Conclusion: Activation of CRF2 receptor increases the mechanosensitivity of GVAs. This may contribute to the stress and CRF2 receptor associated changes in feeding behavior and gastric function, possibly contributing to the hypersensitivity of GVAs in chronic stress conditions.

248 | Fecobionics assessment of biofeedback efficacy

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Background/Objectives: Anorectal disorders such as fecal incontinence and constipation are common. Biofeedback is a behavioral therapy used to treat people with constipation or fecal incontinence, which do not respond to standard treatment. The aim of this study is to use Fecobionics (Gregersen et al CGH 2018, CTG 2019) as an assessment tool of anorectal physiological function during therapeutic interventions such as biofeedback. Pressure and expulsion data were compared to balloon expulsion test (BET) and high-resolution anorectal manometry (HRAM).

Methods: Nine fecal incontinence patients underwent biofeedback treatment. Nine female patients aged 61.9 ± 4.0 years were enrolled and assessed with Fecobionics, HRAM, and BET. The subjects filled in the validated Fecal Incontinence Severity Index (FISI), before and after 8 weeks biofeedback treatment.

Results: The FISI score was 34.4 ± 3.8 and 27.1 ± 3.5 pre- and post-treatment. The urge volume for Fecobionics was 26.1 ± 6.1 and 21.7 ± 6.8 mL pre- and post-treatment ($P > 0.5$). The urge volume for BET was 83.2 ± 5.1 and 74.1 ± 6.9 mL pre- and post-treatment ($P > 0.5$). The urge volume was significantly lower for Fecobionics compared to BET ($P < 0.01$). The expulsion duration for Fecobionics was 4.6 ± 1.5 and 5.0 ± 2.0 seconds pre- and post-treatment ($P > 0.5$). The expulsion duration for BET was 30.6 ± 6.5 and 19.3 ± 2.4 seconds pre- and post-treatment ($P > 0.2$). The expulsion duration for Fecobionics was significantly lower than that for BET ($P < 0.01$). The resting pressure for Fecobionics was 17.8 ± 4.9 and 20.4 ± 5.6 cmH_2O pre- and post-treatment ($P > 0.5$). The resting pressure for HARM was 40.9 ± 5.6 and 20.4 ± 5.6 mmHg pre- and post-treatment ($P > 0.5$). The resting anal pressure for Fecobionics was significantly lower than that for BET ($P < 0.01$). The max squeeze pressure for Fecobionics was 70.5 ± 8.9 and 69.3 ± 8.1 cmH_2O pre- and post-treatment ($P > 0.5$). The max squeeze pressure for HARM was 127.9 ± 5.6 and 103.4 ± 11.0 mmHg pre- and post-treatment ($P > 0.4$). The data for Fecobionics were lower than those for BET for the maximum squeeze pressure ($P < 0.01$). The improvement in FISI score correlated better with Fecobionics expulsion duration than with BET expulsion duration (r = 0.69 vs 0.36)

Conclusion: Biofeedback resulted in modest improvement (20%) of the FISI score. Differences were found between HRAM-BET and Fecobionics data. Fecobionics data correlated best with the improvement in FISI score.

249 | Gastric vagal afferent satiety signals are attenuated during pregnancy

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Background: Gastric vagal afferents (GVAs) play an important role in the regulation of food intake. Pregnancy is characterised by increased maternal food intake, in part due to hormonal changes and reduced central satiety signalling. However, despite the fact that appropriate maternal nutrition during pregnancy is essential for normal fetal growth, maximising progeny survival and health, pregnancy related adaptations in GVA function are unknown. We therefore investigated the effect of pregnancy on GVA satiety signalling.

Methods: Female C57BL/6J mice (10-12 week) were mated and randomised to study in early (day 6.5, N = 7), mid (day 12.5, N = 7) or late (day 17.5, N = 9) pregnancy, or used as age matched, non-pregnant controls (N = 7). Mice were singularly housed in Promethion metabolic cages for assessment of food intake. The mechanosensitivity of GVAs was determined using an in vitro single fibre recording preparation with tissue collected at 07:00 h.

Results: Pregnant mice were heavier from day 6.5 ($P < 0.05$) and ate more during the light phase on days 6.5-7.5 and 12.5-15.5 ($P < 0.05$), predominantly due to an increase in meal size. There was no difference in food intake during the dark phase. The response of GVA tension receptors to stretch (0.5-5 g) was significantly reduced in late-pregnancy compared to non-pregnant mice ($P < 0.05$). The response of GVA mucosal receptors to mucosal stroking (10-1000 mg) was unaltered during pregnancy.

Conclusions: In mice, GVA tension receptor signalling is reduced in late pregnancy and associated with increased meal size during the

light phase. These adaptations may play an important role to ensure energy demands are met during pregnancy, enabling healthy fetal growth.

250 | Panometry using esophageal functional luminal imaging probe (FLIP) in Korean achalasia patients who underwent per oral endoscopic myotomy

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Background: Esophageal functional luminal imaging probe (FLIP) panometry has been used to evaluate clinical characteristics of esophageal motor disorders. However, its clinical application and usefulness are not fully validated. We investigated the clinical usefulness of FLIP panometry using a single tertiary center database of achalasia patients who underwent peroral endoscopic myotomy (POEM).

Methods: From October 2017 to December 2018, achalasia patients who underwent FLIP panometry before POEM were enrolled. Follow-up included esophageal manometry and symptoms questionnaires. FLIP data were analyzed after interpolating with the cubic spine method using MATLAB software.

Results: Records of 33 patients (M:F = 17:16, age = 51.09 ± 16.03) were included. Manometric diagnoses were achalasia type I (N = 8), type 2 (N = 19), type 3 (N = 5), and esophagogastric junction (EGJ) outflow obstruction (N = 1). FLIP panometric diagnoses were spastic achalasia (N = 16), achalasia without contractility (N = 5), EGJ outflow obstruction (N = 1), spastic motor disorder (N = 8), and absent contractility (N = 3). Eckardt symptom scores showed significant improvement after POEM (from 5.61 ± 2.61 to 1.52 ± 1.28, $P < 0.01$). Achalasia subtype did not predict the responsibility to POEM; however, patients with spastic motor disorder or absent contractility identified by FLIP panometry showed marginal symptomatic improvement after POEM ($P = 0.04$).

Conclusions: The FLIP provides an alternative and complementary method in HRM for evaluation of esophageal motility disease. The FLIP panometry showed a potential in the prediction of clinical course after POEM. However, It still needs more data to validate its clinical meaning.

Keywords: Achalasia, peroral endoscopic myomectomy(POEM), esophageal FLIP panometry

251 | Anorectal function in patients with incontinence-predominant low anterior resection syndrome

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Background/Objectives: Colorectal Cancer is the commonest cancer diagnosed in Hong Kong. Up to 80% of patients who has undergone low anterior resection (LAR), develop severe bowel dysfunction postoperatively. Many studies report that the majority of patients experience long-term changes in quality of life after LAR. However, the cause of LAR symptoms (LARS) is often multifactorial and difficult to define. We used Fecobionics (CGH 2018, CTG 2019) and high-resolution anorectal manometry (HRAM) and balloon expulsion test (BET) to assess anorectal function post-LAR to understand the condition better.

Methods: Six patients underwent LAR surgery for anorectal cancer (2 F/4 M, age 63.5 ± 4.87) and subsequently had developed LARS with predominant incontinence symptoms. The LARS patients and six normal subjects (2 F/4 M, age 62.3 ± 4.5) were enrolled and assessed with Fecobionics, HRAM and BET.

Results: The LARS score was 34.2 ± 4.5 in the patients. All LARS patients were capable of expelling Fecobionics but 2 LARS patients did not expel BET. All normal subjects were capable of expelling Fecobionics and BET. The Fecobionics pressure signatures differed between the groups. The urge volume for Fecobionics was 19.2 ± 6.8 and 51.7 ± 8.6 mL in LARS patients and normal subjects ($P < 0.05$). The urge volume for BET was 56.2 ± 3.7 and 111.2 ± 13.3 mL in LARS patients and normal subjects ($P < 0.01$). The expulsion duration for Fecobionics was 10.5 ± 3.6 and 18.5 ± 4.5 seconds in LARS patients and normal subjects ($P > 0.5$). The expulsion duration for BET was 107.2 ± 55.7 and 30.2 ± 14.7 seconds in LARS patients and normal subjects ($P > 0.5$). The resting anal pressure for Fecobionics was 13.6 ± 2.0 and 39.9 ± 8.2 cmH_2O in LARS patients and normal subjects ($P < 0.05$). The resting pressure for HRAM was 48.3 ± 6.6 and 60.5 ± 5.7 mmHg in LARS patients and normal subjects ($P > 0.5$). The max squeeze pressure for Fecobionics was 83.4 ± 6.9 and 132.2 ± 12.2 cmH_2O in LARS patients and normal subjects ($P < 0.02$). The max squeeze pressure for HRAM was 241.2 ± 40.2 and 296.3 ± 57.2 mmHg in LARS patients and normal subjects ($P > 0.5$). The correlation coefficient between symptoms and expulsion duration for Fecobionics and BET were 0.53 and 0.42.

Conclusions: The LARS patients showed signatures consistent with fecal incontinence. Fecobionics were closer associated with the symptom score than HRAM-BET.

252 | Functional classification of enteric glial cells of the mouse colon

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Background: Enteric glial cells (EGCs) are established regulators of gastrointestinal (GI) function. EGCs play important roles in the control of motility, secretion and barrier function. However, the precise cellular mechanisms that govern the role of EGCs in GI function are unclear. Current understanding of the EGC population is limited to their location and the expression of standard markers, which does not correlate to their function or reliably distinguish between EGC subtypes. We have used functional expression of receptors to define EGC populations.

Methods: We performed calcium imaging of myenteric wholemounts of the mouse colon. Preparations were then immunostained to identify EGCs.

Results: The TRPV4 agonist GSK1016790A evoked a mix of oscillatory and sustained Ca^{2+} waves in 59 ± 6% of Sox10-expressing type I EGCs (n = 10, 1295 EGCs). Of these, 16 ± 6% showed an immediate response to GSK1016790A (<5 seconds). Spatiotemporal correlation analysis of Ca^{2+} responses indicated highly coordinated activity between EGCs at intercellular distances less than 115 μm. The gap junction inhibitor carbenoxolone altered the Ca^{2+} response profile and EGC network connectivity. Cells that responded within 15 seconds had a significant reduction in the frequency of oscillations and a sustained Ca^{2+} response compared to delayed responders (15% vs 4%, n = 8; 936 EGCs). The Ca^{2+} responses were unaffected by cyclopiazonic acid, tetrodotoxin, and suramin and PPADS. Acute TNBS colitis resulted in a significant reduction in the amplitude and frequency of EGC responses, the percentage that were responsive to GSK1016790A, and to a loss of coordinated activity.

Conclusion: We have identified a functionally distinct population of type I EGCs within the mouse colon that can rapidly respond to TRPV4 activation, leading to a gap junction-dependent activation of the EGC network. Inflammation disrupted the coordinated activity of these EGCs. Our studies indicate that type I EGCs are a functionally diverse population and highlight the need for approaches to better characterise these cellular populations.

253 | Anatomical, neurochemical and functional characterisation of sensory endings in the mouse bladder

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Background/Objectives: The bladder is innervated by spinal afferent neurons with cell bodies in the lumbosacral and thoracolumbar dorsal root ganglia (DRG) which project to the bladder via splanchnic-pelvic and lumbar-hypogastric nerves. The objective of this study was to characterize anatomically, immunohistochemically and electrophysiologically the endings of sensory neurons in the wholemounts of the mouse bladder.

Methods: Both *in vivo* and *ex vivo* anterograde tracing techniques with dextran biotin and biotinamide were used to reveal lumbosacral and thoracolumbar sensory endings in the detrusor and suburothelium. Dextran biotin was injected either into L5-S2 DRG or T13-L3 DRG. Anterograde tracings were combined with immunohistochemical labelling of calcitonin gene-related peptide (CGRP) and other sensory nerve markers, and vesicular acetylcholine transporter (VAChT) and tyrosine hydroxylase (TH). Single unit extracellular recordings were made from fine nerve trunks of the vesical plexus in urothelium-free, flat sheet bladder preparations *ex vivo*.

Results: In both *in vivo* and *ex vivo* anterograde tracings, three major types of lumbosacral afferents, identified as simple, complex and branching types were scattered over different bladder regions. In contrast, thoracolumbar afferent endings appeared to be predominantly confined to the trigone area. The majority of sensory endings identified *in vivo* were immunoreactive for CGRP, lacking markers for VAChT and TH. In biotinamide-filled nerves *ex vivo* both sensory and autonomic nerve fibres/endings were labelled in the muscle layer and suburothelium. In the detrusor, 38% of nerve fibres were immunoreactive for CGRP, 37% for VAChT and 15% for TH. In electrophysiological recordings, low- and high-threshold stretch-sensitive bladder afferents were identified. When recordings were combined with anterograde tracing, branching type of afferent endings was revealed in close proximity to mechanically sensitive hotspots of low-threshold stretch-sensitive afferents.

Conclusions: The data show the complexity of the sensory innervation with three distinctly different morphological endings revealed in the mouse bladder. Preliminary data suggest branching-type endings represent receptive field of low-threshold stretch-sensitive afferents in the detrusor.

255 | Calcium transients underlie electrical slow waves in the stomach

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Background & Aims: Interstitial cells of Cajal (ICC) in the myenteric plexus region (ICC-MY) of the gastric corpus and antrum are pacemakers that generate rhythmic depolarizations known as slow waves. A new mouse, express GCaMP6f, was used investigate the role of Ca^{2+} transients in slow waves.

Methods: Imaging was performed on intact gastric muscles isolated using spinning disk confocal microscopy. Microelectrode recording of transmembrane potential was performed simultaneously.

Key Results: Ca^{2+} waves spread through ICC-MY networks at the frequency of slow waves. Higher resolution showed that Ca^{2+} transients were not uniform across cells, but consisted of localized transients initiated by a sharp and rapid event that traversed the network. ICC-MY displayed dozens of discrete firing sites with highly localized Ca^{2+} transients. These events were organized in clusters lasting 2 seconds or longer. Simultaneous microelectrode recordings during Ca^{2+} imaging, revealed that electrical slow waves occurred in phase with the clusters of localized Ca^{2+} transients. Intramuscular ICC (ICC-IM) were also imaged. These cells also generated clustered Ca^{2+} transients, and initiation of these events lagged slightly behind events in ICC-MY. Ca^{2+} release activates Ca^{2+}-activated-Cl^- channels, encoded by *Ano1*, in ICC. Activation of Ano1 appears to be responsible for the plateau phase of slow waves, the major electrical component regulating excitation-contraction coupling and phasic contractions. Summation of Ca^{2+} transients in ICC-MY and ICC-IM correlated with the duration of slow waves recorded from cells within fields of vision. Thus, the clustered Ca^{2+} transients in ICC and activation of Ano1 channels define the mechanism of the plateau phase of gastric slow waves. The sharp propagating Ca^{2+} transients that initiated the clusters of localized Ca^{2+} events were found to be due to Ca^{2+} entry through voltage-dependent Ca^{2+} channels, and the localized Ca^{2+} transients were due to release from intracellular stores. CPA and thapsigargin both reduced the duration and amplitude of the clustered events. The duration of the clustered events was also reduced by the Orai antagonist GSK-7975A, suggesting that store-operated Ca^{2+} entry maintains the store-dependent source of Ca^{2+}.

Conclusions: Ca^{2+} handling mechanisms provide the mechanisms for generation, propagation and complex waveform features of gastric slow waves.

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256 | Submucosal ICC calcium dynamics underlie mixing behaviors in the colon

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Background and Aims: Interstitial cells of Cajal (ICC) at the submucosal border (ICC-SM) of the circular muscle in the colon are pacemakers, but the unique behaviors of these cells are not understood because previous studies have evaluated their function from integrated responses of smooth muscle cells (SMC), ICC-SM and intramuscular ICC (ICC-IM). Ca^{2+} transients in ICC are coupled to activation of Ca^{2+}-activated Cl^- channels (CaCC). Therefore, patterns of Ca^{2+} transients in ICC impact electrophysiological, contractile and motility events. Ca^{2+} handling behaviors of ICC-SM in colonic muscles were investigated to learn how these events define motor activity.

Methods: Ca^{2+} transients were recorded from ICC-SM in proximal colon muscles of Kit-Cre-GCaMP6f and Kit-Cre-GCaMP6f/Acta-RCaMP1.2 mice. Contractions of muscles from mice and monkey colons were measured in standard organ baths.

Key Results: Ca^{2+} transients in ICC-SM were rhythmic (17-23 cpm) and activity was reduced by L- type Ca^{2+} channel antagonists nicardipine and isradipine. Ca^{2+} transients propagated across the ICC-SM network. Hyperpolarization by pinacidil (K_{ATP} channel agonist) enhanced propagation and increased dependence upon T-type Ca^{2+} channels. Z-944 and NNC-55-0396 (T-type Ca^{2+} channel antagonists) reduced ICC-SM Ca^{2+} transients. Removal of Ca^{2+} from the external solution abolished Ca^{2+} transients. Ca^{2+} transients are known to activate CaCC (encoded by *Ano*1) in ICC. CPA and thapsigargin reduced the duration and amplitude of Ca^{2+} transients. ANO1 antagonists (Ani9, Benzbromarone and CaCCinh) inhibited contractions in murine and monkey colons. ICC-SM can enhance or suppress activity in adjacent SMCs, and simultaneous, dual-color imaging of ICC-SM and SMCs revealed a strong correlation between Ca^{2+} transients in ICC-SM and activation of SMCs adjacent to ICC-SM. ICC-SM Ca^{2+} transients were followed by SMCs Ca^{2+} transients and then muscle displacement occurred. ICC-SM showed no response to ACh or Substance *P* and no inhibition of Ca^{2+} transients to Nonanoate or increase in the presence of L-NNA (nNOS inhibitor) or ODQ (guanylate cyclase inhibitor).

Conclusions: ICC-SM Ca^{2+} handling mechanisms are likely responsible for the timing of excitation-contraction coupling and generation of colonic mixing behaviors in colonic motility. ICC-SM appear to be devoid of neural regulation and just serve to regulate the timing of phasic contractions. (Supported by NIH Grant DK 120759)

257 | A high throughput machine-learning driven analysis of Ca2+ spatio-temporal maps

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Background: High-resolution Ca^{2+} imaging to study cellular Ca^{2+} behaviors has led to the creation of large datasets with a profound need for standardized and accurate analysis. To analyze these datasets, spatio-temporal maps (STMaps) that allow for 2D visualization of Ca^{2+} signals as a function of time and space are often used. Methods of STMap analysis rely on a highly arduous process of user defined segmentation and event-based data retrieval. These methods are often time consuming, lack accuracy, and extremely variable between users.

Aims: Develop an efficient software method to achieve automated, fast and accurately characterize spatio-temporal Ca^{2+} activity patterns

Methods: The code for The Plugin was written in Java release version: 9. The plugin incorporates Fiji Application Program Interfaces (APIs) provided by the National Institutes of Health, U.S. Department of Health and Human Services.

Key Results: We designed an automated machine-learning based plugin for the analysis of Ca^{2+} STMaps (STMapAuto). The plugin includes optimized tools for Ca^{2+} signal preprocessing, automated segmentation, and automated extraction of key Ca^{2+} event information such as: duration, spatial spread, frequency, propagation angle, and intensity in a variety of cell types including the Interstitial cells of Cajal (ICC). The plugin is fully implemented in Fiji and able to accurately detect and expeditiously quantify Ca^{2+} transient parameters from ICC. The plugin's speed of analysis of large-datasets was 200-fold faster than the commonly used single pixel-line method of analysis.

Conclusions: The automated machine-learning based plugin described dramatically reduces opportunities for user error and provides a consistent method to allow high-throughput analysis of STMap datasets. (Supported by NIH grant R01 DK-120759).

258 | Depolarizing synapses can be inhibitory in enteric nervous system: Computational modelling

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Background/Objectives: The gut's enteric nervous system contains many synapses mediating various synaptic potentials including fast excitatory post-synaptic potentials (fEPSPs) and slow EPSPs (sEPSPs). Several neurotransmitters including acetylcholine, γ-aminobutyric acid (GABA), 5-HT, and ATP contribute to these synaptic potentials, but how their interactions generate post-synaptic action potentials has not been quantitatively explored. We addressed this using computational simulation, testing predictions using Ca^{2+} imaging.

Methods: A computational model of a network of enteric neurons was constructed using conductance-based neuron models with various voltage-dependent currents including Nav1.3, Nav1.7, Kv7.2, delayed rectifier K current and A-type K current. Neurons are connected through sEPSP and fEPSP synapses. sEPSPs are modelled based on activation, inactivation and summation of cAMP and protein kinase A-dependent second messenger cascades. Interactions of fEPSPs and responses to GABA acting on $GABA_C$ receptors were also analysed. Ca^{2+} imaging used myenteric plexus preparations from *Wnt1*-Cre;R26R-*GCaMP6f* mice (either sex), which express a calcium indicator in all enteric neurons. Single focal electrical stimuli applied to local nerve trunks excited fEPSPs and trains of 20 pulses (20 Hz) excited sEPSPs. We compared Ca^{2+} transients evoked by a single pulse before a train with those 10 and 20 seconds after the train.

Results: The model predicted that sEPSPs increase postsynaptic firing initially, but fEPSPs evoked during sEPSPs have reduced amplitude and no longer trigger action potentials. Inhibitory effects of sEPSPs were confirmed for some neurons in Ca^{2+} imaging experiments that showed a marked reduction in excitation produced by a single pulse stimulus in many neurons, excitability of other neurons was increased. The model also showed that activation of $GABA_C$ receptors depolarizes enteric neurons, but inhibits excitation produced by fEPSPs.

Conclusions: Interactions between synaptic events in enteric neurons are more complex than simple summation of membrane potentials with some synaptic depolarizations inhibiting firing produced by conventional fEPSPs. The results highlight the value of computational models for understanding neural information processing.

259 | TRPV4 is expressed by lymphatic endothelial cells and promotes histaminergic signaling

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Background: Colonic lymphatics are essential for fluid homeostasis, immune surveillance, and removal of cellular and metabolic waste. Emerging evidence implicates a role for the lymphatic system in intestinal inflammation. Extensive and sustained lymphangiogenesis is associated with inflammatory bowel disease, and disruption of lymphatic flow leads to inflammation. The initial endings of lymphatic vessels are closely associated with myenteric ganglia. Therefore, changes to lymphatic function may significantly influence

the chemical and mechanical microenvironment in which the enteric nervous system is located, potentially impacting gastrointestinal function. In this study, we report that the stretch-sensitive ion channel TRPV4 is functionally expressed by lymphatic vessels of the gastrointestinal tract. We demonstrate that TRPV4 is a major driver of GPCR-dependent signaling in lymphatic endothelial cells (LECs).

Methods: Lymphatic architecture of the mouse colon was examined by immunofluorescence. Calcium signaling was examined in primary human LECs and in wholemounts of the mouse and monkey colon. Activation of the transcription factor NFATc1 was quantified by microscopy. Changes associated with acute DSS-induced colitis were defined in the mouse colon.

Results: Acute inflammation was associated with greater lymphatic complexity, extensive sprouting from blind endings towards macrophages within the myenteric region, and with increased lymphatic density in the mucosa. LYVE-1 positive initial lymphatics of the mouse and monkey colon responded to the TRPV4 activator GSK1016790A in a TRPV4-dependent manner. TRPV4-mediated signaling was suppressed in LECs of the acutely inflamed mouse colon. The potency and efficacy of histaminergic signaling in primary LECs was enhanced by TRPV4. Exposure to histamine receptor agonists sensitized subsequent responses by LECs to GSK1016790A. Histamine receptor agonists stimulated translocation of the transcription factor NFATc1 to the nucleus in a TRPV4-dependent manner.

Conclusion: We have demonstrated that extensive remodeling of the initial lymphatic network of the myenteric region occurs in colitis. TRPV4 is expressed by LECs, where it contributes to histaminergic signaling and transcriptional regulation and may influence histamine-dependent effects on lymphatic function.

260 | Clinical response of intra-pyloric botulinum toxin injection and pyloric dilation for children and adolescents with gastroparesis

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Background: Current medical and dietary treatments for gastroparesis in children are sub-optimal, with response rates as low as 22%. While pyloric botulinum (botox) injection has been reported to be effective for the treatment of pediatric gastroparesis, there are no studies evaluating the effect of combined pyloric injection and balloon dilation. At our center, we have performed endoscopic pyloric botox injection with balloon dilation in children with gastroparesis in the past ten years. This involves injecting 100 Units of diluted botulinum toxin divided in 4 quadrants of the pyloric muscle followed by CRE balloon dilation up to 20 mm Hg for 2 minutes.

Aim: To assess clinical response to pyloric botox injection and balloon dilation.

Methods: We reviewed the CCHMC EMR database to gather demographics, symptoms, diagnoses gastric emptying information and follow up up to 12 months. Cases were patients with gastroparesis who had pyloric botulinum injection and pyloric balloon dilation in addition to medical management. Controls were patients with gastroparesis who were on medically management alone. Symptomatic response was defined as having no change, partial or complete improvement. In order to eliminate confounding effect of other variables, we applied propensity score matching to pair cases and controls based on age, gender and symptom duration.

Results: Seventy-nine cases and 83 controls were identified. After a 10% propensity caliper matching, 63 patients were included in each group. Before matching, 61 (78%) cases showed partial or complete improvement compared to 42 (51%) controls (P = 0.001, Figure 1). After propensity matching, 47 (76%) cases showed partial or complete improvement compared to 31 (49%) controls (P = 0.004).

Conclusion: In our propensity matched analysis, combined intra-pyloric botulinum injection and pyloric dilation in addition to medical management clearly showed a benefit over medical management alone in children and adolescents with gastroparesis.

261 | 3D-high definition anorectal manometry findings in a Chilean cohort of symptomatic subjects

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Background: 3D- high definition anorectal manometry may aid the diagnosis of functional anorectal disorders, such as chronic constipation and fecal incontinence. We aimed to report a descriptive epidemiologic study of 79 3D- high definition anorectal manometry of symptomatic patients, performed between 2016-2018, according to the protocol proposed by the European Society of Neurogastroenterology and Motility.

Methods: Symptomatic subject were stratified by age and sex. Those with fecal incontinence or chronic constipation were included according to anorectal symptom questionnaires (Wexner and KESS, respectively).

Results: A total of 79 subjects (86% female, 14% male, mean age 44 years) were included. In the group of subjects with chronic constipation, 11% of anorectal manometries were normal, 18% had dyssynergic patterns, 15% presented anal hypertension, 8% anal hypercontractility and 48% other disorders. In the group of subjects with fecal incontinence, only 1% of anorectal manometries were normal, 10% had dyssynergic patterns, 16% presented anal hypotension, 11% anal hypocontractility and 62% combined anal hypotension with hypocontractility.

Conclusions: The most common manometric finding in chronic constipated subjects was dyssynergic defecation. In fecal incontinence, the majority of patients had alterations on internal and/or external anal sphincter pressures (anal hypotension and/or anal

hypocontractility) and only 1% had normal anorectal manometry. This is the first Chilean cohort of 3D high definition anorectal manometry findings.

262 | 5-HT3 receptors mediate fast excitatory synaptic potentials in excitatory motor neurons and NOS+ neurons in mouse proximal colon

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Background: The role of serotonin (5-HT) in the control of gastrointestinal motility is still being debated. We investigated the role of 5-HT in synaptic transmission within the mouse proximal colon myenteric plexus using intracellular recording correlated with morphology and Ca imaging of neural activity, focusing on 5-HT_3 receptors.
Methods: Proximal colon myenteric neurons (C57B6 male mice) were impaled with microelectrodes containing biocytin for *post hoc* morphological analysis. Single electrical stimuli were applied to intermodal strands evoking fast excitatory synaptic potentials (fEPSPs). Effects of blocking nicotinic receptors (hexamethonium, 200 μM) and then 5-HT_3 receptors (ondansetron, 3 μM) were examined. Ca^{2+} imaging used myenteric preparations from *Wnt1*-Cre;R26R-*GCaMP6f* mice which express a calcium indicator in all enteric neurons. Single electrical stimuli were applied to internodal strands before and during exposure to ondansetron. Preparations were fixed and processed to reveal nNOS.
Results: Hexamethonium abolished fEPSPs in 9 of 22 neurons and depressed them in 13 neurons, ondansetron with hexamethonium reduced (n = 7) or abolished (n = 2) fEPSPs in 9 neurons (control amplitude 35 ± 6 mV, hex 8 ± 1 mV, hex + ond 3 ± 1 mV, $P = 0.001$). Ondansetron alone reduced fEPSP amplitude by >25% in 2 of 2 neurons. Four neurons with ondansetron-sensitive fEPSPs projected orally with terminals in circular muscle. 401 neurons were examined via Ca^{2+} imaging; significantly more single pulse-evoked responses were abolished by ondansetron than time controls (control: 5/85, ondansetron: 18/101, $P = 0.01$). Ondansetron depressed the overall amplitude of single pulse-evoked Ca^{2+} transients ($\%\Delta F_i/F_0$ control: 101 ± 5% n = 85; ondansetron: 75 ± 6% n = 101, $P = 0.0007$), some affected neurons were NOS+.
Conclusions: Most proximal colon myenteric neurons exhibit fEPSPs mediated by acetylcholine acting at nicotinic receptors. However, neurally released 5-HT can mediate fEPSPs via 5-HT_3 receptors in some excitatory motor neurons and NOS+ neurons confirming that neurally released 5-HT can regulate colonic motility.

263 | Apurinic/apyrimidinic endonuclease 1/redox factor-1 as a novel therapeutic target for inflammatory bowel disease

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Inflammatory bowel disease (IBD) has been associated with oxidative stress and inflammation induced damage to the enteric nervous system (ENS) leading to GI dysfunction. Apurinic/Apyrimidinic Endonuclease 1/Redox Factor-1 (APE1/Ref-1) is a vital dual functioning protein that is an essential regulator of cellular oxidative stress. Increased expression of APE1/Ref-1 in colon sections from IBD patients is associated with an enhanced pro-inflammatory response. To characterise the role of APE1/Ref-1 in enteric neuropathy in IBD patients and the effects of an APE1/Ref-1 redox inhibitor, APX3330, in the *Winnie* murine model of spontaneous chronic colitis, *Winnie* mice received APX3330 over a two-week treatment regimen. Data obtained were compared to sham-treated *Winnie* and C57BL/6 control mice. *In vivo* GI transit and *ex vivo* organ-bath colonic motility was completed to assess changes to GI functions. Concluding the treatment mice were sacrificed and colon tissues were collected for immunohistochemical and protein analysis. Neuronal markers, APE1, DNA damage, mitochondrial superoxide production, high mobility group box 1, were examined in both animal models and human IBD and control colon biopsies. APX3330 demonstrated neuroprotective effects, improved clinical symptoms, reduced immune cell infiltration, alleviated oxidative stress and ameliorated GI dysfunction in the *Winnie* mice. The study provided novel insight to the expression of APE1 in human enteric neurons. Furthermore, animal models used in the study displayed similar protein changes and expression to the human IBD patients. These studies are the first to investigate the application of APX3330 for the treatment of chronic colitis. Targeting APE1/Ref-1 redox pathway in a clinically relevant model may lead to the use of APX3330 in a clinical setting as an effective IBD therapy.

265 | Altered mucosal structure and barrier function in the neuroligin-3R451C mouse model of autism

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Background: Gastrointestinal (GI) disorders are an important co-morbidity in autism patients. GI dysfunction is reported in autism patients expressing a point mutation (R451C) in the Nlgn3 gene encoding the Neuroligin-3 (NL3) synaptic adhesion protein. Although Nlgn3 expression is well characterised in the brain, this has not been examined in the gut. The intestinal mucosal barrier separates the external environment from the interior of the body and when compromised, the translocation of antigenic materials into the body can cause GI disorders.

Methods: We first evaluated mucosal barrier function in the ileum by assessing transepithelial resistance, paracellular permeability and mucosal secretion using an Ussing chamber. We then developed a dual immunofluorescence and RNAscope *in situ* hybridization-based method to localise Nlgn3 mRNA in the ileal mucosa. An RT-PCR array of 84 tight junction protein genes was performed to evaluate mucosal epithelial structure. To investigate whether the R451C mutation affects submucosal neuronal populations, neurons expressing Choline Acetyltransferase (ChaT) and Vasoactive intestinal peptide (VIP) were quantified using immunofluorescence in WT and $NL3^{R451C}$ mice.

Results: We observed increased intestinal permeability ($P = 0.001$) and decreased transepithelial resistance in $NL3^{R451C}$ (n = 8) ileal tissue compared to controls (n = 8; $P < 0.0001$). We also found that mucosal secretion was decreased in $NL3^{R451C}$ mice through a nicotinic receptor-mediated pathway (n = 4 WT, n = 4 $NL3^{R451C}$ mice, $P = 0.01$). In the submucosal plexus, $NL3^{R451C}$ mice had more VIP-positive neurons (WT (n = 4), 54.3 ± 1.5%, NL3 (n = 6) 61.9 ± 0.9%; $P = 0.0029$) and fewer ChaT-positive neurons (WT (n = 4), 45.2 ± 1.5%, NL3 (n = 6) 38.3 ± 1.2% $P = 0.012$) compared to WT.

Conclusions: In the ileum, Nlgn3 mRNA is predominantly expressed in submucosal neurons, glia and in PYY, GLP-1 and 5-HT-containing epithelial cells. These findings reveal that Nlgn3 is expressed in mucosal neural circuitry and regulates neuronal numbers and mucosal barrier function in mice.

266 | Gas insufflation in the sigmoid colon induces localised contractile activity without a flatal urge

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Background: While computed tomography imaging has demonstrated that gas accumulates in the sigmoid colon after a meal(1), the colonic motility patterns responsible for gas transit are largely unknown. Using gas insufflation and impedance-manometry, our aim was to characterise the relationships between intraluminal gas and colonic motility.

Methods: After an overnight fast and bowel preparation, a high-resolution, solid-state impedance-manometry catheter (32 circumferential pressure sensors, 10 mm spacing; 16 impedance channels, 20 mm spacing; Sandhill, USA) was positioned under colonoscopic guidance. Sensors were positioned in the descending colon, sigmoid colon, and rectum. After a 1 hour control period, room air was infused in the sigmoid colon via a 1.67 mm diameter tube (30 mL/2 minutes; 3 × 20 minute intervals). Impedance, pressure, and symptoms were recorded. Abdominal x-ray was performed to confirm catheter position.

Results: Nine healthy adults participated in the study (n = 7 women; median age 56, range 21-65 years). Gas insufflation induced increased intraluminal phasic pressure activity (mean change = 2.1× increase, 95% CI 1.8-2.4, $P < 0.0001$) and increased impedance (mean change = 2.1× increase, 95% CI 1.9-2.3, $P < 0.0001$). This included a 12% increase in 1 minute time epochs occupied by the cyclic motor pattern (2-8 cpm). Phasic activity was induced at a mean 63 ± 75 seconds post commencement of gas insufflation (Figure 1). During insufflation, the participants were asymptomatic and experienced no flatal urge. While gas pooled in the sigmoid colon, the motor patterns were identified immediately proximal, distal, or co-located with gas, with no significant correlation between the location of gas pooling and phasic activity ($R^2 = 0.19$, $P = 0.26$).

Conclusions: Gas insufflation in the sigmoid colon of healthy adults induces an increase in localised contractile activity, without a flatal urge. These findings may support the hypothesis that rectosigmoid motility can mediate rectal filling.

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268 | Expression and function of GABA in the enteric circuitry of mouse jejunum

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Background: The role of the enteric nervous system (ENS) in antibiotic-associated diarrhoea (AAD) is largely unexplored, despite extensive evidence demonstrating that disturbed ENS function underlies pathogenic hypersecretion. Microbially-produced neurotransmitters, notably γ-Aminobutyric Acid (GABA), alter susceptibility to AAD. The jejunum is the primary site of neurogenic secretion, but little is known about the expression and function of GABA in mouse jejunum. This study examined both.
Methods: Immunofluorescence was used to examine expression of neuronal subtypes in mouse jejunum. We employed Wnt1-Cre;R26R-GCaMP6f mice, expressing a fluorescent calcium indicator in enteric neurons and glia, for Ca^{2+}-imaging.
Results: Overall, antibodies to neuronal markers present in the ENS-calretinin, calbindin, choline acetyltransferase (ChAT), Vasoactive intestinal peptide (VIP), neuronal nitric oxide synthase (nNOS) and GABA, revealed immunoreactive (IR) cell bodies in the myenteric (MP) and submucosal plexus (SMP). 8% of all myenteric neurons were identified as GABA-IR; some co-expressed calretinin (1 ± 0.5%) and nNOS (2 ± 1%) (n = 5 animals). GABA-IR terminals were identified surrounding Calr-IR and nNOS-IR neurons within myenteric ganglia and in muscle. In the SMP, 17 ± 2% of all neurons expressed GABA (n = 3 animals); most were also IR for calretinin (96 ± 4%). GABA-IR fibres were observed within submucosal ganglia. Submucosal neurons (n = 59) were examined via Ca^{2+}-imaging where bicuculline reduced the overall amplitude of single pulse-evoked Ca^{2+} transients (1P $\%\Delta F_i/F_0$ control: 103 ± 9% n = 18; $\%\ \Delta F_i/F_0$ bic: 45 ± 10% n = 15, P = 0.0001). Ca^{2+} transients evoked by 20 pulse 20 Hz trains were unaffected by bicuculline (20 pulse $\%\Delta F_i/F_0$ control: 109 ± 5% n = 28; $\%\Delta F_i/F_0$ bic: 116 ± 9% n = 31, P = 0.49)
Conclusions: We conclude that GABA is present in enteric neurons of the mouse jejunum and electrically-evoked release of endogenous GABA acts at $GABA_A$ receptors in the SMP to mediate fast synaptic transmission.

269 | Gut-derived glucagon regulates colonic motility

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Background/Objectives: Glucagon is a classical fasting hormone released by pancreatic α cells in response to hypoglycaemia. For decades, it was believed the endocrine pancreas is the body's only source of glucagon. However, contrasting the other islet hormone insulin, which is completely absent in patients that had undergone total pancreatectomy, glucagon remains detectable in these patients. As there is evidence suggesting colonic sensory neurons within the ENS express glucagon receptor (GCGR), we hypothesized the colon epithelium is a source of glucagon and is implicated in the regulation of intestinal motility.
Methods: An ex vivo preparation of human mucosa for secretion assays was developed from surgically resected human colon sections. 15 minute static incubations of the preparations in Krebs buffer with or without different glucagon secretagogues were performed. Glucagon content in the secretion supernatants were assayed using a validated sandwich ELISA. An *ex vivo* mouse colon preparation was used to investigate the effects of glucagon on colonic motility.
Results/Conclusions: Glucagon released from human colonic epithelia (N = 8) was detectable at basal and was reliably triggered by IBMX/FSK, high potassium and arginine. However, glucagon was not detectable in any of the colonic preparations that were collected from specimens proximal to the sigmoid colon. While 100 nM of glucagon exerted no significant effects on the frequency or amplitude of colonic migrating motor complexes (CMMCs) in the *ex vivo* mouse colon preparation, the selective GCGR antagonist (Skyrin, 50 μM) significantly reduced the frequency, but not the amplitude of CMMCs (N = 10). These results support the notion that the colonic epithelium is a source of glucagon that it has a regulatory role in ENS function underlying colonic motility.

270 | Simultaneous whole-cell patch-clamp and calcium imaging on cultured mouse myenteric neurons

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Background: Live Ca^{2+} imaging is a proxy for electrophysiological measurements and a valuable tool to analyze activity in multiple cells simultaneously. In the enteric nervous system (ENS), two main electrophysiological classes of neurons exist (AH and S). Although they

have different Ca^{2+} handling mechanisms, they are rarely considered separately in Ca^{2+} imaging studies.

Aim: To investigate whether the characteristics of a $[Ca^{2+}]_i$ transient reflect the electrophysiological differences between murine AH and S neurons.

Methods: Primary ENS cultures were made from adult Wnt1-Cre;R26R-GCaMP6f mice. After 4-5 days in vitro, simultaneous Ca^{2+} imaging and patch-clamp recordings were performed. Cells were depolarized by either 10 or 500 ms current pulses or high K^+ (75 mM). DMPP (10 μM), a nicotinic receptor agonist, was used to mimic fast cholinergic transmission.

Results: We recorded from 40 neurons, classed as AH (16) and S (24) based on the presence or absence of an "inflection" in the action potential's (AP) repolarizing phase. Unlike previous studies performed in guinea pig, S neurons exhibited a prominent $[Ca^{2+}]_i$ transient accompanying a single AP. In response to a 10 ms depolarization pulse, both the AP and $[Ca^{2+}]_i$ transient amplitudes were significantly larger in AH neurons. However, with a 500 ms depolarization pulse or high K^+, no significant difference persisted. Interestingly, when tetrodotoxin (1 μM) was applied to block APs, a reduced but distinct $[Ca^{2+}]_i$ transient remained following the 500 ms depolarization pulse. In 10/12 AH neurons, DMPP did not elicit a membrane potential change or a $[Ca^{2+}]_i$ transient. In 14/16 S neurons, DMPP triggered both a $[Ca^{2+}]_i$ transient and either an AP or sub-threshold membrane potential change.

Conclusions: $[Ca^{2+}]_i$ transients were found to accompany single APs in both AH and S neurons. Although sub-threshold membrane depolarizations could also elicit $[Ca^{2+}]_i$ transients, these were generally amplified if an AP was present. The $[Ca^{2+}]_i$ response to DMPP was the most reliable way to optically distinguish between AH and S neurons.

272 | Functional and regional heterogeneity of glial cells in the enteric nervous system

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Enteric glia is a unique type of peripheral neuroglia localized in the intestine that controls homeostasis in the enteric nervous system. Several different functions have been assigned to enteric glia, but whether these are performed by specialized subtypes with a distinctive phenotype and function remains unknown. We tested the hypothesis that enteric glia displays functional heterogeneity in different gut regions and between individual cells that is reflected by differential responsiveness to neuromodulators. Functional responses to the neuromodulator cholecystokinin (CCK, 100 nM) and the glial agonist adenosine diphosphate (ADP, 100 μM) were assessed in enteric glia by *in situ* Ca^{2+} imaging in whole-mount preparations of myenteric plexus from *Sox10*^{CreERT2};*Polr2a*$^{Tn(pb\text{-}CAG\text{-}GCaMP5\ g,\text{-}tdTomato)Tvrd}$ mice that express the optogenetic reporter GCaMP5 g in enteric glial cells. Comparable percentages of glia responded to ADP and CCK both in the duodenum (63.2% and 50.06%, respectively; n = 212 glial cells) and colon (90.8% and 79.5%, respectively; n = 174 glial cells), but more glia responded to both neuromodulators in the colon than in the duodenum. This is related with higher peak Ca^{2+} responses to ADP and CCK in the colon than duodenum, although CCK resulted in a greater response in the duodenum than in the colon when it is compared to ADP response peak (21.07% and 38.4% of ADP-induced response in the colon and the duodenum). Interestingly, blocking neuronal activity with tetrodotoxin (300 nM) increased the percentage of responding glia (+14.94% in the duodenum vs +2.6% in the colon) and potentiated glial responses to CCK in the duodenum, but not in the colon (+7.73% and +53.2% of ADP-induced response in the colon and the duodenum). Profiles of glial responses reveal eight functional categories of glial responses that can be distinguished by their differential response to ADP and CCK, before and after tetrodotoxin treatment. These data show that enteric glial responses to ADP and CCK differ between the duodenum and colon and that responses differ among glial cells within the same gut region. These results suggest an extensive heterogeneity of enteric glia and a possible region-specific mechanism to locally modulate gut functions.

273 | Can standardized testing protocol for anorectal manometry by international anorectal physiology working group be applied for a low-volume hospital?

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Background/Objectives: Results from one hundred and seven complete responses from 30 countries have revealed that significant discrepancy was in methods for data acquisition, analysis, and interpretation of anorectal manometry (ARM). The international anorectal physiology working group (IAPWG) introduces the standardized testing protocol of high-resolution anorectal manometry (HR-ARM) by a combined consensus approach. This study was to investigate the availability of this protocol by IAPWG at low-volume centers (performing ≤2 studies per week).

Methods: A retrospective analysis of HR-ARM reports from Sep 2016 to Oct 2019 at the Dongguk University Ilsan Hospital was performed. 55 cases were included (48 cases of fecal incontinence and 7 cases of dyssynergia). The values and elapsed time from 3 times of squeeze/push by suggested protocol and additional trials were compared by Paired-t test.

Results: Male to female ratio was 21-34. Mean age was 62.7 ± 16.8 years. Mean trial numbers of resting, squeezing, push and RAIR were 1.5, 5.5, 5.5 and 4.9, respectively. Mean overall test time was 22.8 ± 8.7 minutes. The application of protocol by IAPWG

saved 4.7 ± 2.6 minutes ($P < 0.01$). Moreover, maximal squeeze pressure (152.9 ± 61.1 mmHg vs. 153.4 ± 63.5 mmHg, $P = 0.87$), maximal squeeze pressure increase (59.9 ± 33.0 mmHg vs. 62.9 ± 38.5 mmHg, $P = 0.22$), squeeze duration (6.4 ± 1.8 seconds vs. 6.3 ± 2.2 seconds, $P = 0.68$), push residual pressure (42.4 ± 18.0 mmHg vs. 41.0 ± 17.2 mmHg, $P = 0.13$), Mean rectal expulsive pressure (39.1 ± 20.0 mmHg vs. 38.1 ± 19.8 mmHg, $P = 0.32$), and mean rectal expulsive pressure increment (3.4 ± 9.5 mmHg vs. 4.7 ± 11.8 mmHg, $P = 0.23$) were not respectively significant.

Conclusions: Despite the testing protocol for HR-ARM by IAPWG may come from the experts of high-volume centers, it could be applicable to low-volume center. 3 times measurement of squeeze and push by protocol were reasonable and additional trials did not affect the results.

274 | The defecation habits of people in Central China: A cross-sectional survey and a literature review

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Background/Objectives: Our current understanding of normal defecation habits in China is limited. Available data are collected from specific groups but not from the general population, making it difficult to characterize normal bowel patterns of Chinese. The current study aims to (1) to evaluate the bowel movements and stool form among health population in Central China, (2) to review the differences of stool habits between people in Central China and other countries.

Methods: A cross-sectional study, using a reliable and valid questionnaire was carried out in unselected subjects aged 16 years and above in Wuhan City, China, and only included people with normal bowel patterns (N = 1286). Data regarding defecation frequency; stool form (using the Bristol Stool Form Scale); recognition of abnormal stool forms; and demographic factors were analyzed. Published data from other countries were also used to conclude the characteristics of Chinese bowel habits.

Results: 81.2% of subjects reported once daily habits and 97.9% of subjects reported between 4 and 14 times per week. 83.9% of subjects claimed Bristol type IV as predominant type and 94.5% of subjects claimed type III to V. the elderly were found to have a less and harder stool than young people ($P<0.001$) but their bowel habits were still within normal range. The distribution of Central Chinese defecation frequency and stool forms were both more concentrated than other countries.

Conclusions: Healthy Chinese passed stool once per day and the predominant form was Bristol type IV. The distribution of Chinese defecation frequency and stool forms were both very concentrated. Thus, we suggest to establish a stricter definition of "healthy stool habits" for Chinese while different criteria for different ages or genders were unnecessary. Finally, this study provided a solid basis for further understanding of Chinese defecation.

275 | Changes in conduit emptying and function following oesophageal reconstruction do not mediate improvements in appetite and gastro-intestinal quality of life

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Background: Conduit function and emptying appear post-oesophagectomy are postulated to be critical mediators of recovery of quality of life and nutritional status. Recovery of conduit contractility has been proposed to be essential for emptying. In contrast, our previous data have proposed a paradigm of gravity mediated drainage as a central conduit clearance mechanism. We hypothesised that the recovery of conduit motility and propulsive function over time correlate with improvements in gastrointestinal symptoms and quality of life.

Methods: We recruited disease-free post-oesophagectomy (gastric conduit) patients in this prospective longitudinal study. We undertook a protocolised nuclear scintigraphy imaging (90 minutes dynamic images in supine position and a static image captured after a further 15 minutes mobilisation) assessing intra-conduit transit and emptying along with SF-36 quality of life assessment and structured symptom scores at 6 months, 12 months and 24 months post-operatively. Cross sectional analysis at distributed time points (non-sequential) analysis was performed using intra-conduit manometric measures and real time concurrent fluoroscopy was also undertaken.

Results: Twenty-eight patients were recruited: Mean age 66.0 ± 9.5 years, 79% male, pre-operative BMI 27.6 ± 6.4 kg/m^2. Reflux score and physical component score (SF-36) improved over time improved ($P = 0.02$ and $P = 0.03$). Median conduit emptying half time were prolonged but did not differ significantly (266.0 (IQR 360.25) minutes at 6 months vs 205.5 (IQR 782.8) minutes at 12 months vs 237.5 (IQR 549.3) minutes at 24 months, $P = 0.83$). Significant gravity-mediated emptying observed and appeared unchanged over time (percentage of the overall content excited at 15 minutes upright; 50.5% at 6 months vs. 49% at 12 months vs. 45.9% at 24 months, $P = 0.851$). No difference in conduit peristaltic contractions or correlation with emptying events was

observed on dynamic fluoroscopy and manometry in patients at different time points.

Conclusion: Markedly delayed transit was observed post-oesophagectomy with reliance on gravity mediated drainage. This did not appear to improve over time. Improvement in gastrointestinal symptoms and quality of life after 12 months appeared to occur despite unchanged conduit motility and emptying.

276 | A novel surgical technique to investigate the topographical distribution of thoracolumbar and lumbosacral spinal innervation to the mouse uterus

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Background: Pelvic pain in women can manifest from a range of different pelvic conditions, including those associated with the uterus. Pain-generating stimuli are detected by the uterus via sensory nerves that project from the thoracolumbar and lumbosacral spinal cord. However, the anatomical distribution and extent to which these spinal afferent pathways innervate the uterus is currently unknown.

Methods: For the first time, unilateral transections of dorsal root ganglia (DRG) at thoracolumbar T13-L1 (n = 5) and bilateral lumbosacral L5-S1 (n = 5) spinal cord were performed *in vivo* on adult female C57BL/6 mice. The contralateral uterine horn and sham surgery animals (n = 4) served as controls, respectively. From 9 days post-procedure, uteri were processed immunohistochemically for expression of the nociceptive neuropeptide, calcitonin gene-related peptide (CGRP). Tissues were then imaged, and the density of CGRP immunoreactivity quantified.

Results: Mean expression of CGRP in uterine horns ipsilateral to the T13-L1 DRG transection [1.8 ± 0.9% SD] was reduced compared to contralateral tissues [3.8 ± 1.2%]. This represented a ~52%, 54% and 38% depletion in CGRP-immunoreactivity in the oviduct, mid, and body regions, respectively. Mean CGRP expression in uteri from animals subjected to bilateral L5-S1 DRG transection [1.9 ± 0.5% SD] was also reduced compared to sham [2.8 ± 0.5%]. Regionally, CGRP-immunoreactivity was depleted by ~28% (oviduct), 37% (mid) and 32% (body).

Conclusions: Thoracolumbar spinal afferent nerves provide greater innervation to the mouse uterus than lumbosacral spinal afferents. Moreover, a topographical arrangement of spinal afferents exists within the uterus, where thoracolumbar inputs typically innervate the oviduct-mid region, while most lumbosacral nerves are distributed in the uterine mid-body. These data indicate that pain signals from the uterus may be transmitted via distinct populations of spinal afferents depending on the stimulus location.

277 | The magnitude of acceleration of gastric emptying in acute hypoglycaemia in health is dependent on the level of hypoglycaemia

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Background: The rate of gastric emptying (GE) of carbohydrate is a major determinant of postprandial glycaemia. 'Marked' hypoglycaemia (~2.6 mmol/L) accelerates GE substantially in both health and Type 1 diabetes likely representing an important counter-regulatory mechanism increasing the rate of carbohydrate absorption. There is no information about the effect of 'mild' hypoglycaemia (between 3.0-3.9 mmol/L) on GE, which is more common in clinical practice.

Materials and Methods: 14 healthy males (mean age: 33.9 ± 1.0 years; BMI 24.6 ± 7.0 kg/m^2; HbA1c: 5.3 ± 0.6%) underwent concurrent measurements of GE of a solid meal (100 g ^{99m}Tc radiolabelled minced beef) by scintigraphy and arterialised venous blood glucose by glucose oxidase analyser on 3 separate occasions during 'mild' and 'marked' hypoglycaemia, and 'euglycaemia'. Hypoglycaemia (~2.6 or ~3.6 mmol/L), established using a glucose-insulin clamp at t = −15 minutes, was maintained for 60 minutes (t = 60 minutes) after meal consumption. GE (Over 120 minutes) was expressed as % retention over time. Data are shown as mean ± SEM (Figure). A maximum-likelihood mixed effects model was used to estimate the difference in area under the curve (AUC) for gastric retention. *P*-value <0.05 was considered significant.

Results: There was an overall effect of glycaemia on gastric retention, reflected by $AUC_{0\text{-}120\ min}$ ($P < 0.001$). Pairwise comparisons showed GE was significantly faster during both mild ($P = 0.011$) and marked ($P = 0001$) hypoglycaemia when compared to euglycaemia and faster during marked when compared to mild hypoglycaemia ($P = 0.008$). Gastric retention at 100 minutes at 6 mmol/L positively correlated to magnitude of change in emptying at 3.6 mmol/L ($r = 0.57$, $P = 0.030$) and 2.6 mmol/L ($r = 0.76$, $P = 0.0014$).

Conclusion: The effect of hypoglycaemia to accelerate GE is dependent on the degree of hypoglycaemia in health. Hypoglycemia-induced acceleration in gastric emptying is related to the gastric emptying rate during euglycaemia.

278 | The structure of vagal nociceptive nerve fibers in the mouse esophagus

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The vagus nerves provide afferent nerve fibers with nociceptive sensory properties to the esophagus. However, it is unknown which esophageal layers are innervated by these vagal nociceptive fibers. We hypothesized that the vagal nociceptive TRPV1-positive afferent fibers innervate esophageal mucosa. We evaluated our hypothesis by using genetic neuronal tracing in several mutant mice including two new strains we generated. The wholemount preparations of separated esophageal mucosa and muscle were immunostained and imaged by spinning-disk confocal microscopy. In the TRPV1-Flp/RC:FLTG mice (N = 8) in which TRPV1-expressing cells express tdTomato the TRPV1+ fibers with varicose appearance densely innervated both the mucosa and the muscle. In the mucosa the TRPV1+ fibers were located just beneath the epithelium, run in parallel registers of two or more fibers, had craniocaudal orientation and branched repeatedly. In the muscle the TRPV1+ fibers formed a dense network in the myenteric plexus innervating virtually all myenteric ganglia forming intraganglionic varicose endings (IGVEs). Many TRPV1+ fibers connecting IGVEs run in parallel to the fibers of outer and inner striated muscle. In the TRPV1-Flp/Tac1-Cre/RC:FLTG mice (N = 4) in which GFP is selectively expressed in cells positive for both TRPV1 and the marker of esophageal nociceptors derived from neural crest Tac1, the esophageal innervation by TRPV1+Tac1+ fibers mimicked the dense innervation by TRPV1+ fibers. In contrast, in the TRPV1-Flp/P2X2-Cre/RC:FLTG mice (N = 4) in which GFP is selectively expressed in cells positive for both TRPV1 and the marker of esophageal nociceptors derived from placodes P2X2, the TRPV1+P2X2+ fibers were almost absent in the esophagus. Finally, in the TRPV1-Cre mice unilaterally injected with AAV9-FLEX-EGFP into vagal ganglia (N = 3) in which only the vagal TRPV1-positive fibers express GFP, the vagal TRPV1+ fibers had the same pattern as TRPV1+/Tac1+ fibers, but lower density and patchy distribution. Our data indicate that vagal nociceptive TRPV1+ fibers innervate both the esophageal mucosa and myenteric ganglia and originate from neural crest-derived jugular portion of the vagal jugular-nodose ganglia. Supported by NIH SPARC.

279 | Mechanisms of oesophago-gastric transit following sleeve gastrectomy

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Introduction: Patients who undergo sleeve gastrectomy have substantially altered anatomy and demonstrated rapid gastric emptying. The physiological underpinnings of the post-sleeve gastrectomy state, particularly oesophago-gastric transit, are incompletely delineated and would be of significance. We hypothesised that dynamic oesophageal function is a critical transit and reflux mechanism following sleeve gastrectomy.

Methods: Post-sleeve gastrectomy patients with optimal outcomes, determined by a structured interview, were recruited. Obese controls were used as comparison. Protocolised nuclear scintigraphy with high definition (Xeleris™ 4 DR) imaging, high resolution manometry were performed. Targeted stress barium was used to specifically delineate the mechanism of transit.

Results: Twenty-six patients were identified; mean age 48.9 ± 11.7 years, 19 females, excess weight loss was 56.2 ± 45.2% and duration from surgery was 15 ± 23 months. Gastric emptying half time post gastric sleeve was 25.4 ± 12.5 minutes compared to the 70.7 ± 44.7 minutes in controls ($P < 0.001$). Post-operatively, prolonged oesophageal substrate retention with graduated co-dependence clearance of the oesophagus and stomach was observed. Triggered deglutitive reflux events noted in 65% of patients during semi-solid swallows. Stress barium illustrated 2 patterns of emptying: continuous (69%) or cyclical (31%), with a consistent 5 stage cyclical pattern involving likely repeat oesophageal peristalsis, incisural opening, transpyloric flow with pan-compartmental pressurisation of the vertical compartment. Sustained increases in proximal gastric pressure (35 ± 18 mm Hg) were demonstrated to precede trans-pyloric flow and reflux events.

Conclusion: Following sleeve gastrectomy; delayed oesophageal transit and rapid gastric emptying are observed. An ordered oscillatory pattern of compartmental filling and subsequent transpyloric flow, leads to bolus transit rather than regulated emptying. The mechanism of rapid gastric emptying is via transmitted oesophageal pressurisation of the vertical compartment generating reflex antral contractions and subsequent trans-pyloric flow.

280 | Identifying novel mechanisms of gastroesophageal reflux in sleeve gastrectomy using concurrent high-resolution oesophageal manometry and pH monitoring

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Background/Objectives: Gastroesophageal reflux disease (GORD) post sleeve gastrectomy (SG) is a critical issue due to potential for severe symptoms, lifelong medical treatment, and reoperation. Transient lower oesophageal sphincter relaxations (tLOSRs) are proposed to be the primary reflux mechanism in the normal population, however the pathophysiology remains incompletely delineated in SG patients. We aimed to identify candidate mechanisms for reflux in SG patients.

Methods: SG patients were prospectively recruited and underwent extended, concurrent high-resolution oesophageal manometry and pH monitoring. Monitoring included one hour fasting and one hour following a high-caloric drink. Patients continued with 24-hour ambulatory pH monitoring.

Results: 10 SG patients were included. Baseline demographics were: 70% female, age 54.0 ± 11.5, percentage of excess weight lost 55.07 ± 26.5%, follow-up duration 10.1 ± 8.8 months, and 40% symptomatic reflux. Periods of high intragastric pressure (HIGP) were observed (median 18 (5-29.75 events)). These were ubiquitous across fasting and postprandial periods, and asymptomatic and symptomatic patients. 52.0% of HIGP coincided with oesophageal acidification. Prolonged acidification events were observed with minimal oesophageal effort in response. Minimal tLOSRs were seen (0.6 ± 0.92) and did not differ significantly whether fasting and postprandial (0 vs 0, $P = 0.5$). Axial separation of the diaphragm and lower oesophageal sphincter was identified in 3 patients. These were associated with isobaric pressurisation between components of the oesophagogastric junction (OGJ).

Conclusions: Key mechanisms for reflux in SG patients include HIGP and prolonged acidification events in the absence of oesophageal reaction. Axial separation of the OGJ also induces isobaric pressurisation. tLOSRs, which are the primary mechanism of reflux in the normal population, appear to have a minimal role. Given the altered anatomy and physiology of the sleeve, these novel mechanisms appear to be driving reflux.

281 | Analysis of synaptic baskets onto myenteric neurons of human colon revealed by multi-layer immunohistochemistry

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Background: A multiplexed immunohistochemistry protocol was used to apply 14 antisera for common neuronal markers (HuC/D, NOS, ChAT, 5-HT, SP, ENK, CGRP, VAChT, SOM, Calb, Calret, NF200, VIP & NPY). These were applied successively, in 5 layers, to specimens of ascending colon from 6 patients. This identified the chemical coding of myenteric nerve cell bodies and also revealed dense baskets of varicosities surrounding some myenteric nerve cell bodies.

Methods: Six preparations of ascending colon (1303 myenteric neurons) were analysed to identify quantitatively which enteric neurons received visually dense baskets of varicosities, initially comparing "nitrergic" NOS+/ChAT- (558/1303; 42.8% of all cells), with "cholinergic" NOS-/ChAT+ neurons (595/1303; 45.7% of all cells). Remaining NOS+/ChAT+ and NOS-/ChAT- cells were excluded from this quantitation.

Results: SP-immunoreactive baskets surrounded 354 cells (out of 1303). Of these, 276 target cells (78.0%) were cholinergic, whereas only 30 targets cells were nitrergic NOS+/ChAT- (8.5%). Thus, SP-immunoreactive baskets strongly favour cholinergic neurons. VAChT-immunoreactive baskets were apparent around 318/1303 neurons. Of these, 25 were around nitrergic NOS+/ChAT- cells (7.9%) but 260 surrounded cholinergic cells (81.8% of all VAChT baskets). Again, cholinergic varicosities preferentially target cholinergic neurons. Enkephalin baskets surrounded 156 cells but only 4 were nitrergic NOS+/ChAT- cells (2.6% of ENK baskets) whereas 128 (82.1% of ENK baskets) were around cholinergic NOS-/ChAT+ cells, again showing the same preferential targeting of cholinergic pathways. Double labelling studies revealed partial overlap between SP, VAChT and ENK-immunoreactive baskets. This is consistent with myenteric nerve cell body coding; 97.9% of SP cell bodies were ChAT+, and 100% of ENK neurons were ChAT+.

Conclusions: The multi-layer, multiplexed immunohistochemistry protocol provides detailed quantitative data on connectivity in the enteric nervous system. ENK, SP and VAChT immunoreactive neurons preferentially synapse with cholinergic pathways in the colonic myenteric plexus.

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282 | Metabotropic glutamate receptor 5 is involved in neural control of colonic motility

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Background/Objectives: Group I metabotropic glutamate receptors (mGluRs) comprise mGluR1 and mGluR5 and are involved in synaptic function in the brain. Group I mGluRs may also be involved transmission between enteric neurons, but their role in colonic motility remains unknown. We assessed this using video-imaging of migrating motor complexes of mouse colon (CMMCs) *in vitro*.

Methods: Colons from adult C57BL6 mice were dissected and placed in a heated organ bath containing Krebs solution. A video camera recorded four 15-minute videos of CMMCs in the presence of the Group I mGluR antagonist PHCCC (30 μM), or specific antagonists against mGluR1 (BAY-36-7620, 10 μM) and mGluR5 (MPEP, 10 μM). Videos were converted into spatiotemporal maps and analysed for CMMC frequency, length, and speed.

Results: PHCCC significantly decreased CMMC frequency (Control: 4.10 ± 0.47 CMMCs/15 minutes; PHCCC: 2.20 ± 0.46 CMMCs/15 minutes, n = 10, P = 0.01), length (Control: 37.96 ± 1.92 mm; PHCCC: 21.61 ± 4.36 mm, n = 10, P = 0.01), and propagation speed (Control: 1.18 ± 0.42 mm/s; PHCCC: 0.74 ± 0.23 mm/s, n = 10, P = 0.01). Blocking mGluR5 with MPEP significantly decreased CMMC frequency (Control: 4.86 ± 0.96 CMMCs/15 minutes; MPEP: 3.43 ± 0.81 CMMCs/15 minutes, n = 7, P = 0.03), but not length (40.17 ± 2.90 mm; MPEP: 37.08 ± 3.98 mm, n = 7, P = 0.11) or propagation speed (Control: 1.45 ± 0.21 mm/s; MPEP: 1.16 ± 0.08 mm/s, n = 7, P = 0.18). When mGluR1 was blocked with BAY-36-7620, the change in CMMC frequency fell short of significance (Control: 8.25 ± 0.99 CMMCs/15 minutes; BAY-36-7620: 5.13 ± 1.23, n = 8, P = 0.05), as did CMMC length (Control: 43.51 ± 1.28 mm; BAY-36-7620: 39.35 ± 2.28 m, n = 8, P = 0.07) and CMMC speed (Control: 1.18 ± 0.16 mm/s; BAY-36-7620: 1.87 ± 0.43 mm/s, n = 8, P = 0.88).

Conclusions: mGluR5 is involved in the initiation of neurally-mediated colonic contractions in the mouse. As mGluR1 is expressed in the ENS, additional studies are needed to ascertain their role in enteric function.

284 | Colorectal cancer: Role of the cholinergic system in mechanisms of cancer evasion from the host's immunity

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Background: Colorectal cancer (CRC) is the third leading cause of cancer-related death worldwide. Tumour cells have evolved to express immunosuppressive molecules enabling their evasion from the host's immunity. These molecules include programmed death ligands PD-L1 and PD-L2. Cancer cells can also produce acetylcholine, which plays a role in tumour progression. Moreover, tumour innervation can stimulate vascularisation leading to tumour growth and metastasis.

Methods: We evaluated the expression of immunosuppressive (PD-L1 and PD-L2) and cholinergic (muscarinic receptor 3 (M3R), alpha 7 nicotinic receptor (α7nAChR) and choline acetyltransferase (ChAT)) markers in specimens from stage I-IV CRC patients. The effects of atropine and M3R blocker, 4-DAMP, on cancer growth and spread were tested *in vitro* and *in vivo*. Molecular mechanisms and signalling pathways involved in these effects were examined.

Results: A significantly high expression of PD-L1 and PD-L2 was observed at CRC stages III-IV compared to I-II. Similarly, M3R and ChAT were elevated at stages III-IV compared to early stages. However, there was no significant difference in the expression of α7nAChR at all stages of CRC. PD-L1, PD-L2, M3R and ChAT expression was associated with a poor survival outcome. No significant correlation between the expression of these markers and patient's gender, age and metastasis was found, with exception to M3R. Furthermore, PD-L1 expression correlates with M3R, while PD-L2 correlates with α7nChR. This suggests that PD-L1 expression can cross-talk with M3R and PD-L2 with α7nChR. *In vitro* results demonstrated that atropine and 4-DAMP decreased the expression of immunosuppressive and cholinergic markers via inhibiting EGFR, pSTAT3, and pERK½ signalling pathways in human CRC cells. Blocking M3R *in vivo* decreases the expression of immunosuppressive, cholinergic and angiogenic markers leading to an improved immune response against cancer.

Conclusion: Enhanced expression of PD-L1 and PD-L2, M3R and ChAT associates with the advanced stages of CRC and poor survival outcomes. The expression of these markers was abolished by atropine and 4-DAMP treatments, both *in vitro* and *in vivo*. These data suggest that there is a cross-talk between cholinergic and immune systems in cancer development; the evasion of cancer from the host's immunity is influenced by the cholinergic system.

285 | Pharmacological and electrophysiological analysis of polarised enteric neural pathways activated by balloon distension in the guinea pig colon

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Objectives: Propulsion of individual faecal pellets in the guinea pig colon involves excitatory and inhibitory enteric pathways (1). The final inhibitory motor neurons to the circular muscle in the guinea pig colon may utilise multiple transmitters. We tested antagonists to the three putative transmitters, ATP, NO and VIP, on anal inhibition elicited by distention.

Methods: Segments of distal colon, taken from adult guinea pigs euthanized according to the Flinders university AWC, were placed in an organ bath containing carbogenated Krebs solution at 36.5°. The segments were set up over a small tube with an intraluminal flaccid balloon (14 mm long) connected to a syringe for manual distension to 6 mm maximum diameter. Spatiotemporal maps of distensions from video recording were combined with localized intraluminal force, oral and anal to the balloon, and combined with smooth muscle electrical activity recordings by suction electrodes.

Results: Transient balloon distensions for 10 seconds elicited oral increase in intraluminal force preceded by a discharge of action potentials and anal inhibitory response. Hexamethonium (100 μM) inhibited the oral excitation but only reduced the anal inhibition. After hyoscine (1 μM) the oral excitation was reduced, and an anal inhibition of intraluminal pressure was preceded by a fast hyperpolarisation followed by a sustained hyperpolarisation. MRS2500 (1 μM), blocked the initial compound inhibitory junction potential while L-NNA (100 μM) blocked the slower sustained hyperpolarisation. No hyperpolarisation remained after these two antagonists. VIP antagonists (VIP 10-2, 1 μM) had no effect on the hyperpolarising responses.

Conclusions: Luminal distension of the guinea pig colon activates the descending inhibitory reflex which involves release of ATP and NO from final inhibitory motor neurons.

1. Marcello Costa, Lauren J. Keightley, et al, J Physiology (2019); https://doi.org/10.1113/jp278284.

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286 | Chemical coding of myenteric neurons of human colon revealed by multi-layer immunohistochemistry

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Background: The enteric nervous system (ENS) comprises multiple classes of nerve cells distinguishable by combinations of immunohistochemical markers ("chemical coding"). The chemical coding of ENS of human colon has not been characterised in detail.

Methods: we used 5-layer multiplexed immunohistochemistry to apply 13 antisera for common neuronal markers (HuC/D, NOS, ChAT, 5-HT, SP, ENK, CGRP, SOM, Calb, Calret, NF200, VIP & NPY) on the same 200+ myenteric plexus neurons in each tissue sample. 1303 neurons were characterised in ascending colon (n = 6) and 1087 in descending colon (n = 5).

Results: HuC/D labelled all myenteric nerve cell bodies, which were scored for the 12 other markers. Chemical codes were surprisingly numerous; of 4096 possible codes, 108 were present in ascending colon and 112 in descending colon. However, 40 codes accounted for more 90% of neurons. There was a strong correlation between the rank order of chemical codes in ascending and descending colon (ranked by abundance; Rs = 0.884[39]; $P < 0.001$) indicating that there are very similar ENS populations in ascending and descending colon. In ascending colon (n = 6, 1303 cells) 686 cells (52.6%) were immunoreactive for NOS; 723 (55.5%) for ChAT; 128 (9.8%) were immunoreactive for both ChAT and NOS and 22 cells (1.7%) lacked both markers. The next most abundant markers were NF200 (50.0%), VIP (31.3%) and calbindin (25.1%). A few codes accounted for very large proportions of neurons. For example, putative inhibitory motor neurons, immunoreactive for NOS (with none of the other 11 markers), accounted for 192 of 1303 neurons (14.7%). Dogiel type II neurons (identified by NF200 morphology) were large cells (1795 ± 729 μm^2, 25 cells) with NF200, ChAT and SP, most of which express either Calb or Calret (or both) or SOM.

Conclusions: multi-layer multiplexed immunoreactivity reveals a wealth of information about the human colonic ENS, making possible systematic analysis of classes.

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288 | Bisacodyl excites extrinsic sensory neurons to the gastrointestinal tract

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Background: The stimulant laxative, bisacodyl is a pro-drug that is converted to an active form, bis-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM). It has local effects on colonic secretion, absorption and smooth muscle tone/contractility but how it triggers the coordinated motor activity of defecation is not clear.
Methods: Effects of Bisacodyl and BHPM were studied on extrinsic sensory nerves to the gastrointestinal tract using conventional extracellular recording, ex vivo.
Results: Both Bisacodyl (50 μM) and BHPM (10 μM) caused a significant increase in multi-unit firing (from 1-12 minutes after application) in murine rectal nerves. Single unit analysis showed that approximately half of the rectal afferents were activated by Bisacodyl and BHPM. Stretch-sensitive afferents were disproportionately activated (Pearson Chi squared test for exact significance; $P = 0.032$) as were afferent units with shorter action potentials. After an initial peak response, elevated afferent firing continued for at least 60 minutes, even with prolonged washing. Responses to repeated application of 50 μM Bisacodyl or 10 μM BHPM showed marked tachyphylaxis. Bisacodyl (50 μM) and 10 μM BHPM evoked excitation which persisted in Ca^{++}-free Krebs solution (6 mM Mg^{++}, 1 mM EDTA). Excitation of afferents was also recorded in preparations from which the mucosa had been removed, indicating that deacetylation of Bisacodyl did not require mucosal epithelium or luminal bacteria, as previously suggested. There was no correlation between afferent sensitivity to capsaicin and Bisacodyl/BHPM responses. Extrinsic afferents in small intestine and oesophagus (the latter being vagal afferents) were also excited by Bisacodyl and BHPM.
Conclusions: Bisacodyl and BHPM activate stretch-sensitive rectal extrinsic afferents; this may activate spinal visceral motor pathways which generate coordinated motor patterns of defaecation.

289 | Comorbidity and association between irritable bowel syndrome and asthma: A meta-analysis

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The relationship between asthma and irritable bowel syndrome (IBS) is controversial, for previous studies in other parts of the world have shown that there is neither a link nor a link. There is no meta-analysis or system review that discussed the comorbidity and association between them so far.
Objective: The aim of this study was to systematically examine the evidence regarding the comorbidity and association between asthma and IBS through a meta-analysis.
Methods: Following the Cochrane guidance, we conducted a systematic literature search using the PubMed, Ovid and Web of Science databases for original articles published in any language, and we did not limit the publication time of the articles. Then we systematically reviewed the articles to find out the observational studies (cohort, case-control and cross-sectional studies).
Results: A total of 10 091 items were retrieved from the three databases (PubMed, Ovid and Web of Science). After the two researchers screened them independently, twenty articles of observational study met the criteria of the meta-analysis. Four cohort studies and three case-control studies, which used "asthma" as expose factor, were assigned to the group I. In addition, only one cohort study and one case-control study that focused on the prevalence of asthma in the IBS population, were assigned to the group II. All remaining cross-sectional studies were assigned to group III. Group showed that asthma patients are more likely to develop irritable bowel syndrome (OR: 1.618, 95% CI: [1.361,1.923]). After combining the effect values of the group II, no significant association between the two disease was found (OR: 1.306, 95% CI: [0.940,1.817]). The result of combining the effect values of the cross-sectional studies supported the association and comorbidity between the diseases (OR: 1.538, 95% CI [1.242,1.905]). The results of publication bias and sensitivity analysis confirmed the reliability and stability of this meta-analysis. *see figure below.*
Conclusions: There were comorbidity and association between IBS and asthma, and asthma factor could increase the incidence of IBS in people.
Keywords: irritable bowel syndrome, asthma.

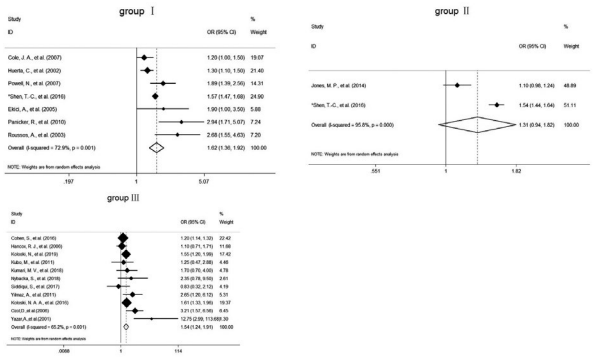

Figure – Meta-analysis-association and comorbidity between IBS and asthma

290 | Mechanosensing Piezo channels in human and mouse Enterochromaffin cells

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Background/Objectives: Enterochromaffin (EC) cells are scattered along the gastrointestinal (GI) tract mucosa and sense GI contents through both chemical and mechanical pathways. EC cells produce ~95% of the body's serotonin (5-HT). It is well established that EC cells secrete 5-HT concurrent with GI contractions, and that 5-HT is a key regulator of colonic migrating motor complexes (CMMCs). Recent evidence demonstrates that the ion channel, Piezo2, is important for EC cell mechanotransduction. However the mechanism involved in human EC cell mechanosensing capabilities has not yet been described. The impact of EC cell mechanosensing on gut motility also remains unidentified. To determine if Piezo2 is involved in the mechanosensing properties of human EC cells, and its role in GI motility, we measured mechanically-stimulated 5-HT release from human tissue, and mouse CMMCs in the presence of a Piezo antagonist (D-GsMTx4).
Methods: To determine Piezo2 and EC cell co-localisation in humans, intestinal tissue was immune-stained for 5-HT and Piezo2. 5-HT release from human intestinal tissue in the presence of 5 μM D-GsMTx4 was measured using the highly sensitive electrophysiological technique of carbon fibre amperometry. Mechanical stimulation of the intestinal tissue was performed by lowering the electrode into the mucosa using a micromanipulator. CMMCs were measured from C57/Bl6 mouse colon using three force transducers attached along the length of flat-sheet colon preparations, over a baseline period followed by addition of 5 μM D-GsMTx4.
Key Results: We observe Piezo2 staining co-localised in human EC cells. Mechanically stimulated peak and steady state release of 5-HT was decreased significantly by blocking the mechanosensing Piezo ion channels in human ileum and colon (n = 8). Baseline frequency and amplitude of mouse CMMCs were significantly reduced by blocking Piezo channels (n = 4).
Conclusions: Piezo is an ion channel significantly involved in the mechanosensitivity of human EC cells of the small and large intestine. Piezo channel inhibition reduces the frequency of CMMCs in mouse colon preparations, demonstrating that mechanosensing capabilities of EC cells play a role in colonic contractions.

291 | Examining cellular metabolism in the enteric nervous system

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Introduction: In the central nervous system, neurons rely on oxidative metabolism, while some astroglia, fibroblasts, and other proliferative cells preferentially utilize predominantly anaerobic glycolysis. Furthermore, cellular metabolism has been found in many tissues (and pluripotent stem cells) to be key to both stem cell maintenance and cell fate decisions. The metabolic processes involved in enteric neural progenitor cell maintenance and neuronal and glial lineage commitment have not been previously investigated. In this project, we investigated the metabolic state of enteric neural progenitors, and whether it is altered during differentiation.
Methods: ENS progenitor cells were isolated from E14.5 Wnt1cre;RosaYFP mouse embryos and cultured *in vitro* for 2 days as progenitors ("early" cultures), or 9 days in differentiation conditions ("late" cultures). Metabolic activity was then analysed using the Seahorse extracellular flux bioanalyser, which can determine metabolic activity of cells *in vitro* in real time. This is done by measuring the oxygen consumption rate (OCR), which is indicative of mitochondrial respiration/oxidative phosphorylation, and the extracellular acidification rate (ECAR), which is indicative of the amount of lactate produced, a product of glycolysis.
Results: Preliminary Seahorse extracellular flux analysis indicated that with ENS differentiation and maturation *in vitro* coincided with upregulation of mitochondrial oxidative phosphorylation. Differentiated cells ("late" cultures) had both higher basal and maximal OCR than progenitors ("early" cultures). On the other hand, there were no significant differences between the glycolytic profiles of the two cultures. The increased oxidative metabolism in "late" cultures correlated with the appearance of GFAP+ glia, a higher percentage of NOS+ neurons, the formation of ganglion-like structures with neuronal aggregates, and the development of dense neurite bundles; all indicative of differentiation and maturation.
Conclusions: Preliminary analysis of cellular metabolism in the ENS indicates a metabolic switch associated with progenitor cell differentiation. Current studies aim to isolate and examine pure populations of progenitors, neurons and glia from different developmental stages, and assess whether manipulation of cellular metabolism can influence progenitor cell maintenance or lineage commitment.

292 | The role of the circadian rhythm on enteric neural plasticity and gut motility

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Introduction: During the 24 hour circadian cycle, there are prominent changes to gut function as it receives food, digests nutrients, and expels wastes. It has been shown that there is plasticity in the enteric nervous system (ENS) through the day/night cycle as expression of neuronal nitric oxide synthase (nNOS) fluctuates in the gut (1). However, it is unclear whether expression of other neurotransmitters also changes, and most importantly, how this neuronal plasticity is triggered is unknown. In this study, we investigated changes in neurotransmitter expression and neuronal function in the murine ENS during the 24 hour circadian cycle.

Methods: Mice were maintained on a standard 12 hour day/12 hour night cycle and fed ad libitum. Gut tissue was collected at 6 hourly intervals (at ZT1, ZT7, ZT13, and ZT19, where lights are on at ZT0 and off at ZT12), the mucosa and submucosa were discarded, leaving the myenteric plexus and muscle layers. Tissue was homogenised and RNA extracted. Gene expression was analysed using digital droplet PCR (ddPCR).

Results: Expression of several neurotransmitter synthetic enzymes was increased in the first hour of night (ZT13), when mice are starting to be active and feeding. We observed increased expression of genes encoding nNOS, vAChT (vesicular acetyl choline transporter), and CGRP (calcitonin gene related peptide; n = 2, Table 1) at ZT13 compared with other times of day.

Conclusions: There appears to be increased expression of genes involved in neurotransmission during the night as mice begin to feed. We are continuing experiments to investigate changes in enteric neural activity using live calcium imaging.

Reference: (1) W. A. Hoogerwerf et al., Gastroenterology 135, 2019-29 (2008).

Table 1: Gene expression (shown as #copies/µl/100ng RNA):

Gene	ZT1 (day)	ZT7 (day)	ZT13 (night)	ZT19 (night)
NOS1 (nNOS)	299.3	292.8	407	143
Slc18a3 (vAChT)	94.9	91.8	160	64
Calcb (CGRP)	187.8	195.5	328.3	136.5

293 | The role of colonic mucosal mast cell in irritable bowel syndrome and its association with abdominal pain

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Background/Objectives: Irritable bowel syndrome (IBS) is characterized by abdominal pain associated with altered bowel habit without an organic cause. Increased colonic mucosa mast cells were found in IBS patients. However, the association between mast cells expression and abdominal pain was not well studied in IBS. We aimed to investigate the hypothesis whether colonic mucosal mast cells expression can help distinguish IBS patients (ROME IV criteria) from asymptomatic controls (AC), and also correlated the density of colonic mast cells with abdominal pain.

Methods: There were 23 IBS patients (9 men, 45.2 ± 11.7 years old) (Type: constipation/diarrhea/mixed/unclassified: 3/10/7/3) and 16 AC enrolled (11 men, 44.9 ± 11.8 years old) for colonic biopsies from the right side (ascending and transverse) colon and left side (descending, sigmoid, and rectal) colon. The density of mast cells in the colonic mucosa was evaluated by immunohistochemistry (per high power field). Abdominal pain was evaluated using the "present pain intensity-visual analogue scale" (PPI-VAS).

Results: The density of mast cells in the colonic mucosa increased in IBS patients (11.6 ± 3.8 cell/HPF) compared with AC (9.0 ± 3.1 cell/HPF, P = 0.029). There was no significantly group difference regarding age, gender, and body mass index. The density of mast cells correlated positively with abdominal pain scores in all subjects (r = 0.407, P = 0.005).

Conclusions: More colonic mucosal mast cells were observed in patients with IBS, suggesting altered mucosa immune response with mast cells plays a role in the pathophysiology in IBS. This work indicates that number of mast cells appears to be associated with abdominal pain; however, abdominal pain in patients with IBS seems to be multifactorial regardless of the expression of mast cells in the colon.

294 | Infused acid in the duodenum increases Prostaglandin E2 level in rat

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Background: Functional dyspepsia (FD) is a disease that causes unpleasant upper gastrointestinal symptoms, even though there are no obvious organic abnormalities.

Recently, altered visceromotor function of the stomach has been found to exist in the pathophysiology of FD and induces duodenal events. The microinflammation in the duodenal mucosa and the accompanying increase in mucosal permeability are now presumed to cause the physiological alternation and the duodenal contents (acid, bile acid, lipid, etc.) may play some role. However, the molecular mechanism in this event is not clear.

Aim: The purpose of this study was to investigate the involvement of prostaglandin E2 (PGE2), one of the representative inflammatory mediators in the duodenum of the rats.

Method: Both male and female SD rats were used. A 4Fr catheter was inserted into the duodenal lumen, from the gastric vestibule, in 7-11 week old rats, and Hydrochloric acid was injected from the tip of the catheter, and Hydrochloric acid (HCl) (pH 1, 0.2 mL) was injected from the tip of the catheter. The duodenum was removed after acid injection and contained PGE2 level (pg / mg protein) was measured using ELISA.

Result: After the HCl exposure into the duodenum, PGE2 level in the duodenum was significantly increased both in the male and female rats compared to the control (n = 4, Male: Saline vs HCL 555.8 ± 42.1 vs 1171.4 ± 253.9, $P < 0.05$ Female: 316.3 ± 12.0 vs 785.6 ± 89.4, $P < 0.05$)

Conclusion: As a result of exposing the duodenal lumen to acid, PGE2 increased in the duodenum in male and female rats. We have assumed the possibility of PGE2 as a candidate for the causative agent of duodenal microinflammation.

295 | Simultaneous video imaging and electrophysiological recordings reveal a new mechanism of the enteric nervous system underlying both propulsive and non-propulsive movements in the mouse colon

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Recently, we revealed a new mechanism in the enteric nervous system (ENS) whereby large populations of excitatory and inhibitory neurons fire in rhythmic bursts to generate rhythmic depolarisations in the smooth muscle in isolated full-length sheet preparations of colon (Spencer et al. 2018; J. Neurosci). Here, we determined whether this rhythmic ENS firing pattern is responsible for the propulsion of liquid content along tubular colonic preparations. Extracellular electrophysiological smooth muscle recordings were made from two independent recording sites while simultaneously video imaging the colonic wall movements. This allowed us to correlate the electrical activity in the smooth muscle (and indirectly the ENS) at the same time as propulsion of fluid content along the colon. Colonic motor activity was evoked either by transient intraluminal fluid, or an immobile metal rod. Fluid distension evoked neurogenic spike bursts which was associated with anterograde fluid propulsion. The metal rod also evoked ongoing neurogenic spike bursts, without propulsion. The coherence of action potentials or subthreshold cholinergic depolarisations between two recording sites was high during neurogenic spike bursts regardless of the intraluminal stimulus, or longitudinal electrode separation (1-30 mm) (n = 9). Coherence was low in quiescent periods. The figure shows that in an intact tubular preparation, large amplitude excitatory junction potentials (EJPs) were highly correlated in time when the two recording sites were separated by 30 mm in the longitudinal (rostro-caudal axis). Synchronised EJPs in the smooth muscle over large spatial fields were abolished by hexamethonium (n = 5). The findings show that the ENS can temporally synchronise the firing of enteric neurons (at ~2 Hz) along the full length of the colon, independent of contractile velocity in both propulsive and non-propulsive motor events.

Supported by ARC grant # 190103628 to NJS.

Reference: Spencer et al. J. Neurosci 38(24):5507-5522.

296 | Nutrient-sensing mechanisms of the gastric ghrelin cell

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Background/Objective: The ability of the gastrointestinal tract to detect nutrients is critical in the regulation of gut hormone secretion, food intake and postprandial control of blood glucose levels. Ingested nutrients are detected by a wide range of specialised nutrient sensors located on enteroendocrine cells. Limited information is available on the nutrient-sensing capabilities of the gastric ghrelin cell. This study aimed to investigate the expression of nutrient sensors within the murine gastric ghrelin cells and their involvement in the secretion of ghrelin.

Methods: Immunohistochemistry was performed to assess the degree of co-expression of ghrelin and selected nutrient sensors, specifically GPR93 (receptor for protein digestion products), FFAR4 and CD36 (both fatty acid sensors), in the stomach of eight-week-old male C57BL/6 mice. Osmolality-controlled *ex-vivo* ghrelin release experiments investigated the effect of nutrients (5% peptone solution, 2 mM linolenic acid, 5% fat emulsion (Intralipid®)) on the release of total and acyl-ghrelin from mice gastric mucosa. Antagonists for GPR93 (TC LPA5, 10 μM), FFAR4 (AH 7614, 10 μM) and CD36 (SSO, 400 μM) were used to assess the involvement of nutrient sensors in the secretion of total and acyl-ghrelin.

Results: Approximately 66% of gastric ghrelin-positive cells expressed FFAR4, and over 80% expressed GPR93 and CD36. Peptone potently stimulated the release of total ghrelin (25.3-fold) and acyl-ghrelin (2.3-fold), an effect inhibited by TC LPA5. Linolenic acid inhibited acyl-ghrelin (32.0% decrease), an effect prevented by the presence of SSO. Intralipid inhibited total ghrelin (32.7% decrease) in an FFAR4 and CD36-independent manner, while stimulating acyl-ghrelin (1.7-fold) via FFAR4 and CD36 activation.

Conclusion: Nutrient sensors are highly expressed within the ghrelin cell population of the murine stomach. Gastric ghrelin cells adjust total and acyl-ghrelin secretion in response to specific nutrient signals via activation of these nutrient sensors. Accordingly, gastric nutrient sensors may be potential targets for the modulation of ghrelin levels and its associated physiological activities, including the regulation of postprandial glucose levels and food intake.

297 | Quantitative PCR as a novel approach to determine small intestinal bacterial load in health and disease

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Background: Small intestinal bacterial overgrowth (SIBO) may play a role for the manifestation of a variety of gastrointestinal (GI) and non-GI diseases. We aimed determine the small intestinal mucosal bacterial load in various patient cohorts and control subjects.

Methods: During endoscopy biopsies were collected from the second part of the duodenum from 237 patients, 63 with "asymptomatic" controls without any structural abnormalities, 84 with functional gastrointestinal disorders (FGID) and 90 with inflammatory bowel disease (IBD) utilising the Brisbane Aseptic Biopsy Forceps to avoid the luminal and working channel contamination of tissue, and total DNA was extracted. Bacterial load was assessed and normalised to human DNA in each sample by qPCR, using Bacteria-domain 16S rRNA gene-specific primers, and primers targeting the human beta-actin gene.

Results: Utilising tissue collected with the aseptic biopsy forceps; bacterial load was significantly greater in FGID patients (0.22 ± 0.03) compared to controls (0.07 ± 0.05; P = 0.007) and IBD patients (0.01 ± 0.03, P = 0.0001), (Figure 1). PPI users had significantly larger bacterial load on duodenal tissue as compared to non-users (P < 0.05), and PPI use did not explain the difference between the FGID patients and controls. Results from samples taken with standard biopsy forceps (and thus susceptible to cross contamination) only poorly correlated with the results produced from matched samples obtained with the aseptic biopsy device (r = 0.036; P = 0.74).

Conclusions: FGID patients had higher bacterial loads as compared to controls and IBD patients, and this difference could not be explained by PPI use, although PPI therapy was associated with significantly higher bacterial loads. Our data suggest that qPCR to assess small intestinal mucosal bacterial load is potentially a valuable test in the routine clinical setting.

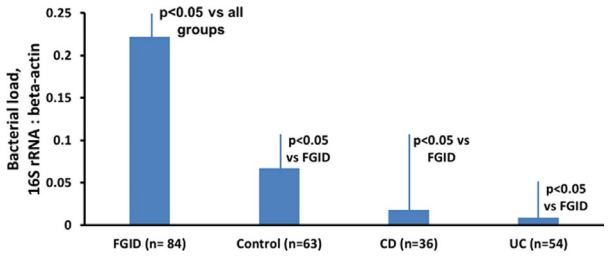

Figure 1: Bacterial load on the proximal small intestinal mucosa in different patient cohorts and controls

298 | Complex functional gastrointestinal disorders represent a high healthcare utilisation group

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Patients with complex functional gastrointestinal disorders (FGID) have severe symptoms which affect gut motility and sensitivity necessitating supplemental nutrition such as enteral feeding and total parental nutrition (TPN) for either symptom control or loss of weight. Complex FGID is a debilitating and poorly understood condition.

Methods: This is a retrospective study which identified patients with complex FGID at a quaternary referral gastrointestinal service (patients requiring a period of supplemental nutrition such as enteral feeding and TPN). Demographic, clinical characteristics and markers of health care utilisation were taken from 2010 onwards.

Results: 46 patients were included. 42 (91%) were female with a median age of 35 ± 2 years. The primary diagnosis was idiopathic gastroparesis in 26 patients, generalised slow gastrointestinal transit in 6 and functional abdominal pain in 4. Ehlers Danlos Syndrome was co-existent in 9 patients and another 5 had features of hypermobility. The number of admissions/year was 2.2 ± 0.3 with a length of stay of 6.5 ± 0.3 days. For each patient, 7.6 ± 1.0 gastroscopies were conducted. Average time spent on either enteral nutrition or TPN was 36 ± 3.6 months. The number of gastroenterology clinic reviews was 14.2 ± 1.6, with 8 dietitian reviews and 6 with nutrition nurses. Each patient had an average of 6 treating specialists (see Figure 1), 13 patients had regular intravenous fluids, and 7 had regular TPN. Regarding TPN use, female sex and younger age (both $P < 0.001$) were the most useful predictors necessitating long-term TPN.

Conclusions: Patients with complex FGID represent a challenging group to manage. Whilst they account for frequent procedures and outpatient encounters, their admission frequency and duration are not as burdensome as thought. Although these patients do access to multidisciplinary specialists, there should be further emphasis on the role of pain and psychiatrists in the management of these patient.

%

Figure 1: Specialist involvement for patients with complex functional gastrointestinal disease.

299 | Coordination of enteric viscerofugal neuron firing with smooth muscle voltage oscillations during the colonic motor complex in mouse

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Viscerofugal neurons project from gut via extrinsic nerve trunks, forming reflex arcs with autonomic neurons that can regulate gut motility or secretion. Large populations of enteric neurons fire synchronously in rhythmic bursts of ~2 Hz to generate rhythmic smooth muscle depolarisations in the murine colonic motor complex. Thus, viscerofugal neurons may be involved in this behaviour. Here, electrophysiological recordings were made from rectal nerve trunks in mouse colorectum in flat sheet, full-length colon preparations (n = 3). An extracellular suction electrode was applied to the serosa within ~5 mm of rectal nerve entry to detect motor complexes. To assist recordings, colorectal sensory innervation was reduced with bilateral surgical removal of L5-S1 DRG, allowing 7 days recovery before recordings. Consistent with sensory denervation, immunohistochemical analysis showed a significant reduction of CGRP in the distal colons of DRG-lesioned mice (n = 3) compared to controls (n = 4). Consistent with viscerofugal neurons, rectal nerve recordings in DRG-lesioned preparations revealed ongoing firing that was activated by the nicotinic agonist, DMPP, but not capsaicin (n = 3). Ongoing motor complexes were detected every 2.6 ± 0.6 minutes, comprising voltage spikes and oscillations in smooth muscle at ~2 Hz (n = 3). Motor complexes were temporally associated with a transition from a relatively disorganised pattern in rectal nerve firing to discrete action potential bursts synchronized with muscle voltage oscillations at ~2 Hz. This suggests the ENS rhythmic discharge pattern underlying motor complexes is transmitted by viscerofugal neurons to extrinsic autonomic neurons, and raises the question whether motor complexes alone can initiate autonomic reflexes to control other gut regions.

300 | The discharge of enteropancreatic nerves is altered in diabetic rats

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Enteric neurons of the proximal duodenum projected out of the gut to pancreas through enteropancreatic nerves. The enteropancreatic nerves have been suggested to play a role in pancreatic function modulation. However, the discharge pattern of enteropancreatic

nerves and its correlation to gut motility are still unknown. In this study, we investigated the discharge of the enteropancreatic nerves and its correlation to gut motility in normal and diabetic rats.

Methods: Normal control and streptozotocin-induced T2DM Sprague-Dawley rats were used. The enteropancreatic nerve discharge was recorded using platinum electrodes.

Results: Interdigestive enteropancreatic nerve discharge consisted of alternate silent period and discharge burst period. In normal control rats, the proportion of the discharge burst period was 50.2% on average. In the diabetes rats, the proportion of the discharge burst period was 53.7%, which had no significant difference compared to the control. In normal control rats, the frequency and amplitude of discharge were 7.46 ± 0.75 per second and 196.9 ± 45.3 μV (n = 7) in the discharge burst period. In diabetic rats, the frequency and amplitude of discharge in the discharge burst period were 2.77 ± 0.41 per second ($P < 0.01$, compared to the control, n = 8) and 280.39 ± 37.67 μV ($P < 0.01$, compared to the control). To test whether the discharge of enteropancreatic nerves was corresponded to the gut motility, we fed the normal control rats and measured the discharge. The enteropancreatic nerve discharge with feeding also consisted of silent period and discharge burst period. The frequency and amplitude of discharge in the discharge burst period were 14.84 ± 1.93 per second and 321.9 ± 41.3 μV at 30 minutes after feeding (n = 8), 9.71 ± 1.15 per second and 280.6 ± 43.9 μV at 60 minutes after feeding(n = 10), and 8.59 ± 1.87 per second and 261.5 ± 44.8 μV at 120 minutes after feeding (n = 8).

Conclusion: Interdigestive enteropancreatic nerve discharge in diabetic rats had lower burst frequency and higher discharge amplitude. The discharge of enteropancreatic nerves reflects the gut motility and the discharge change after feeding appears to mirror the insulin secretion pattern.

301 | Diagnostic limitation of the Lyon gastroesophageal reflux disease consensus criteria for gastroesophageal reflux disease in Korea

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Background/Objective: The recently published Lyon gastroesophageal reflux disease (GERD) consensus defines parameters for esophageal testing that conclusively establish the presence of GERD. However, the usefulness of the Lyon consensus criteria (LCC) has not been validated in the Asian population. We aimed to validate the LCC in the Korean population by comparing them with the GERD Questionnaire (GerdQ) in terms of accuracy for clinical diagnosis of GERD.

Methods: Patients with suggestive GERD symptoms were enrolled in the study. They underwent pH monitoring, upper endoscopy, and completed the GerdQ between 2018 and 2019. The results of the pH monitoring and the endoscopic grade of esophagitis were analyzed using the LCC.

Results: A total of 178 patients (male-to-female ratio, 69:109; median age, 57 years) were included, of whom 112 (62.9%) had a GerdQ score of >8. The endoscopic findings indicated that 0.6% of the patients had grade C or D esophagitis, 14.6% had grade A or B esophagitis, and 1.1% had Barrett's esophagus. In accordance with the LCC, the evidence for the diagnosis of GERD was conclusive in 26 patients (14.6%), negative in 99 (55.6%), borderline or inconclusive in 51 (28.7%), and adjunctive or supportive in 2 (1.1%). GerdQ showed a sensitivity of 73.6% and specificity of 59.7% when the cutoff value was 8. In the LCC, when the cutoff value yielded conclusive evidence of GERD, the sensitivity was 21.5% and the specificity was 100%. When the cutoff value yielded borderline or inconclusive evidence of GERD, the sensitivity was 52.1% and specificity was 75.4%.

Conclusions: Despite the high GerdQ scores > 8, the proportions of the patients with conclusive evidence of GERD and higher reflux esophagitis grade were low. With the current LCC, the incidence of GERD can be underestimated. Moreover, the current LCC showed a limitation in the diagnosis of GERD in the Korean population. However, by expanding the criteria to include borderline or inconclusive GERD findings, the LCC showed a diagnostic capability comparable with that of GerdQ. The application of the LCC in Korean patients with GERD in the clinical setting might need careful consideration.

302 | Oral local budesonide treatment improves both jejunal and colonic function in a spontaneous rat model for functional gastro-intestinal disorders

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Objective: Functional gastro-intestinal disorders (FGID) are characterized by a low-grade inflammation and increased permeability in the GI tract, but their role in symptom generation is still unclear. Our group identified the Biobreeding diabetes-prone rat (BBDP) as a spontaneous model of FGID with colonic hypersensitivity associated with an increased permeability and mucosal eosinophilia from d90 (Meleine et al. 2019). We aim now to elucidate the relationship between low-grade inflammation and the other features by studying the effect of a local anti-inflammatory steroid treatment on symptoms and barrier function.

Methods: Adult male control (BBDR) and BBDP rats (n = 6-8) were treated for 28 days with a suspension of budesonide(bud) at 0.5 mg/kg/d or vehicle (veh) by daily oral gavage from 90 days. Colonic sensitivity was assessed before and after treatment by measuring the

visceromotor response (VMR) to isobaric (from 15 to 60 mmHg) distensions. At the end of the treatment, colonic and jejunal permeability, eosinophil infiltration, were assessed by Ussing chamber experiments, Chromotrope 2R staining respectively.

Results: Budesonide decreased the eosinophil density in the jejunum in treated rats compared to controls (220.1 ± 43.7 per mm² (BBDRbud) & 133.7 ± 53.9 (BBDPbud) vs 561.7 ± 55.3 (BBDRveh); both $P < 0.05$) as well as in the colon (78.1 ± 14.43 (BBDRbud) & 54.76 ± 14.67 (BBDPbud) vs 128.9 ± 14.24 (BBDRveh); both $P < 0.05$). This was associated with a normalization of small intestinal and colonic permeability, which was similar to the control groups after budesonide treatment in BBDP (210.5 ± 50.07 pmol/cm² (BBDRbud) & 225.2 ± 64.49 (BBDPbud) vs 284.1 ± 74.94 (BBDRveh); both $P > 0.1$). Colonic sensitivity was not altered by budesonide in the BBDR rat and decreased in the BBDP group (Figure 1).

Conclusion: Oral treatment with an oral treatment with a locally acting steroid preparation improved both colonic and jejunal inflammation and permeability and normalized colonic hypersensitivity in a rat model for FGID. These preclinical data support a role for eosinophils in the pathophysiology of FGID and identify anti-inflammatory treatments, including budesonide, as potential novel treatment options for FGID.

303 | Anti-inflammatory properties of a bacterial long chain fatty acid

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Clinical data suggest that certain probiotic bacterial strains modulate colonic inflammation. Nonetheless, these data differ considerably among studies due to the probiotic bacterial strains used and the poor knowledge of their mechanisms of action. The aim of our study was to decipher the role of bacterial long chain fatty acid (LCFA) in probiotic activity. Amongst all the LCFA quantified by mass spectrometry in *Escherichia coli* Nissle 1917 (EcN), a probiotic used for the treatment of multiple intestinal disorders, the concentration of 3-hydroxyoctadecaenoic acid (C18-3OH) was increased in EcN compared to other *E. coli* strains tested. Oral administration of C18-3OH decreased colitis induced by dextran sulfate sodium (DSS) in mice. In order to determine if other bacteria within the gut microbiota are able to produce C18-3OH, we changed the gut microbiota by feeding mice with prebiotic fructooligosaccharides (FOS). The anti-inflammatory properties of FOS were associated with an increase in colonic C18-3OH concentration. Microbiota analyses revealed that the concentration of C18-3OH was correlated with an increase in the abundance in *Allobaculum*, *Holdemanella* and *Parabacteroides*. In *Holdemanella biformis*, we quantified very high concentration of C18-3OH in bacteria and in culture supernatant. Finally, using TR-FRET binding assay and gene expression analyses, we demonstrated that the C18-3OH is an agonist of the peroxisome proliferator activated receptor gamma (PPARγ). Thus, the production of C18-3OH by bacteria could be one of the mechanisms implicated in the anti-inflammatory properties of both EcN and the prebiotics. The production of LCFA-3OH by bacteria could be implicated in the microbiota/host interactions.

304 | Coexistence of faecal incontinence with constipation: An underappreciated clinical problem in adults. A phenotyping study of 4027 patients

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Objective: Coexistence of faecal incontinence (FI) and chronic constipation (CC) is well recognised in paediatric and geriatric populations. In adults, however, these conditions are often considered separate entities. We aimed to determine: (1) coexistence of these symptoms in adults referred for anorectal physiological investigation; (2) how frequently coexistence is reported by the referring clinician; (3) whether clustering analysis also revealed different clinical phenotypes existing in the study population.

Methods: Consecutive patients (aged 18-80) referred to the Royal London Hospital GI Physiology Unit (study period 2004-16) for investigation of FI and/or CC were included. All patients self-filled a questionnaire incorporating validated scoring systems. Patient-reported symptoms (Rome IV core criteria) were compared to the primary reason for referral stated in the clinicians' referral letter (FI, CC or both). Clustering analysis of self-reported symptoms was performed through an unsupervised approach.

Results: 4027 patients (3370 females [84%]; median age 52 [IQR 41-63]) were included. From questionnaire responses, 807 (20.0%) *self-reported* FI in isolation, 1569 (39.0%) self-reported CC in isolation, and 1651 (41.0%) self-reported coexistent symptoms (Figure A). By contrast, 1640 (40.7%) patients were *referred* for FI in isolation, 2056 (51.1%) for CC in isolation, and only 331 (8.2%) patients for coexistent symptoms (Figure B). Three distinct phenotypic clusters were found, with the majority of patients assigned to the cluster predominantly presenting with coexistent FI and CC (42.2%).

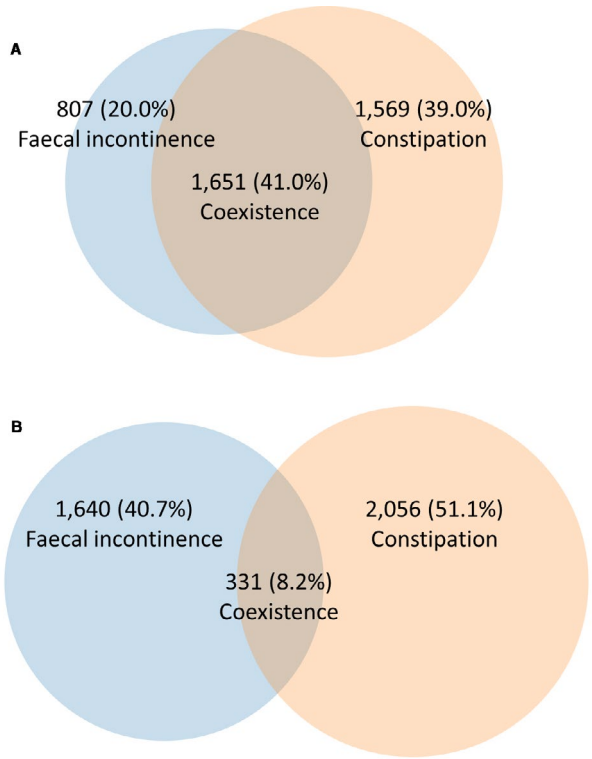
Conclusion: Over 40% of patients referred for anorectal physiological investigation had coexistence of FI and CC, based on validated criteria. Similar coexistence was shown on cluster analysis. This overlap was not acknowledged by the referrer in 80%. Over 50% of patients referred for FI in isolation also had significant CC (Rome IV core criteria positive). Lack of recognition of coexistence of FI and

CC has major treatment implications; therapy may be better directed at the latter, with FI representing a secondary phenomenon.

Figure. Symptoms of faecal incontinence in isolation, constipation in isolation and coexistent symptoms in 4027 patients by patient-reported symptoms based on the Rome IV core criteria (A), and according to the primary reason for referral stated in the clinicians' letter (B).

A. Self-reported symptoms (Rome IV core criteria).

B. Primary reason for referral (clinician's referral letter).



306 | Impact of prenatal stress on visceral sensitivity and intestinal homeostasis in adulthood

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The prenatal period is a delicate time during which intrauterine exposure to environmental factors may modulate the course of development of the intestinal barrier function and the sensory system, thereby increasing the risk of developing functional disorders. We hypothesized that prenatal stress in mice would predispose adult offspring to visceral hypersensitivity and intestinal homeostasis failure. Prenatal stress was induced by using a restriction stress with bright light during 30 minutes, three times a day, in female mice between day 13 and 18 of gestation. In 8 weeks old offspring, visceral sensitivity to colorectal distention and intestinal paracellular permeability were assessed in male and female. Mucus, tight junction proteins, anti-microbial peptides, chemokines and cytokines were monitored by qPCR and intestinal bioactive lipids by mass spectrometry. MiSeq-based microbial taxonomic analysis was used to determine faecal microbiota composition. Prenatal stress induced visceral hypersensitivity and increased paracellular permeability in the majority of male and female offspring. Only in male, we quantified a decrease in 5-lipoxygenase activity and *Tnfα* expression and an increase in *Ttf3* and *Ifnγ* expression. Prenatal stress induced in male and female adult offspring a dysbiosis characterized in male by a decrease in intestinal microbiota diversity, an increase in *Akkermansia* and a decrease in *Desulfovibrio* and in female by an increase in *Akkermansia* and a decrease in *Lactobacillus animalis*. Interestingly, the abundance in *Lactobacillus animalis* was inversely correlated with visceral hypersensitivity. Prenatal stress is sufficient to induce microbiota dysbiosis, visceral hypersensitivity and increase paracellular permeability in adulthood. Thus, prenatal stress could represent a priming event for the development of functional disorders in adulthood.

307 | Peroral endoscopic myotomy for achalasia: Predictors of treatment response

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Background: Peroral endoscopic myotomy (POEM) is increasingly used for treatment of achalasia. There is a lack of knowledge regarding risk factors for reduced response to POEM. Previous studies have focused on dysphagia, retrosternal pain, regurgitation and weight loss (Eckardt-score) as key outcome parameters. The aim of the retrospective analysis of our cohort was to identify manometric predictors for reduced response to POEM and to compare the established Eckardt-score with SAGIS(Structured Assessment of Gastrointestinal Symptoms)-score to capture treatment response.

Methods: Since 2015, 53 POEMs have been performed at our centre by one operator (LFH). All patients underwent independent clinical assessment including endoscopy, barium-swallow and high-resolution-manometry (HRM). Symptoms were assessed by the SAGIS-score. Response was assessed clinically 2-weeks, 4-months and 1-year post-POEM. Patients were offered HRM 12 months post-POEM. We analysed distal contractile integral (DCI), integrated relaxation pressure (IRP) and baseline lower oesophageal sphincter (LES) pressure. Eckardt-score ≥4 post-POEM was considered as a partial response.

Results: The mean post-POEM Eckardt-score decreased from 8.7 ± 2.3 to 1.9 ± 1.9 ($P < 0.02$). In 10/53 patients the Eckardt-score was ≥ 4 at 1-year post-POEM.

At baseline there was a significant difference in the DCI between patients clinically responding to POEM vs those with reduced response (2133 ± 2777 vs 7837 ± 10 430, $P < 0.02$). Similarly, the post-POEM DCI was lower in patients with response vs reduced response

(637 ± 749 vs 1877 ± 1915, $P < 0.02$). There was no difference in the LES pressure or the IRP between responders and non-responders (P all >0.3). Treatment responders had an overall significantly lower SAGIS-score post-procedure (11.7 ± 13.9 vs. 29.6 ± 18.6). SAGIS-score of >50% of responders were within the range of healthy controls.

Conclusions: (1) A high DCI prior to POEM is associated with a reduced treatment response. The data may suggest the need to develop a tailored approach for achalasia patients with a high DCI score. (2) Symptom assessment utilising SAGIS-score is suitable to monitor treatment response to POEM.

308 | TAK-954, a potent and selective 5-HT4 receptor agonist, normalizes GI motility in animal models of post-operative ileus and opioid-induced slow transit

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Background/Objectives: Postoperative ileus (POI), a period of GI dysfunction occurring after surgical bowel manipulation, has symptoms of delayed defecation, pain, nausea and vomiting, and may increase time in hospital. Opioid analgesics may worsen POI. Previously, TAK-954, a highly selective 5-HT_4 receptor agonist reduced GI inflammation and improved motility in a mouse POI model, when preoperatively administered. Here we evaluated the effects of TAK-954 on GI transit following pre-, peri-, and/or post-operative administration in mouse or guinea pig POI models and on morphine-delayed GI transit in guinea pigs.

Methods: POI was induced by gut manipulation following laparotomy in anesthetized animals. In mice, TAK-954 or vehicle was administered 90 minutes before (PRE), and/or immediately after surgery (PERI), and/or 90 minutes before determining transit of an oral FITC-dextran marker administered 22.5 hours after surgery (POST). In guinea pigs, TAK-954 or vehicle was administered 30 minutes after surgery or before morphine administration, and gastro-anal transit (GAT) determined as time between oral carmine red administration and its fecal appearance.

Results: In mice (N = 6-9/group) TAK-954 (0.01 mg/kg, s.c.) increased the geometric center (GC) of FITC-dextran transit *vs.* vehicle-treated controls when administered PRE + PERI (5.673 *vs* 4.925, *ns* by ANOVA), PRE + POST (7.532, $P < 0.05$) or POST (7.658, $P < 0.05$), and maximally when dosed PRE + PERI + POST-operatively (9.267, $P < 0.001$). In guinea pigs (N = 8/group), surgery delayed the median GAT (9-10 hours) or morphine administration (12 hours) versus controls (6-7 hours; $P < 0.005$; log-rank test). TAK-954 (0.003-3 mg/kg s.c.) produced dose-dependent decreases in median GAT prolonged by surgery (6.5-7 hours) or morphine (9 hours; $P < 0.05$). TAK-954-induced attenuation of POI was reversed by the selective 5-HT_4 receptor antagonist, piboserod (3 mg/kg).

Conclusions: TAK-954 dose-dependently reverses delayed GI transit in POI and opioid analgesia animal models, suggesting it may have clinical utility in reducing postoperative gastrointestinal dysfunction (POGD) following abdominal surgery.

309 | Winnie mice: A model to study sarcopenia concomitant with inflammation-induced colitis

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Background: Musculoskeletal dysfunctions are the most common peripheral manifestation in inflammatory bowel disease (IBD). The high incidence of sarcopenia (weakened and atrophied muscles) in IBD patients can lead to adverse outcomes. The mechanisms of IBD-associated sarcopenia have not been well studied, mainly due to unavailability of a reliable animal model. In *Winnie* mice, with intestinal inflammation resulting from intestinal epithelial defect conferred by a mutation in the ***Muc2*** mucin gene, the onset, symptoms and pathophysiology of disease are very similar to human IBD. We hypothesise that this mouse model of spontaneous chronic colitis is sarcopenic. To test our hypothesis, we characterised the muscle phenotype of *Winnie* mice compared to age-matched control C57BL/6.

Methods: As mild spontaneous inflammation in the colon is developed in *Winnie* mice after 6 weeks of age and results in severe colitis at age of 12-25 weeks, we analysed the muscle micro-architecture, muscle mass and oxidative capacity of soleus muscle in 6-, 15- and 25-week-old *Winnie* mice versus controls. Soleus muscles were dissected, weighed and frozen in liquid nitrogen (n = 4-5/group). Transverse sections from the belly of the muscle were cut at a thickness of 12 μm with a cryotome at −20°C. The muscle samples were then subjected to hematoxylin and eosin and succinate dehydrogenase staining procedures.

Results: *Winnie* mice had similar number of fibres, fibre-size and inter-fibre space at 6 weeks of age compared to C57BL6. At 15 and 25 weeks, higher inter-fibre space and smaller muscle area were observed in *Winnie* compared to C57BL/6 mice ($P < 0.05$). Additionally, *Winnie* mice demonstrated lower oxidative capacity at 15 and 25 weeks compared to controls, a difference was not seen at 6 weeks. More pronounced effects (lower muscle fibre size and metabolic activity) were found at 25 weeks Winnies compared to age and sex matched controls.

Conclusion: Mild spontaneous inflammation at 6 weeks of age in *Winnie* mice does not affect soleus muscle micro-structure and muscle mass. However, as this progresses to severe colitis, altered soleus muscle micro-structure, lower muscle mass and reduced

oxidative capacity are observed. Therefore, we conclude that signs of sarcopenia are prominent at the advanced stages of inflammatory disease. Further studies aiming to complete muscle phenotype and functional properties (i.e. strength) are required.

310 | The M1 muscarinic acetylcholine receptor promotes contraction of the mouse colon by activation of myenteric neurons

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Background/Objectives: The M_1 muscarinic acetylcholine receptor (M_1R) is a promising therapeutic target for cognitive decline in CNS disorders such as Alzheimer's disease and schizophrenia. Positive allosteric modulators (PAMs) of M_1R can selectively enhance M_1R signalling and have been tested in preclinical models of cognitive deficit and in human subjects. However, side-effects including those impacting the gastrointestinal tract, have limited the clinical use of M_1R PAMs for CNS disorders. M_1R PAMs have also been proposed as a novel target to treat constipation but the precise location and function of M_1R in the gastrointestinal tract remains largely unknown. We examined the effects of M_1R activation on smooth muscle contractility and myenteric neuronal activity to provide greater understanding of how M_1R exerts its prokinetic effects on gastrointestinal motility.

Methods: Segments of the colon from mice (C57BL/6J or hM_1R-DREADD) were examined. The effects of the M_1R PAM MIPS1780 or clozapine-*N*-oxide (CNO) on circular muscle contractile activity were assessed using C57BL/6J or hM_1R-DREADD tissue, respectively. Ca^{2+} transients in response to MIPS1780 (10 μM) and CNO (10 μM) were examined in myenteric plexus wholemounts.

Results: MIPS1780 and CNO evoked concentration-dependent contractions of colonic circular muscle. MIPS1780-mediated contractions were inhibited by prior incubation with tetrodotoxin (1 μM), consistent with a neurogenic mechanism of action. MIPS1780 and CNO produced Ca^{2+} transients in a subset of myenteric neurons and extraganglionic cells.

Conclusions: Collectively, these results show that M_1R is functionally expressed by myenteric neurons and other cells of the colon. The functional expression that we describe reflects the neurogenic mechanism of action observed in contraction assays. Future studies will delineate the functional distribution of M_1R in the colon and further define the mechanisms through which M_1R influences colonic motility.

312 | The cross-talk between enteric neurons and dendritic cells in the gastrointestinal homeostasis

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Background: The neuro-immune interaction plays a critical role in the maintenance of gut homeostasis. Recently, there are accumulating evidences that enteric neurons (ENs) control functions of various immune cells such as innate lymphoid cells and resident macrophages by releasing neuropeptides and cytokines. Dendritic cells (DCs), which are well known as conductor cells of innate and adaptive immune response, reportedly express some neuronal receptors and transmitters, indicating a bidirectional communication with neurons. Actually, our previous study revealed that the majority of mucosal DCs in the mouse colon were located in proximity to enteric neuronal fibers in the steady state. The purpose of this study is to further elucidate the role of this interaction in the maintenance of intestinal homeostasis under the steady state.

Method: DCs were differentiated from mouse bone-marrow cells using GM-CSF, and mature DCs (CD11c+MHCII+F4/80-) were sorted with FACSAria after the LPS stimulation. Enteric neurons were isolated from LMMP cells of the mouse small intestine, and were cultured in the neuronal medium for 8 days. In immunohistochemical analysis, cross-sections were prepared from the mouse proximal colon.

Results: Immunohistochemically, synapsin-1, a synaptic vesicle marker, was expressed along the mucosal nerve fiber and located in the adjacent to mucosal DC. Moreover, gene expression analysis revealed high expression in the enteric neurons compared to spleen and brain ($n = 5$, $P < 0.05$). In fact, the release of IL-6 protein from the cultured enteric neurons was significantly enhanced by a neuroactivator veratridine stimulation ($n = 5$, veratridine: 216 ± 32 pg/μL, control: 37 ± 7 pg/μL, $P < 0.01$). In addition, we found that IL-6 evoked the increase of calcium concentration in approximately 40% of mature DCs (35.6 ± 12.5%; 132 cells of 371 total cells).

Conclusion: Our results indicate that enteric neurons directly regulate mucosal DCs by the release of neurotransmitters or other bioactive substances. Remarkably, enteric neurons expressed IL-6 mRNA and released IL-6 protein by the neuronal stimulation, suggesting that enteric neurons utilize IL-6 as an effective mediator to modulate the mucosal immune system including DCs.

313 | Neurodegeneration and neuroplasticity in enteric neurons of DSS-colitis mouse colon

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Background/Objectives: Inflammation, infection, dysbiosis, surgery and aging can damage the enteric nervous system (ENS) throughout life, which leads to neurodegeneration in the ENS. In these cases, it is assumed that neurogenesis is induced to maintain the neuronal network not only in embryonic and early postnatal ENS but also in fully developed adult ENS. However, the underlying mechanisms are largely unknown. Thus, we aimed to investigate the neurodegeneration and neuroplasticity in the ENS of DSS-colitis mouse colon.

Methods: Male C57BL/6N mice (12-14 weeks old) were administered 2% DSS in their drinking water for 8-11 days. After DSS treatment, the longitudinal muscle and myenteric plexus (LMMP) preparations from the distal colon were used for immunohistochemistry. The distal colons were mounted in organ baths for motility studies.

Results: In the colonic motility studies, a neuroactivator veratridine (10 μM) induced contractions in the normal mouse colon, but not the colitis mouse colon. Immunohistochemical analyses revealed that the proportion of nitrergic neurons (nNOS-immunoreactive neurons) per ganglion was significantly increased in the distal colon of colitis mice compared to normal mice (Normal mice vs Colitis mice: 34.89 ± 0.89 vs 41.90 ± 0.89%, n = 6-8, $P < 0.05$). Likewise, the proportion of sox2-immunoreactive neurons (HuC/D+sox2+ cells) per ganglion was significantly elevated in the colitis mouse colon (Normal mice vs Colitis mice: 6.62 ± 0.56 vs 10.24 ± 0.66%, n = 4, $P < 0.01$). However, there were no significant differences in total neuron numbers (HuC/D+ cells) per ganglion in the colon between normal mice and colitis mice.

Conclusions: In this study, it is indicated that the colitis caused an imbalance in the enteric neural circuit composed of excitatory neurons and inhibitory neurons in the myenteric plexus, which resulted in the colonic dysmotility in colitis mice. Furthermore, the upregulation of sox2 (neural stem cell marker)-expressing myenteric neurons in the colitis mouse colon may imply that enteric neural stem cells constantly function to maintain the gut homeostasis against damages such as the enteric inflammation.

314 | Esophageal motility in patients with proton-pump inhibitor refractory gastroesophageal reflux symptoms: A study based on high-resolution impedance manometry

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Background/Objective: Refractory gastroesophageal reflux disease (GERD) symptoms despite standard proton-pump inhibitor (PPI) treatment are increasingly common in clinical practice. The underlying pathophysiology is complex and poorly understood. Therefore, with the novel high-resolution impedance manometry (HRIM), we aimed to investigate the esophageal motility in patients with PPI-refractory symptoms.

Methods: From November 2014 to August 2019, consecutive patients with persistent gastroesophageal reflux symptoms despite PPI treatment for at least 8 weeks were prospectively enrolled. All subjects filled out standard questionnaires for GERD severity and frequency. Esophageal motility was evaluated with HRIM and updated Chicago Classification. The reflux profile was evaluated with 24-hours multichannel intraluminal (MII)-pH monitoring.

Results: A total of 130 patients with refractory symptoms were analyzed. The mean age (range) was 50.3 (18-79). Fifty-four (41.5%) were male. Erosive esophagitis was found in 48 (36.9%) and most were mild in severity. The most common esophageal motility abnormalities on HRIM were ineffective esophageal motility (n = 51, 39.2%), followed by absent contractility (n = 7, 5.4%), and esophagogastric junction outflow obstruction (n = 5, 3.8%). Two patients were diagnosed with achalasia. Hiatal hernia was found in 26 patients (20%). Eighteen (13.8%) patients were found to have abnormal bolus transit (incomplete bolus transit in 30% or more swallows). Sixty-five (50%) patients had normal HRIM results. However, symptom profiles were similar between those with and without esophageal dysmotility. Sixty-four patients were studied 'off' and 66 patients 'on' PPI during MII-pH monitoring and 73 patients were diagnosed with functional heartburn accordingly. There was no significant difference of various HRIM parameters between patients with and without functional heartburn.

Conclusion: Based on HRIM, we have found a high prevalence of esophageal motility abnormalities, notably esophageal hypocontractility, in Taiwanese patients with PPI-refractory GERD symptoms. Whether add-on therapy with prokinetic agents could help to improve the treatment response in this group of patients deserve further studies.

315 | Meta-regression analysis of the association between symptom relief and acceleration of gastric emptying rate in gastroparesis

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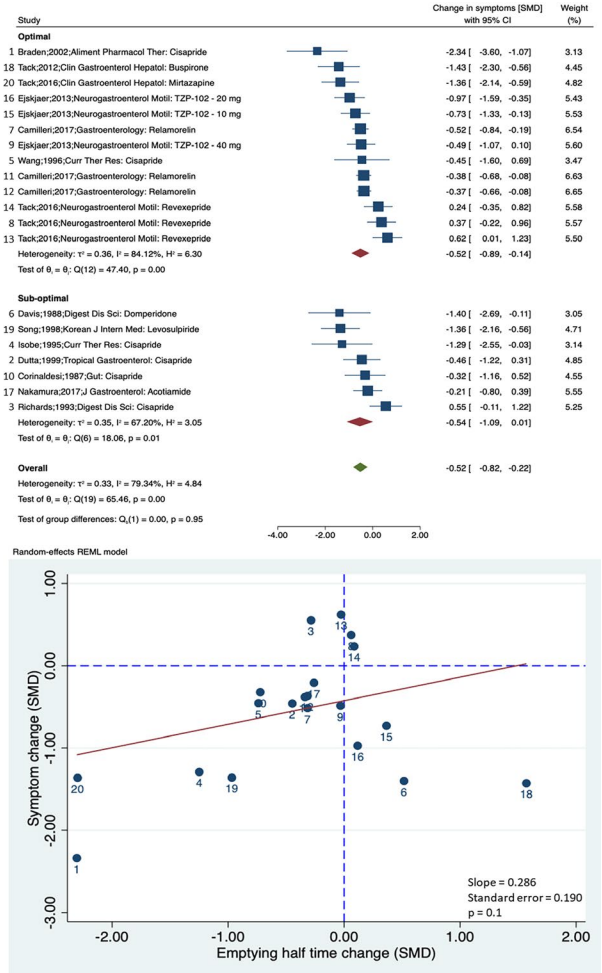
Background/Objectives: Current treatment options for gastroparesis focus on gastroprokinetic therapy, based on the hypothesis that accelerating gastric emptying (GE) will reduce symptoms. In contrast, meta-analyses failed to show a significant relation between both aspects (Sturm 1999; Janssen 2013; Vijayvargiya 2019). However, a positive association emerges when focusing on robust gastric emptying test methodologies and only a selection of prokinetic agents, thus supporting therapy aimed at stimulating gastric propulsive motility (Vijayvargiya 2019).

Aim: To update the analysis of the relation between symptom relief and acceleration of GE with the most recent controlled trial data, and synergising some of the diverging methodologies applied in previous meta-analyses.

Methods: We identified all currently available studies for determining the association between symptom improvement and acceleration of GE due to promotility agents available for long-term use in gastroparesis. Gastric half-emptying times (GET½) of solid test meals obtained by 4 hours scintigraphy or breath tests were considered. The difference in change (post–pretreatment) between treatment and placebo was expressed as standardized mean differences (SMD) for GET½ and symptoms.

Results: We identified 44 trials (5 novel papers since the last meta-analysis). When considering multiple dosing regimens within a study as separate trials, 8 additional trials were identified. From these 52 trials, 20 reported sufficient details for our analysis. As a group, prokinetic agents enhanced GE and improved symptom severity (Figure). The overall meta-regression showed no significant relation between both aspects (slope: 0.286, SE = 0.190, $P = 0.1$) although there was a tendency for accelerated emptying to move with symptom improvement. A sub-analysis restricted to studies applying optimal GE test methodology (as defined by Vijayvargiya 2019) also failed to reveal a significant correlation (slope: 0.284, SE = 0.179, $P = 0.18$).

Conclusions: Applying the methodologies used in the most recently published meta-analyses we failed to find a significant relation between symptom improvement and acceleration of gastric emptying. The observed clinical benefit with prokinetic agents depends on effects other than enhanced GE rate.



Top: Forest plot stratified by robustness of gastric emptying test methodology. Bottom: scatter plot and meta-regression line of the relation between symptom change (negative = lower symptom scores) and change in gastric emptying half time (negative = faster emptying), both expressed as standardized mean differences (SMD). Label numbers correspond with Forest plot

317 | Role of purinergic signalling and oxidative stress in a model of human colitis

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Background/Objectives: Purinergic P2X7 receptor (P2X7R) is considered critical in eliciting inflammatory responses. We previously showed that in a cytokines-induced human model of colitis, selectively blocking P2X7R significantly reduced mucosal damage and preserved tight junctions. Oxidative stress is known to contribute to the development of inflammatory bowel disease. This study aims to investigate the association between purinergic signalling and oxidative stress and their role in colitis.

Methods: The immunoreactivity of 8-oxo-dG, an oxidative stress marker, was determined in the colitis model. The generation of reactive oxygen species (ROS) from Caco-2 and cell viability were measured using the DCF-DA and resazurin assays, respectively. The Caco-2 ***monolayer*** was used cell integrity determination by measuring transepithelial electrical resistance (TEER).

Results: In the colitis model, although inhibiting P2X7R protected crypts from cytokines-induced damage, there were no discernible differences in oxidative DNA damage among groups. ATP, UTP and UDP (all 3 mM) and the selective P2X7 agonist BzATP (300 μM) caused a rapid ROS production within 2-20 minutes ($P < 0.0001$, two-way ANOVA compared to control), and the effect exerted by BzATP and UTP lasted for 48 hours. The action of BzATP was abolished by the non-selective P2X receptor antagonist PPADS (100 μM, $P < 0.0001$), and partially attenuated by P2X7R antagonists A804698, A438079 and AZ1165373 (all 100 μM, $P < 0.0001$). BzATP reduced cell viability, which was reversed by PPADS. Both BzATP and ATP increased cellular permeability, demonstrated by reduced TEERs. BzATP-induced TEER reduction was partially reversed by A-804598. Interestingly, P2X4R antagonists, 5-BDBD and PSB-12062, worsened ATP-induced cell permeability.

Conclusions: The study demonstrated that P2X7R is involved in enhancing epithelial ROS production and cell permeability, although the reduction in epithelial oxidative stress through P2X7R blockade may not be related to the protective effects seen in cytokines-induced colitis. ATP appears to have dual actions in colonic epithelial cells, causing cell damage via P2X7R and exerting cytoprotection to maintain cellular integrity via P2X4R.

318 | Impact of fundoplication following lung transplantation: A cohort study 2004-2018

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Background: Bronchiolitis obliterans syndrome (BOS) is a leading cause of chronic lung allograft dysfunction (CLAD) post-transplant, and gastro-oesophageal reflux (GOR) has been implicated as a trigger through repeated micro-aspirations. However, the only consistent human data linking GOR with BOS are interventional cohort studies which have shown improved lung function and graft survival post-fundoplication. There is no consensus on the type or timing of pH study or fundoplication. We subsequently reviewed our post-transplant fundoplication cohort, with the aim of identifying sub-groups most likely to benefit from fundoplication.

Method: Retrospective study of 431 statewide lung transplant recipients, 2004-2018. 149 with proven GOR and fundoplication included. Standard 24-hour pH monitoring, referred with reflux symptoms, declining lung function, or routine. All positive studies (AET > 4%) referred for fundoplication at one expert centre and underwent laparoscopic fundoplication (159 Toupet, 1 Nissen); 5 did not (borderline study, unfit for surgery). Transplant, fundoplication and forced expiratory volume (FEV1) databases analysed utilising *Stata* v14.

Results: Fundoplication performed median 16.7 months post-transplant (IQR 10-39.8, 2% <3 months, 7.4% <6 months, 36% <12 months). Median follow-up 40.2 (IQR 16.3-94.5) months. Of 149, 39 (9%) met criteria for BOS pre-fundoplication, 37 (8.6%) developed BOS post-fundoplication; versus 75 (17.4%) of 282 in the reference group. No BOS resolved. Time to CLAD as assessed by time-dependent Cox regression survival analysis, was not significantly different between fundoplication and reference groups HR 1.22 (95% CI 0.83-1.78, $P = 0.30$). Time-dependent analysis of graft survival suggested less benefit of fundoplication once BOS is established. Mean FEV1 did not change significantly post-fundoplication in the total (Table 1) or early (<6 months) fundoplication groups.

Conclusion: Our data suggest patients with proven GOR who underwent fundoplication experienced similar clinical outcomes to the reference lung transplant cohort. Selection bias may exist given the evolution of fundoplication practise over the study period, which we are now further investigating.

319 | Immune checkpoint markers in inflammatory bowel disease (IBD) and IBD-associated cancer

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Background: Inflammatory bowel disease (IBD) is a chronic inflammatory autoimmune condition of the gastrointestinal tract, which presents clinically as ulcerative colitis or Crohn's disease. IBD is believed to be of multifaceted origin, however, due to its unknown

Table 1. Forced expiratory volume in 1 second (FEV1) data in the fundoplication group, collected 6, 3 and 0-1 months pre-fundoplication; and 3, 6 and 12-15 months post-fundoplication (mean, SD).

	−6 months	−3 months	Pre-Fundo	3 months	6 months	12+ months
Mean	2.61	2.58	2.49	2.45	2.52	2.49
SD	0.78	0.81	0.91	0.97	0.94	0.91

aetiology, current treatment strategies target the symptoms rather than pathophysiological mechanisms and have limited efficacy, severe toxicity and adverse side-effects. Patients with IBD are at increased risk of developing colorectal cancer (CRC), for which treatment options are similarly limited due to significant gastrointestinal and neurological side-effects experienced by 80-90% of patients. Hence, there is an urgent need to elucidate mechanisms of IBD progression to CRC and identify novel therapeutic targets. Cancer development is a step-wise process involving an escape mechanism from the immune system and altered immune cell functionality. Several immune checkpoint markers have been identified, including cytotoxic T lymphocyte associated antigen 4 (CTLA4), programmed cell death-1 (PD-1/CD279) receptor expressed on the surface of activated T cells, programmed death-ligand 1 (PD-L1/CD274) and PD-L2 are commonly expressed on the surface of dendritic cells/macrophages, and an inflammatory cytokine, tumour necrosis factor alpha (TNFα), expressed on various tumour cells.

Methods: RNA was extracted from the colon tissues from IBD patients and mouse models of spontaneous chronic colitis and colitis-associated cancer and RNA sequencing was performed. Data were analysed using R programming language. The enrichment GO terms were identified using GOrilla and DAVID web-based tools. Upregulated and downregulated genes were compared between all groups of mouse models versus controls and also compared with IBD patients and correlation analysis was performed.

Results: CTLA-4, PD-L1, TNF were highly upregulated. The other upregulated genes were CD74, CD14, CD80, IDO1, Mmp9, Mmp10, Mmp12 and Mmp13. The downregulated genes are CD248, CD163, CD109, CD209. Multiple markers were up and downregulated when compared between controls and spontaneous chronic colitis mouse model. The results correlated with IBD patients' data.

Conclusion: This project brings significant in vitro and in vivo data which identifies novel checkpoint markers at all stages of inflammation and cancer (chronic to severe to cancer), which will lead to advancement of understanding of inflammation-induced cancer and design novel therapies to prevent disease progression.

321 | Compositional changes of gut microbiota according to the response to probiotics treatment in patients with diarrhea predominant irritable bowel syndrome

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Background: We aimed to evaluate compositional changes of gut microbiota after probiotics treatment and examine the differences of gut microbiota between responders and non-responders to probiotics in diarrhea dominant irritable bowel syndrome (IBS-D) patients.

Methods: In our previous study, we had demonstrated the therapeutic effect of probiotic mixture (*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium breve*, *Bifidobacterium lactis*, *Bifidobacterium longum*, and *Streptococcus thermophilus*, 1.0×10^9 CFU) in IBS-D patients. We had collected fecal samples from 45 IBS-D patients consisted of probiotics treatment group (n = 24) and placebo group (n = 21) before and after 8-week treatment. The probiotics group was divided into responders (n = 12), defined as patients who experienced adequate relief on overall IBS symptoms for at least half of the 10-week study period (8-week treatment and 2-week post-treatment), and non-responders (n = 12). Fecal bacterial taxonomic composition and diversity were investigated by Illumina MiSeq and analyzed by the Ezbiocloud pipeline comparing with data from healthy controls (HC, n = 40).

Results: Bacterial community alpha-diversity was significantly lower in IBS-D patients than in HC, and beta-diversity was also significantly different between the two groups. In IBS-D patients, the alpha- and beta- diversities were not significantly changed after treatment in both probiotics and placebo groups, but also not different according to the treatment response. However, the abundance of lactic acid bacteria including *Bifidobacterium* became higher in the probiotics group compared to the placebo group after treatment. The increase of abundance was more prominent in responders than non-responders. After probiotics treatment, *Fusicatenibacter saccharivorans* was more significantly increased in responders compared to non-responders ($P = 0.03$) despite no difference before treatment. In linear discriminant analysis (LDA) effect size algorithm, *Fusicatenibacter saccharivorans* showed the highest LDA effect after probiotics treatment in responders (4.27, $P = 0.03$) without significant change in non-responders. Non-responders showed significantly higher abundance of *Enterococcus faecalis* ($P = 0.02$) and *Lactococcus* lactis ($P = 0.04$) compared to responders and HC before probiotics treatment.

Conclusion: Probiotics can change some bacterial taxa including lactic acid bacteria. *Fusicatenibacter saccharivorans* could lead to novel probiotics for IBS-D patients. *Enterococcus faecalis* and *Lactococcus lactis* can be predictive species for non-response to probiotics in IBS-D patients.

322 | Randomised clinical trial: A double-blind, randomised, active-controlled, phase 3 study to evaluate the safety and efficacy of Tegoprazan in patients with gastric ulcer

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Background: Tegoprazan, a novel potassium-competitive acid blocker (P-CAB), is expected to demonstrate non-inferiority to

lansoprazole in efficacy and safety following oral administration in patients with gastric ulcer (GU).

Aims: To assess the non-inferiority of tegoprazan to lansoprazole in efficacy and safety in patients with GU.

Methods: In this Phase 3, double-blind, active-controlled, multicentre study, 306 GU subjects were randomised to one of three treatment groups: tegoprazan 50 mg, tegoprazan 100 mg and lansoprazole 30 mg once daily for 8 weeks.

Results: The cumulative endoscopic healing rates at week 8 were 100% (88/88) for tegoprazan 50 mg group, 97.85% (91/93) for tegoprazan 100 mg group, and 100% (85/85) for lansoprazole 30 mg group. At week 4, healing rates were 95.45% (84/88) for tegoprazan 50 mg group, 94.62% (88/93) for tegoprazan 100 mg group, and 92.94% (79/85) for lansoprazole 30 mg group. The differences between each dose group of tegoprazan and lansoprazole were statistically significant (P = 0.050) at both time points according to Hochberg method. There was no significant difference in incidence of treatment-emergent adverse events among treatment groups as the percentages were 17.65% (18/102) for tegoprazan 50 mg, 22.55% (23/102) for tegoprazan 100 mg, and 25.00% (25/100) for lansoprazole 30 mg.

Conclusions: The non-inferiority of tegoprazan at both 50 mg and 100 mg to lansoprazole 30 mg was verified in patients with GU.

Keywords: gastric ulcer, peptic ulcer, potassium-competitive acid blocker, tegoprazan

323 | Intercommunication between smooth muscle cells and enteric nervous system cells in tissue engineering of functional muscle layers

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Introduction: Short bowel syndrome (SBS) represents, due to the loss of a large part of the bowel, a devastating and expensive problem in human health care. In such patients the intestine is too short to reabsorb sufficient nutrients. For survival, these patients require daily parenteral nutrition for many years, in several cases for the rest of their life. However, SBS and long-term parenteral nutrition are associated with many severe life-threatening complications. By intestinal transplantation in five to seven years more than 60% of transplants are rejected. The new hope for such patients could be an artificially engineered small bowel.

Materials and Methods: ENS cells were isolated from the gut of rats as previously described (Schäfer K.H. et al, 1997). ENS cells and commercially available Rat Primary Small Intestinal Smooth Muscle Cells were cultured within a different 3D-hydrogel scaffold. The cultures were arranged in individual layers. We tested different environmental factors.

Results: The cells survived and developed interactions between the cells and contraction of muscle cells.

Cultivation of smooth muscle cells in close contact with ENS cells results in a large amount of wide muscle fibres with contractive activity. Besides muscle cells, the muscle fibres consisted from a lot of neurons, which were located in groups of cells, as well as a single cell with long intercommunicated processes. We observed specific ENS-muscle cells intercommunications. Muscle fibers ENS cells are located in the muscle bundles and have not only close contact with muscle cells, but also form the 3D-neuronal net within the muscle layers. Immunohistological studies revealed a close contact between ENS and muscle cells in such manner that ENS cells guide the direction of muscle cells, which are, in our view, crucial for the creation of functional muscle layers.

Conclusion: Intercommunication between smooth muscle and nerve cells in the gut is essential for engineering of artificial functional muscle fibres.

325 | Altered slow-wave pressure activity in low anterior resection syndrome: New mechanistic insight into pathophysiology?

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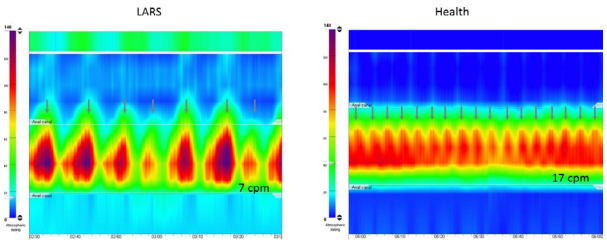
Objective: Up to 90% of patients undergoing low anterior resection for rectal cancer experience post-operative disordered bowel function (e.g. faecal urgency, incontinence, and difficulty in rectal evacuation), termed low anterior resection syndrome (LARS). Pathophysiology of LARS is primarily characterised by impaired (neo)rectal and anal sphincter function. Nevertheless, with regard to the latter, traditional anorectal manometry (ARM) measures (rest and squeeze) are only abnormal in a proportion. Slow-wave pressure activity, reflecting myogenic activity of anal smooth muscle cells, can also be measured using ARM. Hypothetically, iatrogenic damage to the internal anal sphincter might affect slow-waves. The aim of this study was to determine whether slow-waves are altered in LARS patients.

Methods: Twelve consecutive male patients referred to the Royal London Hospital GI Physiology Unit (2014-19) for investigation of symptoms of LARS after low anterior resection for rectal cancer underwent anorectal physiology testing, including high-resolution ARM. Patients with an observed anal slow-wave frequency clearly deviating from the previously published normal frequency of 16-18 cycles per minute (cpm) were selected for detailed analysis. The control group consisted of 37 healthy males.

Results: Aberrant anal slow-waves were demonstrated in 5/12 (41.7%) LARS patients (example: Figure). The frequency was strikingly different qualitatively (i.e. visually) compared to controls (example: Figure). Quantitatively, in controls, peak frequencies were concentrated at 16 cpm and at 3-4 cpm. By contrast, peak frequencies in patients only occurred around 6 cpm. Statistical comparison showed that in patients compared to controls, frequencies around 6 cpm were of significantly greater average amplitude, and higher frequencies (>16 cpm) were significantly reduced.

Conclusion: Aberrant anal slow-wave activity is visualised in some patients with LARS. Whether this results from direct or indirect damage to the internal anal sphincter and impacts function warrants further investigation.

Figure. Example of slow-wave pressure activity in a LARS patient (frequency of 7 cycles per minute) and in a healthy male individual (frequency of 17 cycles per minute).



326 | Dissecting molecular requirements for serotonin release from mouse enterochromaffin cells

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Background/Objectives: Communication between the gastrointestinal tract, the enteric nervous system, the body, and the brain plays an important role in regulating physiology and behaviour, and accordingly, impairments in these signalling routes have been implicated in the pathogenesis of multiple disorders including obesity, inflammatory bowel diseases and irritable bowel syndrome. A better understanding of the fundamental molecular and cellular mechanisms that control these complex biochemical signalling processes in health and disease will therefore be critical for treating these disorders.

Methods: An important group of cells in this context are enterochromaffin (EC) cells, which function as mechano- and chemoreceptors and signal by secreting serotonin, however the molecular control of this release process is poorly understood. To address this question, we have established a multidisciplinary approach combining mouse genetics, intestinal 3D organoid and 2D monolayer cultures, light- and electron microscopy, and electrochemistry, that allow us to monitor the serotonin release process with extremely high spatiotemporal resolution and – importantly – on the single-cell level.

Results: We show here that EC cells release serotonin from large-dense core vesicles that fuse in response to increases in the intracellular calcium concentration with kinetics similar to that of other neurosecretory cells, namely catecholaminergic adrenal chromaffin cells. We further provide functional evidence that the vesicle fusion process is mediated by key molecular regulators of the presynaptic neurotransmitter release machinery of neurons in the brain.

Conclusions: In this study, we present and validate a methodological workflow that will ultimately allow us to monitor the effects of pharmacological agents designed to boost or inhibit serotonin production/release in EC cells with extremely high spatiotemporal resolution. Our research therefore contributes to the ongoing efforts to device new therapeutic strategies aimed at altering serotonin signalling in disease.

327 | Targeting the CCR3-eotaxin axis mitigates enteric neuropathy and gastrointestinal dysfunction in inflammatory bowel disease

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Background: Inflammatory Bowel Disease (IBD) is a severe debilitating chronic intestinal inflammation affecting millions of people worldwide. Current treatments have limited efficacy and associate with significant adverse effects. Our previous findings in chemically-induced colitis provide strong evidence that activation of CCR3-eotaxin axis involved in eosinophil chemotaxis and Th-2 immune response correlates with disease activity and GI dysfunction (Filippone et al., 2018). This study aimed to elucidate the role of eosinophil infiltration in human IBD pathogenesis and in the preclinical *Winnie* murine model of spontaneous chronic colitis closely representing human IBD. The use of a potent CCR3 receptor antagonist (SB328437) on intestinal inflammation and GI functions was investigated in the *Winnie* murine model of spontaneous chronic colitis.

Methods: Colon samples from IBD patients and age-matching controls were obtained from Victorian Cancer Biobank. Colonic samples were processed to determine the role of eosinophils and their products in inducing enteric neuropathy via organotypic culture experiments and immunohistochemistry. Animals were treated with the CCR3 receptor antagonist, SB328437, 3 times a week for a duration

of 2 weeks. Prior to euthanizing animal, a non-invasive radiographic method was employed to record GI transit time. The characterization of infiltrating leukocyte, eosinophils and T cell populations in the colon were performed by flow cytometry. Parameters of intestinal motility were assessed in *ex vivo* whole organ bath experiments.

Results: Our data demonstrated that eosinophil infiltration coincides with loss of myenteric neurons in human IBD and in *Winnie* mice. Ample eosinophil infiltration was inhibited by SB328437 treatment in *Winnie* mice. Enteric neuropathy and alterations to colonic innervation were correlated with eosinophil infiltration adjacent to fibers and myenteric neurons in colon tissues from both IBD and *Winnie* mice. Treatment with SB328437 provided strong neuroprotective and anti-inflammatory effects and alleviated intestinal dysmotility in *Winnie* mice.

Conclusion: Eosinophil-associated damage to myenteric neurons leads to chronic intestinal dysfunctions. Targeting eosinophils and CCR3-eotaxin axis hinders inflammation-induced changes to myenteric neurons in the pre-clinical model of intestinal inflammation.

328 | Characterizing the recto-anal inhibitory reflex parameters in spinal cord injured subjects compared to published healthy control values

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Introduction: The Recto-Anal Inhibitory Reflex (RAIR) contributes to defecation physiology. RAIR is the internal anal sphincter (IAS) response to rectal distention. RAIR is controlled by autonomic parasympathetic response and closely coordinated with the extrinsic somatic neurologic system. Limited data are available exploring RAIR responses in spinal cord injuries (SCI) subjects using High Resolution-Anorectal Manometry (HR-ARM). (i) Compare RAIR parameters in SCI patients vs. published healthy control (HC) values (*Thiruppathy, K. (2012). DDS.*) (ii)Explore correlations and associations between RAIR parameters and SCI injury duration, SCI location, and AIS levels.

Methods: Prospective analysis of SCI subjects who underwent HR-ARM at a single tertiary center, July–September, 2019. RAIR was tested by rapidly inflating 50 mL into the rectal balloon and immediately deflating. RAIR presence = ≥25% of IAS (mmHg) amplitude reduction. RAIR parameters included: IAS resting pressure (mmHg), reflex duration [RD] (seconds), and amplitude reduction [AR] (%). SCI subject's RAIR parameters were compared to published HC. Subjects were categorized by AIS level (cervical vs. thoracic) and completeness of injury (A–E). Bivariate calculations were performed. A *P*-value of ≤0.05 was considered significant.

Results: 22 SCI (cervical = 15; thoracic = 7) subjects underwent HR-ARM. Demographics included: 77.3% male, 95.5% Caucasian, mean age of 46.6 (SD = 13.3; Range: 24-75), and mean BMI of 26.8 (SD = 7.4; Range: 16.9-41.2). The mean duration of SCI was 17.6 years (SD = 12.6 years; Range: 3-45.8 years). AIS levels consisted of: A = 57.1%, B = 23.8%, C = 0%, D = 19.0%, and E = 0%. SCI subjects have significant mean differences, RD (*P* = <0.001), and AR (*P* = <0.001), compared HC; no significant mean differences in IAS pressure. No significant correlations between SCI injury duration and IAS (*r* = -0.17; *P* = NS), RD (*r* = 0.31; *P* = NS) and AR (*r* = -0.09; *P* = NS). No significant mean differences between SCI level and the AIS completeness within RAIR parameters.

Discussion: Cervical and Thoracic SCI subjects elicit RAIR parameters. Minimal scientific evidence demonstrating the use of digital rectal stimulation (DRS) as an effective method of promoting IAS pressure relaxation for stool evacuation despite routine clinical recommendation as a method of bowel management. Findings of altered RAIR response on HR-ARM for SCI subjects provides scientific basis to use DRS for bowel evacuation. HR-ARM may be used to determine the most effective protocol for DRS in bowel management.

329 | Chemotherapy-induced enteric neuropathy and gastrointestinal dysfunction: Mechanisms and novel treatments

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Background: Chemotherapy-associated gastrointestinal side-effects such as nausea, vomiting, diarrhoea, constipation are experienced by 80-90% of patients and limit the dose of chemotherapy reducing

Table 1: Recto-Anal Inhibitory Reflex (RAIR) Parameters in Spinal Cord Injured (SCI) Subjects Compared to Published Healthy Control Values

RAIR parameter	Published healthy controls(n = 21)	Spinal cord injured subjects(n = 22)	*P*-value
Internal Anal Sphincter Resting Pressure (mmHg)	87.0 (SD = 35.0)	87.2 (SD = 37.6)	0.98
Mean Reflex Duration (s), [SD]	5.0 [SD = 2.4]	11.5 [SD = 5.8]	<0.0001
Amplitude Relaxation (%) [SD]	76.0% [33.0%]	54.5% [SD = 22.0%]	<0.001

the efficacy of anti-cancer treatment. Chronic intestinal dysfunction persists more than 10 years post-treatment in most cancer survivors. Persistence of gastrointestinal symptoms long after treatment suggests long-term damage to gastrointestinal innervation. We aimed to study mechanisms underlying damage to gastrointestinal innervation, and test therapies alleviating chemotherapy-induced enteric neuropathy and gastrointestinal dysfunction.

Methods: Fresh colon specimens from non-obstructive colorectal carcinoma patients treated and untreated with chemotherapeutic agents and animal models of colorectal cancer treated with anti-cancer drugs were used in this study. Intracellular electrophysiology, immunohistochemistry, *in vivo* gastrointestinal transit, *ex-vivo* colonic motility and secretion, qPCR and Western blot were used.

Results: Neuronal hyperexcitability and morphological changes of enteric neurons were prominent in colon samples from chemotherapy-treated patients compared to untreated controls. In samples from chemotherapy-treated patients we observed hyperexcitability of myenteric neurons, increased number of neurons with nuclear translocation of Hu protein and increased soma size of nNOS-immunoreactive neurons. Damage and death of submucosal and myenteric neurons were observed in mice treated with chemotherapeutic agents, oxaliplatin, 5-fluorouracil (5-FU) and irinotecan, commonly used in clinic for colorectal cancer treatment. Severe axonal damage, changes in glial cells and in neuromuscular transmission persisted long after cessation of chemotherapy when mucosal damage has subsided. Enteric neuropathy was associated with long-term changes in gastrointestinal transit, colonic dysmotility, changes in neuromuscular transmission, chronic diarrhoea (5-FU, irinotecan-treated), chronic constipation (oxaliplatin-treated), lack of weight gain and pica (equivalent to nausea and vomiting in humans). Co-treatment of cancer-baring mice with oxaliplatin and novel compounds targeting oxidative stress pathway attenuated enteric neuropathy and gastrointestinal dysfunction and potentiated anti-cancer efficacy of oxaliplatin.

Conclusions: Our studies revealed that chemotherapy-induced oxidative stress, direct toxicity and inflammation leading to mitochondrial and nuclear damage contribute to enteric neuropathy in both humans and animal models. Our results provide strong evidence that novel treatments targeting oxidative stress pathway attenuate enteric neuropathy and gastrointestinal dysfunction without reducing efficacy of anti-cancer treatment.

330 | Treatment expectancies, but not psychological distress, predict treatment response to the low FODMAP diet in individuals with functional gut symptoms

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The low fermentable oligosaccharide, disaccharide, monosaccharide and polyol (FODMAP) diet has shown efficacy in functional gut symptoms (FGS) at the group level, but predictors of treatment response at the individual subject level are lacking. This study aimed to investigate whether expectancy about treatment effect and various indicators of psychological distress would positively or negatively predict treatment response. Adults (>18 years) were recruited from dietetic practice clinics and via social media. Participants completed questionnaires at 3 time points; pre-dietitian appointment, immediately post and 4 weeks post initial consult. Figure 1 shows the timeline of data collection and contact with the dietitian.

Baseline depressive, anxiety, somatic and GI-specific anxiety were measured using visual analogue scales (0 = poor/none; 100 = greater) and Likert scales. Expectancy about treatment effects were measured using a modified version of the Credibility/Expectancy Questionnaire. The outcome measure, irritable bowel syndrome symptom severity score (IBS-SSS), was analysed in a linear mixed model including the main effect of the baseline psychological variable, the effect of time, and their interaction effect. 35 participants (85% F) aged 35 ± 13.3 years were included. Levels of depressive, general and GI-specific anxiety and somatic symptoms at baseline were significantly associated with higher baseline levels of IBS symptoms (main effects all $P < 0.005$), however no baseline variables were associated with treatment response (interaction effects all $P > 0.25$). Higher baseline treatment expectancy was associated at trend level with lower baseline levels of IBS symptoms ($\beta = -20.64 \pm 10.93$, $P = 0.068$), and with a stronger treatment response ($\beta = -3.84 \pm 2.22$, $P = 0.093$). Higher baseline treatment expectancy feelings were not significantly associated with baseline levels of IBS symptoms ($\beta = -9.42 \pm 11.21$, $P = 0.41$), but significantly predicted treatment response ($\beta = -4.80 \pm 2.26$, $P = 0.041$). Expectancies about treatment effects predict treatment response to the low FODMAP diet in FGS independent of psychological distress. Boosting treatment expectancies about the low FODMAP diet may boost its efficacy.

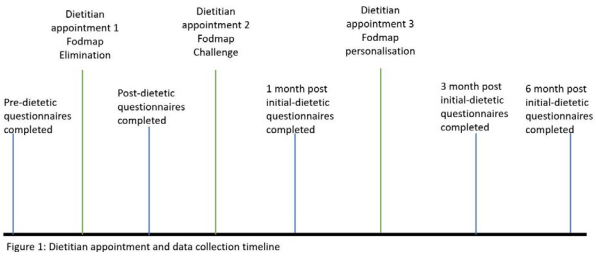

Figure 1: Dietitian appointment and data collection timeline

331 | Discrepancy between symptom and manometric findings in patients with post-myotomy Achalasia

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Background/Objectives: In 1913, Heller performed a myotomy for esophageal achalasia. Recently, new techniques such as laparoscopic surgery or per oral endoscopic myotomy (POEM) are developed. On the other hand, when patients present symptoms such as dysphagia after myotomy, there are currently no guidelines about decision for re-myotomy. To resolve this issue, we examined the clinical features of achalasia patients who presented symptoms after myotomy.
Methods: Achalasia patients who presented symptoms after myotomy were evaluated by high-resolution esophageal manometry (HRM) using a 36ch Unisensor catheter. As a cut-off value for impaired lower esophageal sphincter relaxation, 26 mmHg of integrated relaxation pressure (IRP) was used (Neurogastroenterol Motil. 27: 188-94). This retrospectively was approved by ethics committee (No. 20150081).
Results: 18 cases (male: female = 9: 9, average age 55.2 ± 19.4 years) were analyzed. The methods of myotomy were 1 laparotomy, 11 laparoscopic surgery, and 6 POEM. The average duration between myotomy and HRM was 4.4 ± 8.6 years. According to the findings of HRM, the average value of IRP was 14.6 ± 6.8 mmHg, and only one case exceeded the cut-off value of 26 mmHg. The subclassification of esophageal achalasia based on the Chicago classification were 7 cases of type I, 11 cases of type II, and 0 cases of type III.
Conclusions: In many cases, the effect of myotomy persisted. For decision of re-myotomy, HRM should be performed.

332 | Accurate diagnosis of "Zhang": An overlooked challenge in Chinese clinical setting

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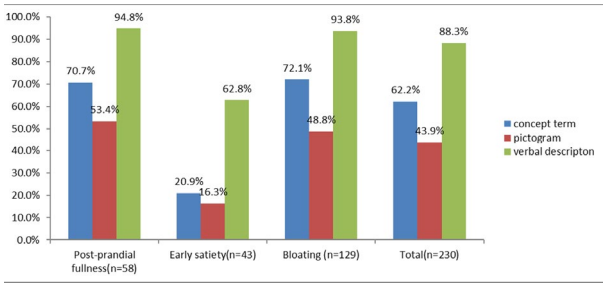
Background: Symptom understanding is of vital importance for diagnosis of functional gastrointestinal disorders. However, understanding of fullness, early satiety, bloating and distention were easily confused worldwide. In China, all the four symptoms were commonly expressed as a single chief complaint, "Zhang".
Aim: To explore the most efficient method to acquire characteristics of symptoms from patients complaining about "Zhang".
Method: Consecutive patients with the chief complaint of "Zhang" were included. Their symptom patterns were determined by face-to-face interview. Patients with single symptom (post-prandial fullness, early satiety, bloating, or abdominal distention) finished the questionnaire assessing their understanding about the symptoms through concept terms, pictograms and verbal descriptions respectively.
Result: A total of 230 patients (121 males, 109 females; mean age 43.7 ± 12.6 years) were involved. Among all subtype symptom groups, verbal descriptions were the best applicable method for patients to identify their real symptom patterns, followed by concept terms, and pictograms. Among different symptom patterns, early satiety was the most difficult to be identified (Only 20.9% by concept term), while fullness and bloating enjoyed apparently higher recognition (70.7% and 72.1% by concept term). Patients with elder ages (>40 years old), poorer education (junior high school education or less) showed relatively poorer understanding.
Conclusion: Patient's understanding of "Zhang" varies greatly. Existing pictograms did not represent comprehension-improving features under Chinese cultural setting. Verbal descriptions may be the best option for doctors diagnosing Chinese patients with "Zhang". More caution and patience were required during history taking from patients with elder age and low educated experience.



333 | Electrical stimulation can delay fluid propulsion during motor complexes in mouse colon

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Background: The propulsion of content along the colon is a well-conserved mechanism that integrates mechanical, neural, and contractile elements [1]. During colonic motor complexes (CMCs) in mouse colon, the discovery of synchronized junction potentials in smooth muscle over 7 mm suggested temporal coordination of enteric neural activity [2]. This was supported by calcium imaging [3], where synchronized neural activity at 2 Hz was found to generate 2 Hz oscillations in smooth muscle synchronized across large spatial

fields. The objective of this study was to determine if the propagation of CMCs persists when the spatial coordination of ENS activity is disrupted by electrical stimulation.

Methods: C57BL/6J mice were killed by isoflurane as approved by the Animal Welfare Committee of Flinders University, and the entire colon was emptied and placed in an organ bath. Fluid distension evoked CMCs, measured by extracellular electrophysiology and diameter-mapping. Extracellular stimulation was applied with 400-μs pulses at 20 Hz for 5 seconds. Contractions were video imaged to calculate apparent and actual velocity.

Results: Electrical stimulation to the colon did not block propagation of the contraction. Stimulation temporarily halted the contraction at onset of stimulation, but propagation resumed when the stimulus train ceased (Figure 1a). Contraction propagation was halted for the duration of stimulation (Figure 1b). The apparent velocity was unchanged with stimulation, however the actual propagation velocity after stimulation was significantly greater than before stimulation (Figure 1c).

Conclusions: Electrical stimulation and disruption of 2 Hz oscillatory patterns did not occlude contraction propagation, however it did temporarily halt propagation for the duration of stimulus. ENS stimulation may by a future application to control GI transit.

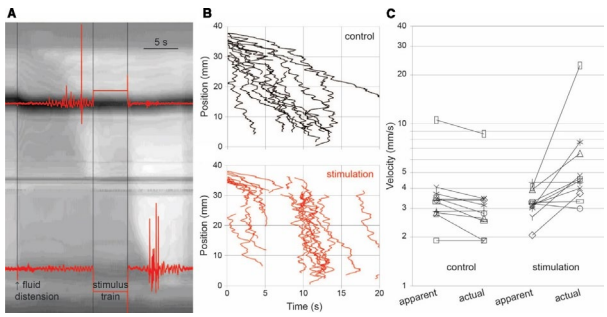

Figure 1. Propagating contraction in the mouse colon during electrical stimulation. (A) Representative recording, (B) individual contraction traces, and (C) quantification of contraction velocity.

References:

1. Costa, M., et al., *J. Physiol*, 2019. 597(20): p. 5125-5140.
2. Spencer, N.J., et al., *J. Physiol*, 2005. 564(3): p. 829-847.
3. Spencer, N.J., et al., *J. Neurosci*, 2018. 38(24): 5507-5522.

334 | Effect of different bitter compounds and route of administration on hunger feelings and orexigenic hormones

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Background/Objectives: As a result of our Western lifestyle, pharmaceutical alternatives for overweight and obesity are indispensable. For over 6 years, bitter tastants were reported to suppress hunger, but the published literature lacks consistency in methods and findings. This study aimed to establish which agent should be used (denatonium benzoate (DB) or quinine hydrochloride (QHCl)); where it should be administered (intragastrically (IG) or intraduodenally (ID)); and whether these effects are explained by changes in motilin and acyl-ghrelin plasma levels.

Methods: Fourteen healthy female volunteers (age: 22 ± 0.7 years; BMI: 21.7 ± 1.2 kg/m^2) participated in a randomized, placebo-controlled, six-visit crossover study. After an overnight fast, DB (1 μmol/kg), QHCl (10 μmol/kg) or placebo were given IG or ID via a naso-gastric or naso-intestinal feeding tube. Blood samples were taken 10 minutes prior to administration and every 10 minutes after for a period of 2 hours. Hunger was scored at the same points on a visual analogue scale (VAS). Delta values were calculated from baseline, and analysed using mixed models. Multiple testing was adjusted by stepdown Bonferroni.

Results: Delta motilin values were unaffected after ID administration of any condition. IG administration showed an inhibition of motilin release, specifically for QHCl at 50-70 minutes after infusion (padj = 0.046), but not for DB (padj = 0.766).

Delta acyl-ghrelin values were unaffected after ID administration, while IG administration suppressed these. DB significantly reduces acyl-ghrelin levels at 50-70 minutes (padj = 0.021), which was not observed with QHCl (padj = 0.132).

Hunger sensations increased progressively during the fasted state. ID administration had no effect on hunger ratings, while IG_QHCl tended to suppress the hunger increase, especially between 50-70 minutes after infusion (padj = 0.098). This effect was less clear for IG_DB (padj = 0.123) (see Figure).

Conclusion: The stomach is the active site for bitter tastants to reduce motilin and hunger. Quinine-like compounds have the highest potential to reduce hunger. The ideal window to affect food intake occurs between 50-70 minutes after infusion, which confirms earlier results from our group.

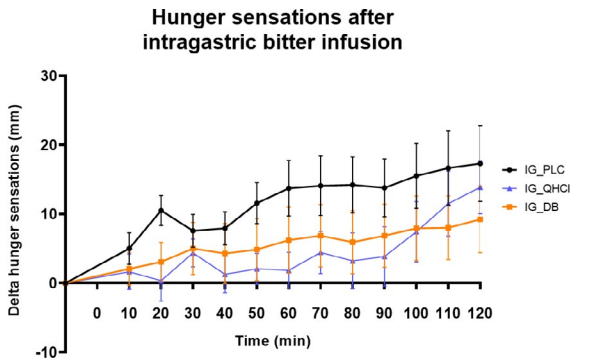

Figure – Effect of intragastric infusion of placebo (IG_PLC), quinine hydrochloride (IG_QHCl) and denatonium benzoate (IG_DB) on hunger sensations (n=14). Data is presented as delta hunger sensations adjusted with time point -10 as baseline, analyzed by mixed models and represented as mean±SEM

336 | Criteria for rumination revisited

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Background: Rumination is defined by the ROME IV criteria as the repetitive, effortless regurgitation of recently ingested food into the mouth. These episodes are preceded by a rise in intragastric pressure (IGP) and mainly occur postprandially. Kessing *et al.* (Am J Gastro, 2014) proposed IGP peaks >30 mmHg as a cutoff to differentiate rumination from reflux events. In clinical practice, we observed that this cutoff, which does not consider esophago-gastric junction (EGJ) resistance, is often not reached and therefore rumination episodes are missed.

Methods: We studied 22 patients with clinically confirmed rumination syndrome (RS) [mean age 38.8, 59% female] and 24 gastro-esophageal reflux disease (GERD) patients [mean age 41.2, 54% female], who underwent a high-resolution impedance manometry with meal ingestion. For each rumination, reflux or abdominal straining episode the following parameters were registered: maximal IGP, IGP and EGJ pressure preceding the respective episodes. We defined the gastro-sphincteric pressure gradient (GSPG) as the difference between IGP and EGJ pressure immediately prior to the rumination/reflux/abdominal straining episode. EGJ resting pressure was measured during both the pre- and postprandial period.

Results: EGJ resting pressure decreases significantly after the meal ($P < 0.0001$), both in RS and GERD patients, without difference in both groups. None of the reflux episodes were characterized by a maximal IGP >30 mmHg. However, also in 117 of the 420 rumination episodes (27%) the maximal IGP did not reach 30 mmHg. Median GSPG was positive for rumination episodes and significantly higher compared to abdominal straining and reflux episodes (3.0 ± 11.2 vs −3.0 ± 11.1 vs −1.0 ± 3.9; $P < 0.0001$).

Conclusion: Applying the previously proposed cutoff of 30 mmHg missed 27% of the rumination episodes. We found that the GSPG differs between rumination episodes (positive GSPG), abdominal straining without rumination (negative GSPG) and reflux events (GSPG around 0). Based on these results we propose a positive GSPG as a new determinant to distinguish rumination from reflux episodes and abdominal straining.

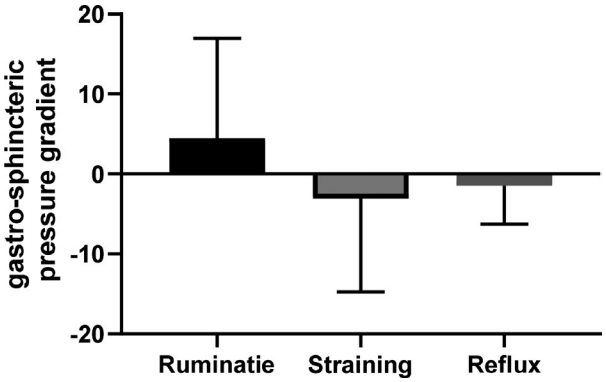

Figure 1. Median GSPG was significantly higher in rumination episodes compared to abdominal straining and reflux episodes ($P < 0.0001$).

337 | Recognising pseudoachalasia on high-resolution manometry

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Background: Pseudoachalasia, with signs & symptoms that mimic primary idiopathic achalasia, is rare. Causes include secondary malignant (70%) or benign (30%) etiologies. Recent cases where manometry raised suspicion of pseudoachalasia beckons examination of risk factors and role of high-resolution manometry (HRM) in recognising pseudoachalasia.

Methods: In this prospective series, patients with a manometric diagnosis of achalasia or pseudoachalasia were analysed for clinical indications, age, duration of dysphagia symptoms, dysphagia score (Dakkak & Bennett, 0-45, 0 = none); weight/ weight loss, surgical history, endoscopic, fluoroscopic and HRM findings. Patients with outflow obstruction in the presence of primary peristalsis or large hiatus hernia were excluded.

Results: HRM was performed in 422 patients, with 10 cases of idiopathic primary achalasia (5 M/5 F; mean age: 48 years, range 20-82) and 7 cases of suspected pseudoachalasia (4 M/3 F; mean age: 66 years, range 49-80). Manometry showed aperistalsis or absence of normal peristalsis and absent or incomplete OGJ relaxation. For three patients, prior surgery made benign pseudoachalasia likely (lap band; fundoplication). At the time of manometry, prior endoscopy found no reason for dysphagia in four patients, so the cause of pseudoachalasia was unknown. Following HRM, review of the oesophago-gastric junction (OGJ) was recommended, including OGJ biopsies; CT chest/abdomen or endoscopic ultrasound for possible underlying pathology. Subsequently, malignant pseudoachalasia was diagnosed in three patients: OGJ adenocarcinoma (58 M); signet cell adenocarcinoma OGJ (80 F) and an atypical, indolent oesophageal adenocarcinoma (61 M). The remaining pseudoachalasia case (72 M) had a negative CT and at operation unusual muscle layers, atypical for primary achalasia. Malignant (cf. benign) pseudoachalasia had shorter history of dysphagia (mean 1.2 vs. 10.7 years); >10 kg weight loss; with similar age (mean 66.3 vs. 65.8 years) & dysphagia severity (mean 32 vs. 25). While HRM achalasia-like manometric patterns did not fit with achalasia subtype 1-3, Chicago classification.

Conclusions: Recognition of pseudoachalasia requires careful appraisal of high-resolution manometry, as it mimics primary idiopathic achalasia imperfectly. Benign pseudoachalasia is recognised by a discordance of manometry findings with a patient's clinical history.

Importantly, patients with unclassified achalasia-like motility, a short history of dysphagia; and significant weight loss warrant prompt investigation for possible malignant pseudoachalasia.

338 | Clinical and immunomodulatory effects of transcutaneous vagal nerve stimulation for idiopathic gastroparesis

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Gastroparesis, a chronic gastrointestinal motility disorder characterized by delayed gastric emptying, chronic abdominal pain, nausea, and vomiting, remains a largely unexplained disease, with a rising 3-fold incidence in hospitalizations and 10-fold associated healthcare costs. Given that autonomic dysfunction and immune dysregulation have been associated with gastroparesis, vagal nerve stimulation (VNS) has been become an attractive therapeutic target for gastroparesis. Unfortunately, prior methods for VNS require invasive surgical placement of electrodes and electric stimulators.

Objective: To test the therapeutic and immunomodulatory potential of the GammaCore cervical transcutaneous vagal nerve stimulator in patients with idiopathic gastroparesis in a pilot prospective study.

Methods: Primary endpoint was symptom improvement assessed by reduction of >0.8 in the gastroparesis cardinal symptom index (GCSI) score. Secondary endpoints included, four hour gastric emptying by breath testing, and immune profiling of the gastric mucosa and blood by cytokine multiplex and flow cytometry before and after therapy.

Results: Fifteen patients have completed the study. Twice-daily bilateral non invasive VNS (nVNS) therapy (median 35 days, 28-45) led to a total improvement in symptom scores (2.56 ± 0.76 to 1.87 ± 1.05; $P = 0.01$), with 6/15 (40%) participants meeting our primary endpoint. Therapy was associated with a reduction in gastric emptying ($T^{1/2}$ 155 minutes vs 129 minutes; $P = 0.053$, CI −0.4 to 45). Immune profiling showed significant increases in myeloid and lymphoid subsets of the gastric mucosa, as well as plasma and tissue chemokines and cytokines. nVNS therapy reduced infiltrating CD4 T cells, and clinical responders were distinguished by higher baseline levels of select cytokines.

Conclusions: Our results are in agreement with prior experiences using implantable gastric stimulators and clinical data showing anti-inflammatory effects of VNS. Our work supports the use of nVNS as a non-invasive alternative and adjunct therapy for idiopathic gastroparesis patients. Future directions include further analysis of the impact of nVNS on mucosal barrier function and its relationship to mucosal immune activation.

340 | Purinergic nerves in the colonic myenteric plexus of guinea pigs and mice

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ATP or related molecule is a neurotransmitter in the enteric nervous system (ENS) where it contributes to excitatory synaptic transmission and inhibitory neuromuscular transmission. We used an antibody against the vesicular nucleotide transporter (VNUT, SLC17A9) to determine the neurochemical phenotype(s) of purinergic nerves in the mouse and guinea pig colon.

Mouse: VNUT-immunoreactivity (ir) was detected in separate nerve fibers in myenteric ganglia and in the circular muscle. VNUT-ir varicose nerve fibers surrounded choline acetyltransferase (ChAT), nitric oxide synthase (nNOS), and calretinin-ir neurons. VNUT-ir was not detected in tyrosine hydroxylase (TH)-ir nerve fibers in myenteric ganglia or in calbindin-ir nerve fibers. VNUT-ir rarely co-localized with any of the tested markers in nerve fibers in the myenteric plexus, tertiary plexus, or circular muscle (CM) layer.

Guinea pig: NOS-ir neurons and nerve fibers were detected in the myenteric plexus. VNUT-ir nerve fibers were also detected in the myenteric plexus but they did not overlap with NOS-ir nerves. VNUT-ir nerve cell bodies were not detected. VNUT-ir nerve fibers surrounded most NOS-ir neurons and VNUT-ir nerve fibers also surrounded NOS-negative neurons. NOS-ir and VNUT-ir did not overlap in any nerve fibers in myenteric ganglia or the circular muscle. VNUT-ir nerve fibers surrounded some ChAT-ir myenteric neurons. Conversely, VNUT-ir and ChAT-ir were detected in separate nerve fibers in the CM layer. VNUT-ir nerves surrounded calretinin-ir neurons but CalR/VNUT-ir neurons or nerve fibers were not detected in the myenteric plexus or circular muscle. VNUT-ir and TH-ir did not co-localize in the myenteric plexus or circular muscle. Our data suggest VNUT-ir neurons are a subclass of myenteric neurons that drive purinergic neurotransmission in myenteric ganglia and the circular muscle. These results also suggest that ATP and nitric oxide (NO) may be released from separate populations of inhibitory motor neurons that release ATP or NO to cause circular muscle relaxation. There are also separate populations of VNUT-ir and ChAT-ir myenteric plexus nerve fibers suggesting that cholinergic and purinergic synaptic transmission are also mediated by separate populations of interneurons.

342 | PAMORAs result in clinically significant improvement in spontaneous and complete spontaneous bowel movements with low risk of side effects in patients with opioid induced constipation: Systematic review and meta-analysis

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Background: Opioid-induced constipation (OIC) is a significant adverse effect of opioid use. Centrally acting opioid antagonists result in opioid withdrawal or aggravation of pain due to inhibition of the analgesic effects of the μ-opioid agonist. This has led to the development and use of peripherally acting mu-opioid receptor antagonists (PAMORAs).

Aim: To evaluate the efficacy and potential for relieving different manifestations of constipation without reducing pain relief in patients with OIC with the available PAMORAs and other treatments approved or proposed for treatment of OIC based on a systematic review and meta-analysis of the literature.

Methods: With the help of an expert librarian, we completed an exhaustive search of multiple databases to evaluate μ-opioid antagonists to counter μ-opioid effects included terms: naloxone, naltrexone, methylnaltrexone, alvimopan, naloxegol, and naldemedine. Different doses of each medication were appraised separately. Results of efficacy are based on appraisal of diverse endpoints including the bowel function index (BFI), number of spontaneous bowel movements (SBMs), combination of >3 SBMs or complete SBMs (CSBMs) plus 1 over baseline, Bristol Stool Form Scale. Additionally, we collected adverse event data and focused on treatment emergent adverse events, serious adverse events, major adverse cardiovascular events, diarrhea, abdominal pain and opioid withdrawal symptoms.

Results: Naldemedine, naloxone, linaclotide, and alvimopan demonstrated clinically significant improvement in change in SBM. Naldemedine had the highest OR for the FDA endpoint for SBM [OR 2.3 (95% CI 1.9-2.8) and CSBM [OR 3.9 (95% CI 2.3-6.7)]. TEAE, SAE, and MACE were not significantly greater in active treatment compared to placebo. Rates of abdominal pain were highest in patients taking lubiprostone [OR 5.6 (95% CI 2-15.4)] followed by methylnaltrexone [OR 3.4 (95% CI 2-5.6)]. Diarrhea was significantly greater in patient receiving lubiprostone [OR 3.5 (95% CI 2.3-5.3)].

Conclusions: Opioid-induced constipation response to treatment is best achieved with PAMORAs, naldemedine, naloxegol and alvimopan, which are associated with low risk of opioid withdrawal and development of pain.

343 | Physiologic distress in relation to gastric emptying rate in patients with dyspepsia-like symptoms

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University of Leuven

Due to the chronic nature and severity of their symptom, gastrointestinal functional and motility disorders are associated with considerable physical, psychological, and socio-economic distress. Little is known about the level of psychological distress in gastroparesis. In this study we studied levels anxiety, depression and somatization in gastroparesis and their relation to the severity of gastric emptying.

Methods: Patients with dyspepsia-like symptoms referred for a gastric emptying (GE) test filled out the State-Trait Anxiety Inventory (STAI, cut-off at least moderate anxiety >19) and the Patient Health Questionnaire (PHQ) to assess levels of somatization (PHQ15) and depression (PHQ9) (cut-off in both, at least moderate >10). The GE was measure by the breath test with ^{13}C-octanoic acid labelled 250 Kcal meal. Data were compared in patients with normal (N) and delayed (D) GE (cut-off for delayed GE T1/2 > 109 minutes) using non-parametric statistical tests, Mann-Whitney test, Chi square test and Spearman correlation.

Results: Of 177 patients (78% F, 39 ± 0.9 years, 22 ± 0.4 kg/m^2), 19% showed a significantly delayed GE and 2 patients had accelerated GE (T1/2<30 minutes). No significant differences were observed in the average scores of anxiety (STAI P = 0.4), depression (PHQ9, P = 0.05) or somatisation (PHQ15, P = 0.08). Levels of moderate to severe anxiety were high in all groups (STAI, D = 100%, N = 98%, P = 0.9). The levels of somatisation were higher in the gastroparesis subgroup (PHQ15, D = 88%, n = 70%; P = 0.007), which also tended to have increased depression ratings (PHQ9, SD = 45%, N = 28%, P = 0.07). The GE rate was significantly correlated to somatisation scores (PHQ15: r = 0.19, P = 0.02). No significant correlation was found for anxiety (STAI r = -0.07, P = 0.4) or depression scores (PHQ9, r = 0.14, P = 0.09) (Figure 1).

Conclusion: Patients with upper gastrointestinal symptoms referred for gastric emptying testing have high levels of anxiety, depression and somatization. Gastroparesis and the severity of GE delay are significantly associated with somatization. The nature of this relationship and its response to treatment need to be addressed in future studies.

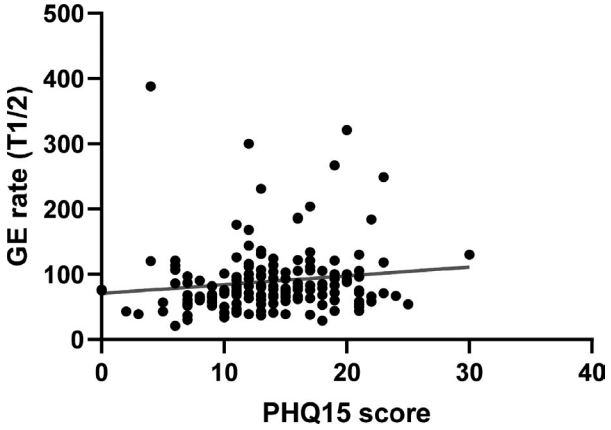

Figure 1

344 | Small intestinal bacterial overgrowth in rheumatic autoimmune diseases

A.M. Madrid; D. B. Vera; Alexandra Sandoval
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A high prevalence of Small Intestinal Bacterial Overgrowth (SIBO) there has been reported in patients with Systemic Sclerosis (scleroderma: SC). The existence of SIBO in other rheumatologic autoimmune pathologies associated with chronic inflammatory process is not still clearly established. The aims were to evaluate SIBO in a group of patients with SC and to compare with a group of patients with Systemic Lupus Erythematosus (SLE) and/or Rheumatoid Arthritis (RA). Methods and patients: Retrospective study in 53 patients with SC, mean age 54 years (range: 21-83) and 49 women. 37 patients with SLE and RA, mean age 52 years (range: 18-78), 34 women. The patients attended to hydrogen breath test with lactulose (HBT) with standardized technique. The SIBO was considered to be more than two cyphers above 20 ppm of the baseline in the first 60 minutes. Orocecal transit time (OCTT) was considered at the arrival of lactulose to the colon marked as the first elevation of the curve in the absence of SIBO. Statistical analysis with chi-square.
SIBO was found in 60% of patients with SC and in 44% of patients with SLE and/or RA, without differences. OCTT without differences between both groups. Pain and bloating were greater in patients with SC ($P < 0.02$). Finally, SIBO is highly prevalent in all autoimmune pathologies. We propose to study SIBO in patients with these pathologies, considering that this condition maintains a state of low-grade chronic inflammation that perpetuating the inflammatory cascade.

345 | Novel therapeutic target for visceral pain: Opioid receptor signaling by endosomes

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Background: Previously we have shown that sustained signaling can occur from G protein-coupled receptors in endosomes and that the activation of opioid receptors (OPr) by endogenous opioids released in colonic tissues from inflammatory bowel disease (IBD) mouse models caused a sustained inhibition of colonic nociceptors. Our aim was to evaluate whether this sustained inhibition of colonic afferents results from OPr endosomal signaling.
Methods: Mouse dorsal root ganglia (DRG) neurons were exposed to supernatants from colonic biopsies taken from healthy controls (HC) or patients with chronic ulcerative colitis (cUC), or δ-opioid receptor (DOPr) agonists (DADLE and SNC80, which elicit DOPr endocytosis, and ARM, a weekly internalizing agonist), or a μ-opioid receptor (MOPr) agonist (DAMGO). We recorded changes in rheobase (minimum current to fire an action potential) by perforated patch-clamp, after washout of drugs, 0 or 30 minutes later. Extracellular recording of mouse colonic afferents were performed and von Frey hair probing was assessed every 15 minutes for 60 minutes after OPr agonists. Neurons or colonic afferents were pre-treated with clathrin or protein kinase C (PKC) or extracellular signal-regulated kinase (ERK1/2) inhibitors for 30 minutes before agonists. One- or two-way ANOVA with Tukey's tests was used to analyze the data.
Results: In patch clamp studies, DADLE, SNC80 and supernatant from cUC colons caused a sustained (30 minutes) inhibition (increased rheobase $P < 0.01$ and < 0.05). However, ARM390 and the MOPr agonist increased rheobase at T0 but not T30 minutes. In colonic afferent nerve recordings, SNC80 but not DAMGO caused sustained inhibition (60 minutes) ($P < 0.05$). This inhibition on DRG neurons and colonic afferents was blocked by the clathrin inhibitor, which inhibits receptor endocytosis, and by inhibitors of the PKC and ERK pathways.
Conclusions: Endogenous opioids in colonic tissue from IBD patients activated DOPr, causing a markedly sustained analgesic effect that is mediated by endosomal signaling, and PKC and ERK pathways. Activation of DOPr in endosomes could provide a novel therapeutic target to treat pain in IBD.

347 | Small intestinal bacterial overgrowth in patients with gastric bypass

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Small Intestinal Bacterial Overgrowth (SIBO) is associated with multiple conditions and alterations of the gastrointestinal tract and bariatric surgery is one known risk factor. Ours aims were to determine the presence of SBI in a cohort of patients undergoing gastric bypass (GB) and compare with a cohort undergoing gastric sleeve (GM) and asymptomatic population.

Methods: retrospective study in 53 patients with a history of BG and 19 MG and 42 asymptomatic controls with hydrogen breath test with lactulose (HBT), standardized technique. SIBO was considered in BG peak >20 ppm of basal of hydrogen greater than two measurements in the first 40 minutes in HBT. In GM and asymptomatic population in the first 60 minutes of HBT. Symptomatic evaluation was classified using criteria Rome IV. Statistical analysis with Shapiro-Wilk test, ANOVA and post-hoc tests.

SIBO was found in 58% in patients with GB, 26% in MG and 24% in controls. SBI was higher in BG than controls ($P < 0.002$), no difference with GM. It was found difference in pain, abdominal distension, interference with activities of daily life between the three groups ($P < 0.001$). The higher scores were for the group GB. 68% patients with BG and SIBO meet criteria for Irritable Bowel Syndrome.

In our series, the patients with GB have high frequency of SIBO. In patients with GB, abdominal pain and interference with activities of daily living are relevant factors to evaluate. It is proposed in the patients with GB to conduct studies since they share symptoms suggestive of Irritable Bowel Syndrome.

348 | The relation between changes in gastric emptying rate and improvement of simultaneously measured symptoms with prokinetic agents; an analysis of 8 treatment trials

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Introduction: Meta-regression analyses have failed to show a significant relationship between improvement of gastric emptying rate (GE) and improvement of symptoms with prokinetic agents in idiopathic gastroparesis (IGP)/functional dyspepsia (FD).However, significant effects emerge when a selection of agents and tests is applied (Janssen 2013; Vijayvargiya 2019). The potentially strongest link between effects on GE and symptoms is found when both are measured simultaneously. We studied this relationship at the individual patient level in treatment intervention studies performed by our unit.

Methods: Data from 6 placebo-controlled cross-over trials and 2 open label studies involving ghrelin, prucalopride, cisapride, botulinum toxin (BT), buspirone, erythromycin, clonidine and octreotide;(2 acute, 6 repeat administration) in 140 FD/IGP patients, were analysed. All patients underwent a GE breath test (13C-octanoic acid) during which the intensity (0-4) of fullness, bloating, nausea, belching, epigastric pain and burning (EB) were scored every 15 minutes for 4 hours. Meal-related severity of individual symptoms (ISS) was obtained by summing all scores for that symptom and the overall meal-related symptom severity (OSS) was defined as the sum of all individual symptoms scores. The relationship between change in GE T1/2 and change in symptom score was assessed via Spearman correlation in individual and pooled studies.

Results: In the pooled studies, GE improved from 123 ± 8 to 95 ± 4 minutes during treatment ($P < 0.001$), OSS (88 ± 7 to 63 ± 6 ($P < 0.0001$)) and all ISS except EB were improved (all $P < 0.005$). OSS as well as ISS did not correlate significantly with GE T1/2, at baseline/placebo or during active treatment (respectively R = 0.01 and R = -0.03). The change in GE T1/2 did not correlate with the change in OSS (Figure 1) or the change in ISS (all R-values <0.12). Individual studies with BT, erythromycin and prucalopride showed significant improvement in GE T1/2, but no significant positive correlation was found for the studies separately (respectively R = -0.42,-0.28 and 0.23) or pooled together (R = -0.13).

Conclusion: Even in the closest apposition between measurement of GE and symptom assessment in FD/IGP, no correlation was found between the degree of symptom improvement and the improvement of GE. The observed symptomatic significant benefit must be attributed to effects other than enhanced GE.

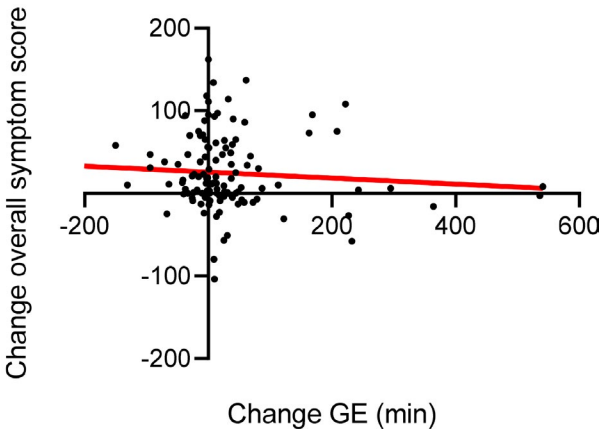

Figure 1

349 | Comprehensive neurogastroenterology evaluation of parkinson's disease: Preliminary results

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Background: Lower and upper gastrointestinal symptoms are common in Parkinson's disease (PD) and precede CNS manifestation, but there is a dearth of knowledge regarding gut dysmotility in PD.
Aim: To examine anorectal sensorimotor function, gastric emptying, small bowel and colonic transit, and presence of SIBO in PD patients.
Methods: PD subjects underwent high-resolution anorectal manometry, balloon expulsion test, wireless motility capsule (WMC), and glucose breath testing. Constipation and dyssynergic defecation were by Rome criteria and SIBO by ≥20 ppm rise in H2 or CH4.
Results: In this interim analysis, 28 PD subjects (17 M, 11 F, mean age 66.9 ± 8.4 years) were enrolled, of whom 16/28 (57.1%) had constipation (Rome IV), and 15/28 (53.6%) had dyssynergic defecation (Rome IV). Of the PD subjects with constipation, 10 (66.7%) had dyssynergic defecation, 2 (13.3%) had slow-transit constipation, 1 (6.7%) had normal transit constipation. Six of 26 (23.1%) subjects had delayed gastric emptying suggesting gastroparesis, five of whom had co-existing dyssynergic defecation. The other PD subject with delayed gastric emptying had delayed small bowel transit time and small intestinal bacterial overgrowth (SIBO) on glucose breath testing. Seven of 23 subjects (30.4%) had SIBO.
Conclusions: Our study demonstrates the majority of PD subjects with or without constipation exhibit dyssynergic or disordered defecation. The majority of other NGM disorders such as gastroparesis and SIBO in PD occur in the presence dyssynergic defecation. These findings suggest that dyssynergic defecation lies at the root of neurogastrointestinal dysfunction and GI symptoms in PD that merits further validation.
Acknowledgement: Parkinson's Foundation Translational Research Grant (PF-TRG-1730).

350 | Optogenetic inhibition of colon epithelium reduces hypersensitivity in a mouse model of inflammatory bowel disease

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Background/Objectives: Visceral pain and hypersensitivity are thought to be mediated by extrinsic primary afferent neurons (ExPANs) innervating the colon, but evidence shows that epithelial cells in the colon may contribute to changes in afferent excitability. Our recent studies showed that optogenetic stimulation of colon epithelial cells alone initiates robust action potential firing in ExPANs and engages pain-like behaviors, suggesting that the epithelium has a considerable role in sensory signaling in the gut. This led us to examine the behavioral implications of epithelial *inhibition*. We tested the hypothesis that inhibiting the epithelium would impact visceromotor responses (VMR) to colorectal distension (CRD) and hypersensitivity in a model of inflammatory bowel disease (IBD).
Methods: Using 2 different transgenic mouse models, the inhibitory yellow-light activated protein archaerhodopsin (Arch) was expressed under the villin promoter (protein specific to intestinal epithelium) or the TRPV1 promoter (specific to ExPANs). A laser-balloon device was custom made to deliver both CRD and yellow laser stimuli in the colon. Visceral sensitivity to these stimuli was measured using electromyographic recording of VMR. Administration of 3% dextran sulfate sodium (DSS) in via drinking water was used to induce inflammation and model IBD.
Results: In Vil-Arch mice, VMR to a noxious CRD stimulus (60 mmHg) was 4.5 ± 0.5 V. When yellow laser was shone concurrently with CRD stimulus, this significantly decreased the VMR to 1.0 ± 0.9 V ($P = 0.04$). In TRPV1-Arch mice, average VMR to CRD was 7.0 ± 2.1 V and with yellow laser was 3.6 ± 1.5 V. Therefore, VMR in Vil-Arch mice was reduced ~22%, whereas VMR in TRPV1-Arch was reduced by ~51%. These experiments were repeated after DSS inflammation and showed similar reduction in VMR responses, although not complete reversal of hypersensitivity.
Conclusions: These data show that inhibition of the colon epithelium can prevent or moderate the ability of distension stimuli to initiate VMR and that this inhibition remains effective even after inflammation.

351 | Bile acids, diet, and cytokines among individuals with irritable bowel syndrome and healthy volunteers

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Background/Objectives: Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder. Elevated secondary bile acid (BA) profiles have been associated with abdominal pain symptoms, mucosal inflammation, and diarrhea in a subgroup of IBS. The purpose of this study was to compare 1) Fecal primary and secondary BAs in women with and without IBS and 2) Women with 'high' and 'normal' fecal BAs (i.e., similar to healthy [HC] women) on diet and peripheral cytokine levels.
Methods: Women (ages 18-45) with IBS (Rome III) and HCs were recruited from healthcare providers or the community. HCs were included if they did not report any current disease, disorder, or syndrome. Participants kept a 28-day symptom diary. During the follicular phase, women completed a 3-day food journal, collected a stool sample and had blood drawn for cytokines. BA levels were determined by mass spectrometry and plasma cytokines by ELISA.

Results: Seventy-three individuals are included in this analysis. Primary BAs did not differ between IBS and HC groups. However, women with IBS had increased levels of several secondary BAs including lithocholic acid ($P = 0.037$), glycodeoxycholic acid ($P = 0.006$), taurodeoxycholic acid ($P = 0.006$), glycolithocholic acid ($P = 0.01$), and taurolithocholic acid ($P = 0.026$). Sixty percent of women with IBS had normal BAs whereas 40% had 'high' BA relative to HC. Women with IBS with 'high' fecal BAs consumed less fiber and vegetable protein and more animal protein compared to women with IBS whose fecal BAs levels were comparable to HCs. Plasma IL-6, IL-12P40, IL-10, and IL-1β levels were significantly increased in the 'high' BA group versus the 'normal' BA IBS and HC groups.

Conclusions: A subgroup of women with IBS have increased levels of secondary BAs compared to HC women. Dietary fiber impacts fecal BA levels in women with IBS. Higher levels of fecal BAs are associated with increased peripheral cytokine levels suggesting an inflammatory response.

352 | Gut microbiota regulates intestinal barrier permeability in rotenone induced Parkinson's disease mice

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor and gastrointestinal (GI) deficits. However, it is not well known whether GI deficits such as disrupted permeability seen in PD are a direct effect of environmental toxin consumption or an indirect effect of toxin that works through the gut microbiota.

Aim: To investigate mechanism of disrupted GI permeability in rotenone induced PD mice.

Methods: Conventionally raised (CR) mice were gavaged either with 10 mg/kg of rotenone dissolved in control solution containing 4% carboxymethylcellulose and 1.25% chloroform or with control solution for six weeks. Motor coordination and strength was measured in rotenone and control group using a rotarod and grip strength test respectively. At the end of sixth week, mice were euthanized, and brain and colon samples were collected. Tyrosine hydroxylase (TH) neurons in substantia nigra (SN) were quantified using IHC to assess motor neuron loss. Proximal colon mucosa-submucosa preparations were mounted on a Ussing chamber to assess TEER and FITC flux. Differences between groups were analyzed either using *ANOVA* or Unpaired -t-test. Data are represented as Mean.

Results: Six weeks of rotenone gavage lead to a decrease in grip strength (0.0104 vs 0.013 lbf/BW; $P = 0.007$, n = 5), decrease in time spent on a rotarod (1600 *vs* 6754 seconds, $P = 0.003$, n = 5) and loss of TH neurons (30.8 *vs* 7.8; $P = 0.003$, n = 4) in SN compared to control gavage mice confirming establishment of PD in CR mice. Chronic rotenone gavage in CR mice did not cause change in TEER but lead to significant increase in FITC flux (1.1×10^3 *vs* 6.9×10^2 ng/mL; $P = 0.004$, n = 3) suggesting an increase in paracellular permeability. No such change in paracellular permeability was observed when rotenone was applied either acutely on virgin CR mice tissues or chronically in germ free mice.

Conclusion: Disruption in intestinal permeability in PD is a chronic effect of environmental toxin interaction with the gut microbiota.

353 | Dihydrocapsaicin test in the assessment of esophageal contractile function in patients with gastroesophageal reflux disease

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Background: Esophageal contractility is impaired in GERD. Provocative tests as multiple rapid swallowing (MRS) and drinking test that have implemented to assess peristaltic reserve. Dihydrocapsaicin (DHC) is a Transient Receptor Potential Vanilloid 1 (TRPV-1) agonist related with an increase in esophageal contractility in small studies with healthy volunteers (HV) after instillation of DHC. There are no data about the effect of DHC swallowing to assess esophageal contractility in GERD.

Aim: To assess esophageal contractility after DHC swallowing test in patients with GERD.

Methods: GERD patients were recruited to perform solid state high resolution esophageal manometry (Medtronic MN, USA). HRM standard protocol consisted in 10 swallows 5 mL water each, 30 seconds apart. Subsequently, 3 MRS (5 swallows 3 mL each) 30 seconds apart. Next, 3 DHC 0.3% swallows 5 mL each were given and finally 3 water swallows 5 mL each were given. Esophagogastric junction (EGJ) resting pressure (RP) was compared before and after DHC stimulation. Data are expressed as median and percentiles (25-75th) and analyzed with SPSS 21.

Results: 23 patients were recruited, 15 women, median age 52.5 years (45-56.5). One patient did not tolerate DHC test; 22 were analyzed. Median 10 water swallows DCI was 701.4 (589.7-1614) mmHg.s.cm. After MRS DCI was 1113.9 mmHg.s.cm (527-1995). After DHC 0.3% 979 mmHg.s.cm (692-1686) and with 3 water swallows post DHC 1291 (740-2408) mmHg.s.cm. When median 10 waters swallows was compared with median 3 water swallows post DHC there was a significant increase in DCI ($P = 0.002$) but not different to MRS ($P > 0.05$). EGJ resting pressure also increased after DHC test stimulation from median of 9.9-19.4 mmHg ($P = 0.0001$). When comparing between gender, men showed higher EGJ RP ($P = 0.004$). One patient with absent contractility showed esophageal contraction after DHC test but not with MRS.

Conclusions: Overall, DHC test was well tolerated. DHC test induces esophageal contractility in swallows performed after stimulation similarly to MRS. There was an increase in EGJ RP. We propose DHC test to assess esophageal contractility and EGJ RP in patients with

GERD. Interestingly, increase in contractility showed latency (after water swallows post DHC). This finding merits further research.

354 | Impedance-guided measurement of the intrabolus pressure domain to quantify esophago-gastric outflow resistance induced by leg lift

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Background: Pressure generated within the intrabolus domain is relevant to normal esophageal bolus transport and may detect abnormal esophageal outflow resistance. This study measured the effect of leg-lift, a protocol designed to artificially increase esophageal outflow resistance.
Methods: Patients (GERD, globus and/or dysphagia history) referred for HRIM (MMS, 2.67 mm solid-state catheter) and diagnosed with 'Normal' motility were included. Supine liquid swallows were tested. Leg-lift was employed to generate esophageal outflow resistance by increasing abdominal pressure. Studies were analysed via Swallow Gateway web application (www.swallowgateway.com). Standard pressure topography metrics and three measures of the intrabolus pressure domain were calculated. These were; i) mid-domain intrabolus distension pressure (DP, mmHg), ii) intrabolus ramp pressure (RP, mmHg/s), quantifying pressure change as the bolus is compressed between the peristaltic wave front and the EGJ and iii) time from mid-bolus distension to contraction (DCL, s). The bolus measures were combined to derive a pressure-flow index composite score of esophageal outflow resistance (PFI = [DPxRP]/DCL). Data are mean (SD).
Results: Thirty-four patients were included (aged 25-73 years, 52% f). Leg lift increased EGJ integrated relaxation pressure from 20 (8) to 31 (21) mmHg ($P = 0.004$) and distal contractile integral increased from 1558 (751) to 2413 (1027) mmHg.cm.s ($P < 0.0001$) consistent with augmented of peristaltic vigor as a physiological response to resistance. All three bolus domain metrics were altered by leg lift. Intrabolus DP increased from 22 (5) to 36 (12) mmHg ($P < 0.0001$), intrabolus RP increased from 5(6) to 8(6) mmHg/s ($P < 0.05$) and DCL shortened from 2.5(1.0) to 2.1(1.0) s ($P < 0.05$). The PFI score increased from 74(145) to 232(248) ($P < 0.001$).
Conclusions: Leg-lift is a simple provocative test that may help to reveal underlying esophageal dysfunction. Impedance-guided measurement of the intrabolus pressure domain can quantify changes in esophageal outflow resistance and may have relevance to the diagnosis of EGJ outflow obstruction.

356 | Is it all in our head-constipation and obesity in medically relevant models of prion disease

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Prion diseases are invariably fatal neurodegenerative disorders associated with the aberrant and transmissible misfolding of the normal prion protein in the central nervous system (CNS). Although predominantly considered a rapidly progressive dementia, variation in disease presentation is a recognised feature of prion diseases, which is attributed to differences in the conformation and location of protein misfolding. We have generated mouse adapted forms of human prion disease to understand the basis of clinical variation in medically relevant prion disease and investigate the effect of this variation on diagnosis, treatment and prevention of disease.
Gastrointestinal dysfunction was observed in late stages of prion disease and was found to be associated with the accumulation of misfolded prion protein and loss of specific neuronal populations in the enteric nervous system (ENS) of prion infected mice. Gastrointestinal dysfunction is also observed in patients with prion disease and our findings suggest that contrary to the assumption that it a consequence of neurodegeneration in the CNS, this clinical presentation is a direct consequence of protein misfolding induced pathology in the ENS.
Obesity was a reproducible and unexpected finding associated with prion disease infection of mice. A review of the literature suggested that obesity could be a clinical feature of human prion disease. To understand the prevalence of this apparently transmissible obesity we reviewed the database of the Australian National Creutzfeldt-Jakob Disease registry and found that weight gain and obesity was significantly associated with the clinical phase of human prion diseases and that this phenotype appeared to correlate with disease of longer duration. The results of investigation on the CNS and peripheral tissues to determine the cause of this obesity will be discussed. Our findings extend the clinical presentation of human prion disease and emphasise the relevance of these mouse models in understanding their pathogenesis.

357 | miR-302 contributes to the gene regulatory network of enteric neural crest cells

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Hirschsprung disease (HSCR) is the most common congenital enteric neuropathy, resultant from incomplete colonization of the enteric

nervous system (ENS) by enteric neural crest cells (ENCCs). Though numerous genes, including microRNAs have been identified that contribute to ENCC biology, there are still large gaps in our knowledge regarding the pathogenesis of this complex and devastating disease. There is hope that in the future, HSCR will be treated with cell therapy instead of extensive surgical resections, however for this to be a reality, the basic biological processes governing ENCC specification, proliferation, migration and differentiation must be understood. This project offers an innovative combination of tissue engineering and animal models to investigate the role of miR-302 in ENS development in an in vivo mouse model and using human embryonic stem cells differentiated into human intestinal organoids and ENCCs. Absence of this microRNA leads to a frank neurocristopathy: mouse embryos deficient in *miR-302* display gross peripheral and ENS defects; notably, near-absence of vagal nerve development. miR-302 is an evolutionarily conserved miRNA that is required during early embryonic development, known to play a role in regulating neural progenitors by suppression of precocious differentiation. EDNRB is upregulated in *miR-302* knockout mice, suggesting an important role for miR-302 in the gene regulatory network (GRN) of ENCC development. These studies will provide valuable insights into the pathogenic mechanisms of miR-302 deficiency, as well as miR-302 contributions to the ENCC GRN controlling normal ENS colonization.

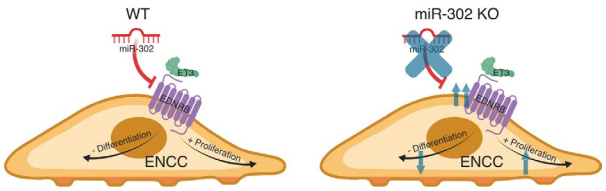

Figure: Proposed miR-302 pathogenesis. In WT, miR-302 negatively regulates EDNRB. Without negative regulation of miR-302 in KO animal, EDRNB is upregulated and leads to increased proliferation of ENCCs and delayed neuronal differentiation.

359 | Involvement of hypothalamic BDNF signaling in the regulation of gastrointestinal motility

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Chronic stress is a major risk factor for the development of hypertension. Increased sympathetic nerve activity results in hypertension by elevating heart rate and peripheral vascular resistance, but sympathetic hyperactivity also has profound effects on gastrointestinal motility and sensation. The paraventricular nucleus of the hypothalamus (PVN) is an important brain area that is involved in the regulation of sympathetic activity. PVN neurons are markedly activated by stress that increases the expression of the neurotrophin, brain-derived neurotrophic factor (BDNF). In turn, BDNF-mediated mechanisms stimulate the hypothalamo-pituitary-adrenal (HPA) axis and induce sympathetic hyperactivity. We examined whether activation of BDNF signalling in PVN neurons is associated with gastrointestinal symptomology.

Our laboratory has previously used stereotaxic injection of BDNF-producing AAV virus into the PVN as a novel model of chronic stress and demonstrated profound increases in blood pressure, heart rate and indices of sympathetic activity in rats (Erdos et al. 2015). We injected either BDNF- or GFP-producing AAV into the PVN of naïve rodents and initially assessed GI symptomology using faecal pellet output whilst developing histology and gut transit assays.

Rats injected with BDNF-producing AAV virus into the PVN gained less weight than control animals that were injected with control virus (AAV-GFP). In addition, they displayed elevated HPA axis activity and altered behaviour both of which are indicative of a stressed phenotype. Faecal pellet output was reduced in BDNF-injected animals suggesting reduced motility and/or reduced orexigenic signalling. Ongoing experiments will pinpoint which aspects of GI function are most affected by BDNF-induced sympathetic hyperactivity.

These findings establish BDNF overexpression into the PVN as a suitable model to study gastrointestinal dysfunction in chronic stress and demonstrate that BDNF signalling in PVN neurons likely play an important role in the central regulation of GI motility.

361 | One-day post graduation education could effectively improve primary care doctors' knowledge of functional dyspepsia

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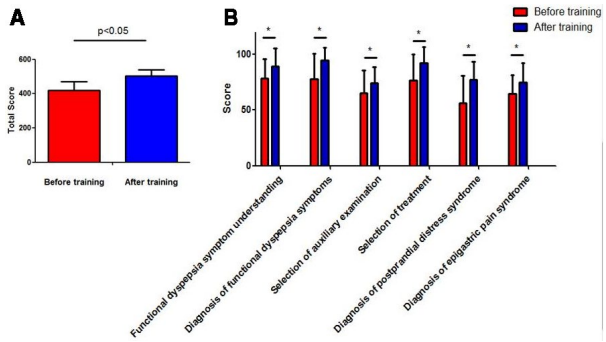
Background and Aims: Functional dyspepsia is the most common brain gut disorders in clinic. Although the Chinese version of Rome IV has been published for two years, the knowledge acquisition of primary care doctors on the definition of functional dyspepsia and Rome IV evidence-based diagnosis and treatment recommendations is still unclear.

The aims of this study are to investigate the knowledge of primary care doctors on the definition, diagnosis, examination selection and treatment of dyspepsia, and to evaluate the improvement of primary care doctors' knowledge through one-day post graduation education.

Methods: 71 unselected primary care doctors were included in the education program. Before training, the knowledge of dyspepsia symptom definition, symptom diagnosis, auxiliary examination selection, treatment selection, epigastric pain syndrome diagnosis and postprandial distress syndrome diagnosis were collected by questionnaire. Each dimension consists of 3-5 questions. The training video was made by experts of gastrointestinal functional diseases collaborative group of Chinese medical association according to Rome IV continuing education course and Rome IV teaching materials.

Results: 64 doctors completed the assessment before and after the training (the response rate was 90.1%). The scores before and after training were 419.2 ± 51.3 and 501.7 ± 37.1 ($P < 0.05$) (Figure 1A). For functional dyspepsia symptom understanding, the scores before and after training were 78.4 ± 17.2, and 89.2 ± 16.0 ($P < 0.05$). For the diagnosis of functional dyspepsia symptoms, the score before training was 78.1 ± 22.4 and after training was 94.3 ± 11.9 ($P < 0.05$). For the selection of auxiliary examination, the score before training was 65.3 ± 20.0 and after training was 74.0 ± 14.7 ($P < 0.05$). For the selection of treatment, the score before training was 76.7 ± 23.3 and after training was 92.2 ± 14.4 ($P < 0.05$). For diagnosis of postprandial distress syndrome, the score before training was 56.0 ± 24.7 and after training was 77.3 ± 16.0 ($P < 0.05$). And for diagnosis of epigastric pain syndrome, the score before training was 64.7 ± 16.7 and after training was 74.7 ± 17.3 ($P < 0.05$) (Figure 1B).

Conclusion: Knowledge of functional dyspepsia in Chinese primary care doctors is insufficient. One day's online training course can effectively improve doctors' knowledge of functional dyspepsia.



362 | Novel viscous material to improve simultaneous measurements of impedance and ultrasound in the esophagus

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Background: Impedance is becoming a standard feature with esophageal manometry technology. Its primary utility has been to observe bolus clearance and reflux events, however with further development, has the potential to estimate the distension of the esophagus. In this study we use simultaneous ultrasound (US) imaging as ground truth to measure distension. A major obstacle in obtaining quality data for both techniques is air noise in the bolus during swallow. We present the use of a novel saline viscous material to reduce air noise which improves impedance and ultrasound readings.

Objective: Explore the use of novel viscous material to improve impedance and ultrasound recordings.

Methods: Studies performed on 8 healthy subjects without esophageal symptoms. Simultaneous recording of impedance manometry (Medtronic HRMZ) and ultrasound (Boston Scientific iLab) in the supine position. Three materials were used: 1) 0.45% liquid saline, 2) novel 0.45% viscous saline, and 3) commercially available Diversatek EFT Viscous™. Ultrasound images were reviewed on NIH ImageJ. Impedance manometry was reviewed on Medtronic ManoView software. Viscosities were measured with a NDJ-8S viscometer.

Results: By visual inspection, the novel viscous saline material presents better ultrasound images than the liquid saline and Diversatek viscous (Figures 1a–3a). The novel viscous bolus presents as a dark oval, with fewer air noise speckles than the liquid saline and the Diversatek viscous. The clear bolus and reduced air noise makes the margins of the esophageal mucosa easier to identify. The manometric peristaltic pattern of viscous saline is similar to Diversatek viscous (Figures 1b-3b). The viscosities of the viscous saline and Diversatek viscous are 8760 mPa sec and 36 800 mPa sec at 37°C, respectively.

Conclusions: This study presents the use of a novel viscous material to improve impedance and ultrasound measurements. This viscous material may reduce the air noise present during swallow of either liquid saline or industry standard viscous material. This could further progress the use of impedance to measure esophageal distension.

364 | Insufficient Rome criteria to diagnose pure functional abdominal pain and epigastric pain syndrome: The need of ruling out chronic abdominal wall pain

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Objectives: There is firm evidence that chronic abdominal wall pain (CAWP) is one of the major causes of chronic abdominal pain. CAWP has been easily ignored and the physician may misdiagnose CAWP as functional abdominal pain syndrome (FAPS) or even as epigastric pain syndrome (EPS). We investigated whether there is a difference between clinical response for local anesthetic injection or a somatic nerve block (a major therapeutic option for CAWP) in CAWP alone and in CAWP-FAPS overlap.

Methods: From January 2012 to April 2017, patients complaining of abdominal pain were undergone relevant studies. CAWP with negative screening exams were enrolled. Greenbaum's criteria were adopted to diagnose CAWP while FAPS was diagnosed according to the Rome III criteria. For eligible patients, Rome IV criteria of EPS were surveyed also if they were Rome III-based EPS. CAWP alone group and CAWP-FAPS overlap group were treated by tap block. Baseline and post-treatment pain sore were recorded by 10-Likert scale. The 0.1% lidocaine mixture was used for the local anesthetic injection. After the local treatment, the responsiveness was assessed and 1 or 2 sessions of additional injection were performed if it is warranted. Clinical responsiveness was defined by over 50% degree reduction in symptom score.

Results: A total of 595 Patients complained with chronic abdominal pain were underwent EGD, colonoscopy, blood exam and imaging study (US or CT scan). Among them, 49 participants without obvious abnormal result to explain the pain were enrolled (11 of overlap, 38 of CAWP only). Mean age was 49.5 ± 11.2 and female were predominant by 1.45 fold. The most frequently affected area was epigastrium followed by right iliac in both groups. Kaplan-Meier analysis of unresponsiveness after the local treatment revealed no significant difference between the two groups (P = 0.781). The responsiveness at 3 months after the treatment also was not significantly different. When we diagnosed with Rome III criteria, EPS-CAWP overlap was less common (P = 0.035, McNemar test).

Conclusions: Clinical responsiveness to the local anesthetic injection was not different between CAWP-FAPS overlap and CAWP alone. Rome III criteria of EPS are more suitable to discriminate EPS from CAWP than Rome IV criteria.

365 | Diet composition of fiber and fatty acids alter the rat gastrointestinal tract functions and behavior in a sex-dependent manner

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Food and diet affect proper functioning of the gastrointestinal tract but may also alter behavior. Furthermore, the effects may be different in males and females. Our aim was to determine the changes induced by diets with different composition in fiber and fatty acids on gastrointestinal tract functions and behavior in male and female rats.

Male and female young adult rats were fed for 6 weeks with standard diet or diet containing lower content of insoluble fiber, supplemented with 7% soybean oil (SOY), SOY+3.5% virgin oil coconut oil (COCO), or SOY+3.5% evening primrose oil (EP). Body weight gain (BWG) and food/water intake were weekly recorded. At weeks 5-6, different experiments were performed to evaluate changes in behavior, gastrointestinal motility, and somatic and colonic sensitivity. At sacrifice (week 7), white adipose tissue (WAT) and gastrointestinal organs were macroscopically evaluated, and samples taken for microscopic analyses.

Compared with males, females under standard diet showed less BWG, food/water intake, WAT weight, caecum size and small intestinal contents, but increased locomotion, grooming (self-care) behavior in the splash test, and sensitivity to somatic (tactile, muscular) stimuli and colonic distension. Compared with standard diet, the low-fiber diets reduced caecum size and small intestinal contents in both males and females, decreased grooming behavior and small intestinal transit in females, and increased colonic sensitivity and number of colonic mast cells in males. EP increased body and WAT weight in males. Colon length was decreased by SOY and COCO in males, and by EP in females.

Modifications in dietary fiber and fatty acids may alter, in a sex-dependent manner, both gastrointestinal functions and behavior.

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366 | Characterisation of anal slow waves and ultra slow waves in health and constipation

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Background/Objectives: Anal slow waves (SW) and ultra slow waves (USW) have been noted on anorectal manometry tracings for many years and recent consensus guidelines suggest documenting their presence. Details of prevalence, characteristics and potential role in pathophysiology, however, are sparse. Our aims were to evaluate SW and USW in health and constipation, and to correlate these with demographics and clinical features.

Methods: Data were collected prospectively in 44 female healthy subjects and 90 patients with constipation at a tertiary referral centre. Symptoms and medical history were recorded using standardised questionnaires. High resolution water perfused manometry was performed using a catheter with 0.5 cm spaced sideholes, radially arranged. Rectal sensory testing and balloon expulsion were performed. Retrospective blinded descriptive and quantitative analysis of SW and USW was performed. Associations of SW and USW with other traits were evaluated via Kruskal-Wallis test for numeric traits and Pearson Chi-Square test for categorical traits.

Results: SW were present in 57% of health and 59% of constipated patients (P = 0.9). A significant difference was found in the prevalence of USW: 2% (1 out of 43) in health vs 30% in constipation (P < 0.0001). No difference was found in the characteristics of SW and USW in both groups including frequency and mean amplitude. Both waves were noted more commonly in the mid portion of the anal canal as demonstrated in channels 3 and 4 compared to manometric ports higher or lower in the anal sphincter complex. Further sub-analysis found no difference between in the presence of SW or USW in patients with dyssynergic defecation (P > 0.1), proctalgia fugax (P > 0.1), or perianal disease (P > 0.5).

Conclusion: SW and USW exhibited a typical frequency and amplitude. The prevalence of USW was much higher in constipation than health, raising the possibility of a potential pathophysiological role of USW in constipation.

Table 1: SW and USW characteristics in healthy subjects vs constipated patients; no significant difference was demonstrated between the 2 groups ($P > 0.5$).

	Slow Waves		Ultra-Slow Waves	
	Health; n = 44	Constipation; n = 90	Health; n = 44	Constipation; n = 90
Prevalence of SW and USW	57% (25/44)	59% (53/90)	2% (1/44)	30% (27/90)
Frequency (number of waves in 60 seconds)	12.8 (±3.2)	13.1 (±3.9)	1.0	1.4 (±0.4)
Mean Amplitude (mmHg)	6.5 (±2.7)	6.9 (±3.4)	10.0	30.6 (±13.3)

367 | Chromosome topology analysis reveals remote super-enhancers for neuronal nitric oxide synthase 1 (Nos1) expression and the mechanisms of their association with Nos1-proximal regulatory elements

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Background/Objectives: The mechanisms of transcriptional regulation of *Nos1* are poorly understood. Previously we found that physiologically low O_2 (physioxia) stimulates *Nos1* transcription by stabilizing hypoxia-inducible factor 1α (HIF1A). By chromatin immunoprecipitation-sequencing (ChIP-seq) and targeted chromosome conformation capture (3C) analysis we discovered 3 *Nos1*-proximal enhancer-promoter pairs (E1-P1, E2-P2, E3-P3) within ~100 kb upstream of *Nos1*. Physioxia/HIF1A stimulated transcriptional initiation at all 3 promoters by relaxing interactions between E1-E3 and P1-P3 and facilitating interactions between cognate enhancers and promoters. Here, we aimed to discover enhancers outside the *Nos1* locus and analyze the structural basis of their interactions with the *Nos1*-proximal regulatory elements.
Methods: $NOS1^+$ N1E115 cells were cultured at high (20%) and physioxic (4%) O_2 for 3 days. Binding of HIF1A, the cohesin component RAD21, the chromatin loop anchor CTCF, and 6 histone marks was determined by ChIP-seq. Chromosome topology analysis was performed by 3C-sequencing (HiC).
Results: HiC identified an ~800-kb region that contained all interactions involving the *Nos1*-proximal regulatory elements. At 20% O_2, ChIP-seq revealed 3 enhancer clusters (super-enhancers; SEs) within this region: 2 upstream (5': SE1, SE2) and 1 downstream (3': SE4) of *Nos1*. At 4% O_2, binding of HIF1A and H3K27ac (a histone mark for active enhancers) increased within these SEs and one remote 5' enhancer gained SE status (SE3). E3, the most *Nos1*-proximal enhancer, interacted with all 3 SE sites at 20% O_2, but only the SE1(5')-E3 and SE4(3')-E3 interactions were RAD21- and CTCF-anchored architectural loops (conventional and coiled, respectively), while others were dynamic/temporary. At physioxia, the SE4(3')-E3 loop dissolved, whereas the SE2(5')-E3 and SE3(5')-E3 interactions became conventional architectural loops; and E1 and E2 formed new dynamic interactions with a HIF1A-bound typical enhancer 5' of *Nos1* and with SE1, respectively.
Conclusions: Physioxia stimulates *Nos1* transcription by reconfiguring structural and functional interactions between *Nos1*-proximal and remote regulatory elements.

368 | Toll-like receptor 4 interaction with enteric dopaminergic pathways in a mouse model of dextran sulfate sodium-induced colitis

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Objective: Changes in dopamine levels, deregulated dopaminergic machinery and altered Toll-like receptor 4 (TLR4) expression have been consistently associated with IBD in patients and in animal models. Thus, we aimed to assess the crosstalk of enteric dopaminergic system and TLR4 signaling in a mouse model of dextran sulfate sodium (DSS)-induced colitis.
Methods: Male $TLR4^{-/-}$ mice (8 ± 1 weeks old; N = 32 mice) received 1.5% DSS in their drinking water for 5 days, then switched to regular drinking water for 3 days. Small intestine inflammation was measured by disease activity index and histological analysis. Gastrointestinal transit was measured by nonabsorbable-FITC-labeled dextran distribution. Changes in ileal muscle tension were isometrically recorded following: i) cumulative addition of dopamine (0.1-300 μM); ii) electric field stimulation (EFS, 4 Hz) in presence of 30 μM dopamine with or without 10 μM SCH-23390 (D1R antagonist) or 10 μM sulpiride (D2R antagonist). Immunofluorescence distribution of the neuronal HuC/D and glial (GFAP and S100β) markers and dopamine β-hydroxylase (DBH) and dopamine transporter (DAT) were determined in longitudinal-muscle-myenteric plexus whole-mounts (LMMPs) by confocal microscopy.
Results: In WT mice, DSS treatment determined a delayed gastrointestinal transit, a reduction of dopamine-induced relaxation (-26%, N = 5, $P < 0.05$), reactive gliosis and 1.2-fold increase in DBH immunoreactivity. After DSS treatment $TLR4^{-/-}$ mice showed a significant increase in dopamine-induced relaxation (+30%, N = 5, $P < 0.01$),

together with a 2.3-fold increase in 4-Hz EFS-elicited contraction (N = 5, $P < 0.001$), sensitive to D1R and D2R activation. Changes in ENS neurochemical coding were evidenced by a reduced number of HuC/D$^+$ neurons (-12%, N = 5, $P < 0.05$), together with a 1.4-fold increase of DBH immunoreactivity and no changes in GFAP and S100β immunofluorescence in DSS-treated TLR4$^{-/-}$ LMMPs.

Conclusions: In mice, TLR4 signaling influences the severity of small intestine inflammation as well as ENS activity and neurochemical coding, markedly increasing small intestine dopaminergic-mediated neuromuscular function.

369 | Involvement of toll-like receptor 4 signaling and dopaminergic neurotransmission in high-fat diet-induced small intestine dysmotility

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Objective: Recent clinical and preclinical studies have shown that high-fat diet (HFD) intake is associated with gastrointestinal dysmotility, gut microbiota dysbiosis, endotoxaemia and colonic neurodegeneration via Toll-like receptor 4 (TLR4). Excessive intake of dietary fats leads to diminished brain dopaminergic function. In this study, we assessed the role of TLR4 in tuning small bowel contractility and dopaminergic signaling pathways affected by HFD-induced obesity.

Methods: Male TLR4$^{-/-}$ mice and wild-type (WT) C57BL/6J male mice (7 ± 1 weeks old) were fed with standard-diet (SD; 18% kcal fat) or HFD (60% kcal fat) for 8 weeks. Changes in ileal muscle tension were isometrically recorded following: i) cumulative addition of dopamine (0.1-300 μM); ii) electric field stimulation (EFS, 4 Hz) in presence of 30 μM dopamine, with or without 10 μM SCH-23390 (D1R antagonist) or 10 μM sulpiride (D2R antagonist). Immunofluorescence distribution of the neuronal HuC/D and glial GFAP markers and dopamine transporter (DAT) were determined in longitudinal-muscle-myenteric plexus whole-mounts (LMMPs) by confocal microscopy.

Results: After 8-week SD, TLR4 deficiency determined a significant reduction in dopamine-induced relaxation response (Emax = -23%, N = 5, $P < 0.001$), 4-Hz EFS-elicited contraction (N = 5, $P < 0.01$) sensitive to D1R activation (N = 5, $P < 0.05$), a 1.4-fold increase in GFAP and DAT immunoreactivity. However, TLR4 deficiency protected against body-weight gain after HFD (+21% in TLR4 mice vs +33% in WT mice, N = 16, $P < 0.05$). In WT mice, HFD caused a marked reduction in dopamine-induced relaxation (Emax = -50%, N = 5, $P < 0.001$), in 4-Hz EFS-elicited contraction (N = 5, $P < 0.05$) sensitive to dopamine and involving D1R together with a 1.5 fold increase of DAT immunoreactivity and onset of reactive gliosis. In TLR4$^{-/-}$ mice, HFD impaired markedly 4-Hz EFS-elicited neurotransmission and D1R-mediated response (N = 5, $P < 0.01$).

Conclusions: In mice, TLR4 signaling appears to partially protect against the detrimental metabolic effects of HFD and to be highly involved in finely tuning enteric dopaminergic neurotransmission controlling neuroglial crosstalk.

371 | Effect of the adaptive immune system on the homeostasis and function of the enteric nervous system

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A complex set of tissues of distinctive embryological origin (such as the intrinsic immune and nervous systems) continuously interact to maintain a normal physiology of the gastrointestinal (GI) tract, which is constantly threatened by several and sometimes severe challenges originated within the wall or the lumen of the gut. The enteric nervous system (ENS) is composed of an intricate network of enteric glial cells (EGCs) and neurons localised into different compartments within the GI wall. These cells are involved in regulating virtually all aspects of GI physiology, including intestinal peristalsis. The ENS shares the same intestinal environment with the highly active immune system, due to its positioning at the interface between the internal and external milieu of the body, and therefore is expected to be influenced by immune responses. However, the methodology to explore these questions and consequently our understanding of neuro-immune communications in the mammalian gut is limited. In this work, we examined the effects of the absence of the adaptive immune system on the homeostasis and function of the ENS in adult animals. We have effectively generated a robust technology to isolate and evaluate the molecular landscape of enteric neurons from the adult gut. Moreover, by using immunodeficient mouse models lacking T and B lymphocytes (*Rag1*$^{-/-}$ and *Rag2*$^{-/-}$) and only T cells (*Tcrα*$^{-/-}$), our experiments revealed that in the absence of the adaptive immune cells, there is an impaired network of EGCs and neurons within the myenteric ganglia. These changes are followed by alterations on the expression of important genes by ENS cells. Besides, the frequency of the neuronal colonic motility (CMMCs) is decreased in mice lacking the adaptive immune system. Together, our data suggest that the adaptive immune system is crucial for maintaining the integrity and functional equilibrium of the ENS.

372 | Constipation stool APP versus paper form stool diary – Randomized study of constipated patients

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Background: Constipation is characterized by irregular bowel habit and/or evacuation difficulty. Typically, for clinical evaluation most physicians rely on recall of stool habit, but this has been shown to be inaccurate, leading to suboptimal care. The availability of smart phones provides a unique opportunity to record symptoms accurately whilst protecting privacy.

Aim: To develop and examine the feasibility and validity of Constipation stool APP and compare its usefulness with validated paper stool diary, in a randomized study.

Methods: In a randomized cross over trial, patients with constipation (Rome IV) recorded their bowel habit on either paper or mobile phone APP for 2 weeks. The diaries assessed 10 questions related to stool frequency, consistency (Bristol Scale), straining, completeness, gas and bloating, use of digital maneuvers, and medications. Data were compared and correlated between weeks 1 and 2, and between APP and paper diaries.

Results: Sixteen patients (F/M = 13/3) participated. The APP was reliable and reproducible, and showed significant correlations for the stool frequency, spontaneous (SBM) and complete spontaneous bowel movement (CSBM), methods used for inducing BM, gas and bloating episodes (ICC > 0.713, Table 1), and stool consistency (ICC = 0.4), between weeks 1 and 2. Also, all stool parameters and bowel symptoms assessed by the APP significantly correlated with those recorded on paper stool diaries (ICC > 0.770), except mean stool consistency (Table 1). APP functionality was comparable to that of paper diary, and more patients preferred stool APP (Table 1).

Conclusion: The Constipation Stool APP appears to be a reliable, valid and practical method of recording bowel symptoms including difficulty with defecation, and showed excellent correlation with validated paper diary. The accompanying electronic summary report as well as daily stool log and computed data on a spreadsheet, further enhance the accuracy of data capture and data analysis. Constipation APP can be a useful tool for assessment of constipation, and its availability on a mobile phone could improve clinical diagnosis.

373 | Dominant negative effect of ACTG2 mutations influences TGF-b signaling in Megacystis Microcolon Intestinal Hypoperistalsis Syndrome

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Background/Objectives: Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) is a rare inherited disorder characterized by bladder hypocontractility, and intestinal obstruction. Mutations in five genes have been identified as its cause, but the majority of cases are caused by de novo heterozygous mutations in the γ-smooth muscle actin gene (*ACTG2*). *ACTG2* encodes for an actin isoform specifically expressed in the intestinal and urogenital tracts. Previous studies by us and others have shown that *ACTG2* mutations disrupt actin polymerization and result in reduced cellular contractility. However, the mechanisms underlying these effects are still unknown.

Methods: In this study, a series of in vitro assays where performed by stably expressing the wild-type and/or mutant ACTG2 proteins in an osteoblastoma cell line (U2OS). The wild-type protein was fused to a GFP tag, while the mutant ACTG2 had a mCherry tag. Transdifferentiation assays were also performed in fibroblasts derived from two MMIHS patients carrying *ACTG2* mutations, and three controls. After differentiation, cells were analyzed by immunofluorescence, qRT-PCR, and luciferase assays.

Results: Actin polymerization assays performed in U2OS cells showed that actin polymerization and cellular contractility was impaired when the mutant ACTG2 protein was expressed alone, or in combination with the wild-type. Furthermore, the transdifferentiation assays showed an up-regulation of TGF-β signaling in the patient derived fibroblasts. This result was confirmed by immunofluorescence, qRT-PCR, and a TGF-β reporter assay.

Conclusions: Our results show that *ACTG2* mutations lead to dysregulation of the TGF-β pathway, and reduce cellular contractility by exerting a dominant negative effect.

374 | Effect of proton pump inhibitors on duodenal pH, bile salts and mucosal permeability in healthy volunteers

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Background/Objectives: Effects of acid suppression with proton pump inhibitors (PPI) on the duodenal microenvironment are unknown. We aimed to study the effect of PPI on duodenal pH, bile salts and barrier function in healthy volunteers (HV).

Methods: Duodenal aspirates were obtained via a naso-duodenal tube after upper GI endoscopy with duodenal biopsies. Bile salt concentrations (BS) were measured in fasting and fed state (1 hour after Fortimel®, 300 kCal) using LC-MS/MS after pH-measurement. Transepithelial electrical resistance (TEER) and paracellular passage of fluorescein-labeled dextran (4 kDa) were studied in Ussing chambers. Study procedures were repeated after pantoprazole 40 mg OD for 4 weeks.

Results: In total, 25 HV (16 female, median (IQR) age 26 (24-32) years) were included. Both duodenal fasting (7.2 (6.9-7.5) vs. 6.2 (4.6-7.1), $P < 0.001$) and fed pH (6.7 (6.5-6.9) vs. 5.9 (5.4-6.4), $P = 0.0001$) increased after PPI. Total BS were higher on- vs. off-PPI during both fasting (4.8 (2.4-7.3) vs. 1.4 (0.7-2.9) mM, $P < 0.001$) and fed state (14 (9.6-17.2) vs. 5.77 (3.4-9.7) mM, $P = 0.002$) with an increase in primary and secondary BS. TEER was similar but a trend for higher paracellular passage was found on- vs. off-PPI (35.6 (23.2-49.6) vs. 32.5 (13-36.3) pmol, $P = 0.06$). Off-PPI, TEER correlated positively with peak pH (r = 0.47, $P = 0.04$) and fed secondary BS (r = 0.53, $P = 0.02$). Conversely, paracellular passage correlated negatively with peak pH (r = -0.47, $P = 0.04$) and secondary BS both in fasting (r = -0.74, $P < 0.001$) (Figure) and fed state (r = -0.52, $P = 0.03$). Correlations were lost on-PPI except for TEER and secondary BS in fasted (r = 0.39, $P = 0.02$) and fed state (r = 0.38, $P = 0.02$).

Conclusion: PPI significantly increased duodenal pH and bile salts in healthy controls both in fasting and fed state and tended to increase permeability. Higher duodenal pH and secondary bile salts were associated with lower paracellular passage off- but not on-PPI, pointing towards the involvement of other factors that may lead to increased permeability after PPI such as the microbiota.

375 | Duodenal bile salts are linked with gastric emptying and symptoms in functional dyspepsia patients via changes in mucosal permeability and inflammation

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Background/Objectives: Despite increasing evidence for duodenal mucosal hyperpermeability and low-grade inflammation in functional dyspepsia (FD), the role of luminal changes has scarcely been studied. We studied duodenal bile salt concentrations and pH in relation to gastric emptying, duodenal mucosal changes and symptoms in FD patients.

Methods: FD patients (Rome IV) with no acid suppression were recruited with aspiration of duodenal fluids via a naso-duodenal tube after upper GI endoscopy with duodenal biopsies. Bile salt concentrations (BS) were measured in fasting and fed state (45 minutes after Fortimel®, 300 kCal) using LC-MS/MS after pH-measurement. Transepithelial electrical resistance (TEER) and paracellular passage of fluorescein-labeled dextran (4 kDa) were studied in Ussing chambers and eosinophils counted on H&E-stained sections per high-power field (HPF; 0.24 mm^2). Gastric emptying for solids (14C-octanoic acid breath test T1/2) and PAGI-SYM scores were determined.

Results: In total, 22 FD patients (19 female, median (IQR) age 28 (23-35) years) were included. Median total BS were 2.1 (1-3.6) mM in fasting state with pH 6.9 (6.4-7.4) and 10.3 (6.1-16.6) mM in fed state with pH 6.6 (6-6.8) and a similar proportion of secondary BS. Median T1/2 was 65.5 (58-79) minutes and PAGI-SYM 2.5 (2-2.9). Fed secondary BS correlated negatively with both T1/2 (r = -0.61, $P = 0.02$) and paracellular passage (r = -0.51, $P = 0.03$), while a positive correlation was found between T1/2 and passage (r = 0.73, $P = 0.002$). PAGI-SYM correlated positively with fed total BS (r = 0.62, $P = 0.005$) and mucosal eosinophils (r = 0.54, $P = 0.03$) (Figure) but not T1/2. A positive correlation was also found between mucosal eosinophils and fed total BS (r = 0.52, $P = 0.04$).

Conclusion: In FD patients, symptoms are associated with postprandial duodenal bile salts and mucosal eosinophils, suggesting a central role of the duodenum in symptom generation. Gastric emptying rate is associated with duodenal secondary bile salts and epithelial permeability but not symptoms, indicating that gastric dysmotility may be secondary to duodenal pathology.

376 | Abdominal pain and high FODMAP meals in school-children

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Background: Abdominal pain (AP) is common in childhood. Some children recognize food as a trigger for their AP. Fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) have been associated with AP in children with IBS. However, the effect of FODMAP loads in healthy school-children has not been studied. We assessed the relationship between FODMAP load, AP severity, and portion sizes consumed in a sample of healthy school-children. We hypothesized that high FODMAP diets trigger symptoms in school-children, higher FODMAP content would increase severity of AP, and children who associate AP with food will limit their portion sizes.
Methods: School-children from two schools in Argentina completed information on past history of AP. All children received a meal with varying FODMAPs loads during lunchtime for 1 week. During 4/5 weekdays the children received either lower (10.72-17.08 g) or higher (38.85-39.45 g) FODMAP content than a standard Argentinian meal (23.81 g). Dietitian's recorded intake and calculated lunch meal portions consumed. Three hours after the meals were provided, children completed questionnaires to assess their GI signs/symptoms. Questionnaires were collected by the research team upon completion.
Results: 146 school-children completed the study and 54 (37%) developed AP with the meals. 50/146 had a history of AP. There was no significant association between: 1) history of AP and development of AP with high FODMAP loads (P = 0.59), 2) the FODMAP load and development of AP (P = 0.28). Greater FODMAP load did not increase AP severity (P = 0.18), or reduce portion sizes consumed (P = 0.89). Children who reported AP with meals limited their portion sizes (P = 0.002).
Conclusions: AP is common amongst children in Argentina. Children experience AP during lunch regardless of their FODMAP load or history of AP. A higher intake of FODMAPs is not associated with higher severity of AP and meal size restrictions. However, children who reported AP were more likely to consume smaller portions than those who did not report AP.

377 | Adjunctive metrics for the diagnosis of gastroesophageal reflux disease in patients with inconclusive reflux test

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Recently, the Lyon Consensus for GERD has established new cutoffs for reflux monitoring tests considering abnormal an acid exposure time in 24 hours (AET) >6%, physiologic <4%, and borderline or inconclusive 4%-6%. In our clinical practice 13% of the reflux studies are inconclusive. Our aim was to evaluate if adjunctive metrics like the mean nocturnal baseline impedance (MNBI), number of reflux episodes and the presence of esophageal motor disorders are useful in clinical practice for the diagnose of GERD. Methods: We studied 80 patients, separated in three groups who underwent to a pH impedance monitoring (MII/pH) off antisecretory therapy and to a high resolution esophageal manometry (HRM) due to reflux symptoms. MNBI was calculated in the distal impedance channel at 3 nocturnal 10 minutes periods. Total number of reflux episodes, the prevalence of esophageal motor disorders and hypotensive esophagogastric junction (EGJ) were recorded. Statistical analysis with Shapiro-Wilk, Kruskal-Wallis and Mann-Whitney tests. Results: 23 patients had a normal MII/pH, 25 an abnormal test and 32 an inconclusive MII/pH. Mean age 45, 53, 51 years, 56%, 56%, 60% female respectively. The median MNBI was 3481 ohms (2348-6683) in the normal group, 929 ohms (670-2745) in the reflux group and 2523 ohms (971-5176) in the inconclusive group (P < 0.0001). 37.5% of patients in the inconclusive group had a MNBI <2292 ohms. 20% and 18.7% of patients in the reflux and in the inconclusive group respectively had a hypotensive EGJ in HRM. Absent contractibility was present in 16% of reflux group, major motor esophageal disorders were not found in the other groups. The percentage of >80 reflux episodes was similar. Conclusions: A lower MNBI and the presence of abnormalities in the HRM are suggestive of the presence of GERD in patients with inconclusive tests, and these findings were seen in approximately one-third of these patients.

378 | Discovering the roadblocks in Parkinson's disease: Preliminary results of bidirectional gut-brain assessment

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Background: Chronic constipation precedes neurological manifestation in Parkinson's disease (PD) by over 15 years, with hazard ratio of >3.3 of developing PD. Abnormal neuronal accumulation (alpha-synuclein), may start from the anorectum and spread towards the CNS, but gut brain function has not been systematically assessed.

Aim: To study anorectal sensory function and bidirectional pelvic floor-cortex and lumbosacral anorectal axis in Parkinson's disease (PD) and healthy subjects.

Methods: Anal and rectal sensory threshold responses to electrical stimulation and ano-cortical and recto-cortical evoked potentials (CEP) were obtained (afferent) by placing a 4-ring EMG electrode probe in rectum. Also, anal and rectal motor evoked potentials (MEPs) were determined following lumbosacral and transcranial magnetic stimulation (efferent) using specialized magnetic coils (Magstim). The latencies for CEP and MEP responses were measured in PD patients, and compared with normative data.

Results: 16 PD subjects (F/M = 7/9) were enrolled and data compared with 31 healthy subjects (F/M = 20/11). 81.3% of PD patients (13/16) had constipation (Rome IV). The anal and rectal CEP latencies were prolonged but not significantly different when compared to healthy subjects (table 1). Bilateral lumbosacro-anal and lumbosacral-rectal MEPs were significantly prolonged ($P < 0.003$) when compared to controls (Table 1). After transcranial magnetic stimulation (TMS), there was significant prolongation ($P < 0.05$) of right cortico-rectal MEP latency, but not other MEP latencies in PD subjects. The thresholds for first and pain sensations in the anus and rectum were significantly abnormal ($P < 0.003$) and higher in PD patients compared to health controls (table 1).

Conclusion: PD patients show significant efferent neuropathy, especially in the lumbosacro-anorectal pathways, and isolated unilateral cortico-rectal pathway. Also, PD patients demonstrate significant anal and rectal hyposensitivity. These neurophysiological abnormalities may help explain bowel problems including constipation and pelvic floor dysfunction and neuropathy in PD patients.

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380 | 'The anal pressure' does not exist

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Background: 'The anal pressure' is frequently reported when evaluating anorectal (patho)physiology, as if the anal canal was a uniform structure. We aimed to illustrate that the pressure in anal canal is very diversely distributed, and talking about just 'the anal pressure' is unjustified.

Methods: This prospective, observational study was performed at the University Medical Centre Groningen. We performed anorectal physiology tests in 52 healthy, asymptomatic volunteers. We measured the anal pressure profile during different physiological conditions: in rest (basal pressure), during squeezing (voluntary contraction), coughing, rapid rectal dilatation (rectoanal contractile and inhibitory reflexes) and during balloon retention test (autonomic continence reflexes). A multisensory catheter was utilized to test the pressures along the anal canal, at every 8 mm, starting from the anal verge. As men and women have different lengths of canal, we analysed their outcomes separately.

Results: The study group consisted of 25 (48.08%) men and 27 (51.92%) women. The pressure was diversely distributed along the anal canal during different physiological conditions. For example, in men (Figure 1A), during coughing, the highest pressure was 235 mmHg (2.2 cm from anal verge), while the lowest was 97.5 mmHg (5.4 cm from anal verge). Furthermore, the range of maximal pressure was very diverse at different levels of the anal canal, during different conditions. In men, the maximal pressure observed at the maximal tolerable volume during balloon retention test, at 4.6 cm was 135 mmHg, the maximal pressure during the maximal relaxation (rectoanal inhibitory reflex), at 0.6 cm from the anal verge was 35 mmHg, while during the other four conditions the maximum value was located at 2.2 cm from the anal verge (see Figure 1).

Conclusions: The location and profile of the 'maximal anal pressure' observed along the anal canal, during different physiological conditions are so extremely varying that talking about just 'the anal pressure' seems to be unjustified.

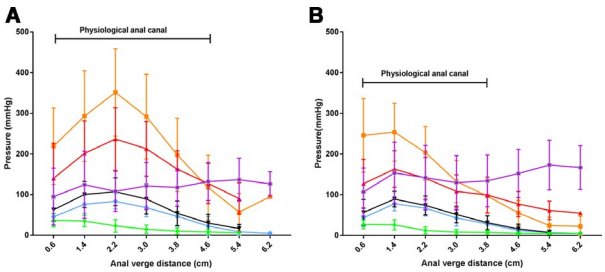

Figure 1. The anorectal pressure profile in men (A) and women (B)

381 | A rapid CRISPR/Cas9 genome editing screen to identify new regulators of neuronal differentiation and specification in the zebrafish enteric nervous system

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The enteric nervous system (ENS) provides the intrinsic innervation of the gastrointestinal tract and plays a central role in all gut functions including gut motility. There is a significant gap in knowledge understanding the molecular mechanisms that regulate neuronal differentiation and specification in the ENS, which has impacted understanding the genetic basis of many ENS disorders. High-throughput testing of candidate genes for developmental processes or candidate

disease genes is very time-consuming, expensive, and relies on easy to interpret read-outs. To address this challenge, we have developed a high throughput CRISPR/Cas9 genome editing screen in zebrafish to be able to test a large number of candidate genes for their role in regulating ENS neuronal differentiation and specification. Pools of guide RNAs targeting up to four candidate genes together with Cas9 protein are injected into one-cell stage zebrafish embryos. Injected embryos are raised to 5 days post fertilization and ENS neuron number is compared to uninjected embryos. If we identify changes in ENS neuron numbers, we de-multiplex positive pools to test if loss-of-function of one or several genes is responsible for the phenotype. In subsequent steps, we quantitatively assess changes in neuron number and neurochemical subtype specification in different regions along the entire gut. Our proof-of-principle experiments targeting two known ENS regulators *sox10* and *ret* show that we can phenocopy stable mutant phenotypes very effectively. This shows that our approach is reliable to identify genes that regulate ENS neurogenesis with high efficiency in G0 guide RNA-injected larvae. We have already identified a reduction in ENS neuron number in a number of candidate genes tested indicating that they may influence enteric progenitor cell differentiation into ENS neurons. Our work will contribute to a better understanding of how ENS neurogenesis is regulated during development and provide a high-throughput screening approach for testing the function of developmental or disease candidate genes.

382 | Multichannel impedance monitoring for distinguishing non-erosive reflux esophagitis with minor changes on endoscopy in children

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Objectives: There are reports about the relationship between baseline impedance level and esophageal mucosal integrity by endoscopic findings such as erosive and non-erosive reflux esophagitis. However, many children with symptoms of GERD have normal findings or minor changes on esophagogastroduodenoscopy (EGD). We would like to examine whether trivial changes in EGD study can be evaluated by multichannel Impedance monitoring as an alternative measure.

Methods: Patients (ages 0-17 years) with symptoms related to GER who underwent EGD and pH-MII monitoring in Women's and Children's Hospital in Adelaide, Australia, between 2014 and 2016 were retrospectively studied and the following data were collected: demographics, pH-MII data, included baseline impedance. Endoscopic findings were classified by Los Angeles grading, LA-N as normal, LA-M as with a minimal change such as the erythematous or pale mucosa, or friability in the mucosa. Patients on proton pump inhibitors were all excluded.

Results: Seventy patients (43 boys 61%) were enrolled with a mean of age of 7.9 years (range 10 months to 17 years). Fifty-one patients (72.9%) were allocated to LA-N, while LA-M evident in nineteen patients (27.1%). Statistical differences were observed in the following parameters: frequency of acid and non-acid reflux, and baseline impedance in channels 5 and 6. The median values of the data were 18.3 episodes, 16.0 episodes, 2461.0 Ω, 2446.0 Ω in LA-N, 36.0 episodes, 31.0 episodes, 2033.0 Ω, 2009.0 Ω in LA-M respectively.

Conclusion: Lower baseline impedance is helpful in predicting minimal endoscopic changes in the lower esophagus. To detect endoscopic slight change, lower esophageal baseline impedances were helpful. The higher frequency of acid and non-acid reflux episodes was also predictive of minimal endoscopic change in the lower esophagus.

383 | The N-Myc downstream-regulated gene 4 is required for enteric nervous system development and function

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Background: We recently described that the N-Myc Downstream-Regulated Gene 4 (*NDRG4*), an established early-detection DNA-methylation marker for colorectal cancer (CRC), is specifically expressed by enteric neurons. So far, our studies focused on how enteric neuronal NDRG4 is involved in the development and progression of CRC. However, the role of NDRG4 in the development and function of the enteric nervous system (ENS) during gastrointestinal homeostasis also remains to be elucidated.

Methods: To explore the role of *ndrg4* in ENS morphogenesis and intestinal motility, we used a (i) morpholino (MO) and (ii) CRISPR-Cas9 knockdown approach in transgenic zebrafish (*Danio rerio*) expressing the fluorescent-kaede-protein in enteric precursor cells and enteric neurons (Tg(-8.3phox2b:kaede) zebrafish). We quantified the number of enteric neurons in the distal intestine and created spatiotemporal maps from video recordings to examine intestinal motility in wild-type and *ndrg4*-knockdown (*ndrg4*$^{-/-}$) zebrafish larvae (5 and 7 days post-fertilization (dpf), respectively).

Results: In agreement with mouse and human expression studies, we found that *ndrg4* is also expressed within the GI-tract of zebrafish larvae. In 5dpf zebrafish, loss of *ndrg4* is associated with a significant reduction in the number of enteric neurons: (i) average number 102.4

control-injected vs. 67.4 *ndrg4*-MO injected ($P < 0.001$) and (ii) average number 110.9 *ndrg4*$^{+/+}$ vs. 97.8 *ndrg4*$^{-/-}$ zebrafish ($P = 0.028$). Although the frequency, velocity and contraction interval of peristaltic waves were not affected in 7dpf *ndrg4*$^{-/-}$ zebrafish, their travel distance was significantly shorter in *ndrg4*$^{-/-}$ as compared to *ndrg4*$^{+/+}$ zebrafish ($P = 0.040$).

Conclusion: This is the first study describing *ndrg4* expression in the zebrafish intestinal tract. Our data suggest that loss of *ndrg4* has consequences for ENS development and function.

385 | Comparison of epidemiological data on functional GI diseases under an herbal therapy from non-interventional studies in the US and Germany

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The comparison of epidemiological data on functional GI diseases from different countries can help to improve the understanding of patients' needs and of therapeutic approaches globally. In two recent non-interventional studies (NIS), one from the US and the other from Germany [1,2,3], data from patients with functional GI symptoms using an herbal medicine [4,5] have been collected, including epidemiological data, symptom quality and severity, medical needs as well as the perceived effect of the therapy.

The two studies, i.e. a study conducted by Grundmann et al. via an internet questionnaire in patients from the US, and the PhytoVIS study, a NIS in patients using natural products conducted via interviews in pharmacies and physician's practices in Germany were accessed based on the original data. For warranting comparability, data on users of the same natural product, consisting from nine standardized herbal extracts including *Iberis amara* (STW 5) were selected and evaluated using descriptive statistics.

The number of patients meeting the inclusion criteria in the US study was 50, thereof 22 (44%) who had finalized the survey and could be evaluated. In the German (DE) study, 1515 patients could be evaluated. 88% of the US resp. 70% of DE participants were female, and 50.0% resp. 34.9% belonged to the age group from 26 to 45 years. Dyspepsia-like symptoms were the largest group in both studies, while IBS could be assigned to 15.6% resp. 4.0% of patients. 50.7% resp. 63.1% rated the treatment very effective, 24.5% resp. 32.4% moderate, 5.9% resp. 5.6% not effective, and 21.7% resp. 0% didn't know. The recommendation to take the product came in 39.4% resp. 61.7% from HCPs, 9.1% resp. 26.6% from friends/family, and 51.5% resp. 11.7% from other sources (incl. internet).

There are, accordingly, considerable similarities in the predominance of female patients and the rating of the treatment effect, while the US patients are, seen overall, younger, with HCP recommendation playing a smaller role. These differences are in accordance to the conduct of the US survey via internet vs. the DE survey in pharmacies and physician's practices. A limitation is the comparatively low number of participants in the US. Nevertheless, these studies turn out to be a valuable tool to study the epidemiology of patients with functional GI diseases and the perceived effect of an herbal treatment and to rate differences and similarities between the patient populations in the US and Germany.

Acknowledgment: The PhytoVIS study is supported by Kooperation Phytopharmaka GbR, Bonn, Germany.

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386 | Handling of complexity: Describing the mechanisms of action of a multi-target therapy in functional GI diseases by multi-step clustering derived heatmaps

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Introduction: In functional gastrointestinal diseases (FGDs), a multitude of concomitant causes and likewise also targets for therapeutic interventions have been identified [1]. Therefore, a multi-target approach is a promising therapeutic strategy. For assessing the underlying complex mechanisms of action, a novel approach has been developed on the example of a complex natural product, a fixed combination of nine herbal extracts [2]. This approach is now applied to another natural product (NP) consisting from standardized extracts of lemon balm leaves, caraway fruits, peppermint leaves, bitter candytuft total plant, chamomile flowers and liquorice roots, which has been studies in a multitude of clinical and preclinical studies.

Aims: To analyze and describe its mechanisms of action of NP and the role of its components in relation to the different forms and causes of functional GI diseases, an evaluation of data is needed.

Methods: All data from studies including the combination NP and its components were retrieved. In a second step, study results were sorted according to types of study models and respective etiologic mechanisms related to FGDs, and multi-step clustering was conducted to reduce data complexity.

In a next step, results were classified in relative classes according to the strength of the effects. These data were then clustered, so giving an overview of the effects of the combination and its combination partners in relation to the different etiologic mechanisms. These transformed and clustered data sets were then visualized in the form of 2D histograms/heatmaps.

Results: The evaluation of the data shows that NP is active in response to multiple etiologic factors involved in FGDs, especially functional dyspepsia and irritable bowel syndrome, like hyper- and hypomotility, acidity, inflammation and hypersensitivity, but also in inflammatory gastrointestinal diseases. The clustering by multiple steps allows the conclusion that all components are, with a respective specific profile of activities, involved in these actions, with the heatmaps allowing an overview of the highly complex body of data within one figure.

Discussion and Conclusions: Multi step clustering allows the transformation of complex data sets necessary to show the multi-target action of medicines, especially those with a complex composition, as has been shown earlier [2]. For NP, it makes the allocation of specific actions to the different components of the medicine manageable and allows their visualization within the context of the overall action of the medicine in FGDs, so also giving support to its clinical use in patients with different symptoms.

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387 | Dependency on sex and stimulus quality of nociceptive behaviours in a conscious visceral pain rat model

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Introduction: Visceral pain, present in numerous gastrointestinal pathologies and disorders, may be influenced by many factors. The aim of this study was to analyze the impact of sex and quality of intracolonic mechanical stimulus on the behavioral manifestations of visceral pain in a preclinical model.

Material & Methods: Male (N = 7-13/group) and female (N = 5-12/group) young adult Wistar rats were used. After sedation, a 10 cm longitudinal line and shorter transverse lines every 2 cm were drawn over the abdomen to better visualize the contractions. A 5 cm long latex balloon lubricated with vaseline was inserted through the anus into the colon so that the tip of the balloon was 7 cm inside the colorectum. Sedation was reverted and behavior was recorded using a video camera located underneath the rat cage.

The pressure of the intracolonic balloon was increased (0-80 mmHg), using a sphygmomanometer. Two different types of stimulation were used:

Phasic: for each pressure value, 3 stimuli of 20 seconds were applied, 1 minute apart.

Tonic: for each pressure value, a single stimulus was applied and maintained for 5 minutes.

Visceral sensitivity was measured as abdominal contractions in response to mechanical intracolonic stimulation. Total time contracting the abdomen and duration of the contractions were also measured.

Results: Both phasic and tonic stimulation produced a pressure-dependent increase of abdominal contractions, but differences between males and females were only found for phasic stimulation: whereas females exhibited higher number of contractions than males, duration of contractions in males (but not in females) increased in a pressure dependent manner.

Conclusions: Although both female and male rats respond to colonic distension, these responses depend on the quality of the stimulus, which produce also sex-dependent differences. These differences must be taken into account in the development of models and treatments for different gastrointestinal pathologies.

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388 | Influence of age on rectal mechanics in men with dyssynergic defecation

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Background: The prevalence of chronic constipation (CC) increases with advancing age. A common cause of CC in the elderly is dyssynergic defecation (DD). Muscle atrophy and changes in the contractile tissue occur with aging. It is unknown if age related differences exist in rectal wall compliance of men with DD.

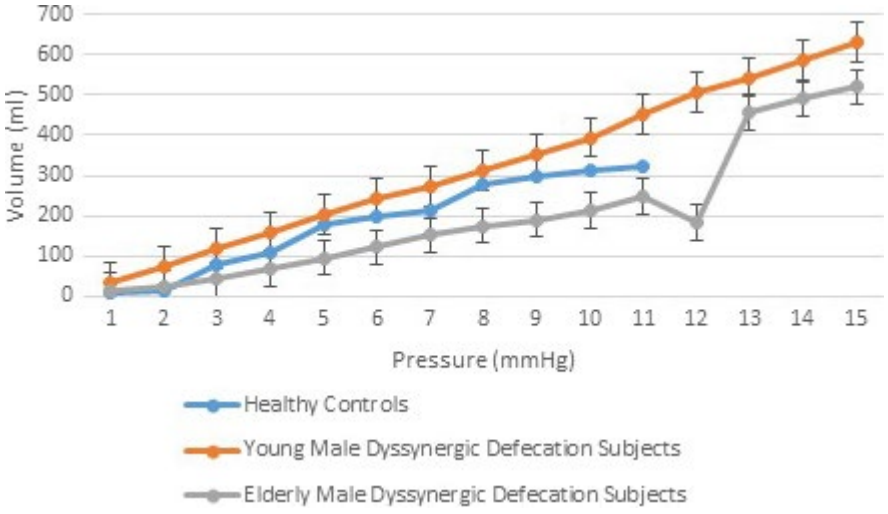
Objective: Compare rectal compliance in elderly men with DD (age ≥65 years) to that of younger men with DD (ages 18-64).

Methods: Prospective analysis of men undergoing high resolution anorectal manometry (HR-ARM), balloon expulsion testing (BET), and rectal wall sensory and compliance testing at a single tertiary care center for CC. DD was defined as a paradoxical contraction of the anal sphincter during valsalva and BET >60 seconds. Those defined with DD by HR-ARM and BET were offered rectal barostat

testing using stepwise distention, 0-60 mmHg, in 4 mmHg/30 seconds or to patient tolerance. Enrollees were divided into those aged 18-64 years (young) and those ≥65 years (elderly). All participants self-report their urge-to-defecate (0-10; higher scores representing a greater desire) at each isobaric distention. Independent t-tests and Chi-square using the Fisher statistics were performed. A *P*-value of less than 0.05 was considered significant.

Results: Ten male patients were enrolled, 5 young and 5 elderly. Young men had mean age of 32.0 and mean BMI of 26.9. Elderly men had mean age of 68.0 and mean BMI of 28.8. Final mean distention metrics were similar between both groups: elderly (53.0 ± 8.7) vs. young (55.2 ± 8.2). All of the elderly men demonstrated decreased rectal wall compliance compared to published healthy controls. The young men demonstrated rectal wall compliance similar to published healthy controls [Graph 1]. Elderly men self-reported significantly higher mean urge-to-defecate responses (8.8 ± 0.89) versus the young men (6.4 ± 0.89) at their last isobaric distention, $P = 0.009$.

Conclusions: Decreased rectal compliance as well as heightened urge to defecate was observed in elderly men compared to young men with DD. This may suggest altered rectal compliance has a contributory role in the development of DD in older men. These preliminary findings warrant further evaluation.



Graph 1. Pressure Volume Relationship During Isobaric Dissentions: Young vs. Elderly Male Dyssynergic Defecation Subjects.

389 | **Effects of gastrointestinal pH on gastric emptying time in non-diabetic patients with symptoms of gastroparesis**

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Gastroparesis is a disease with a complex pathophysiology, in which cellular alterations, motility abnormalities and gastric myoelectrical dysrhythmias seem to be central pathophysiological factors. However, the influence of microbiota in these patients has not been thoroughly investigated. Colonic fermentation products have been shown to influence gastric motility. Being a less accessible organ, the small intestinal microbiotas influence on gastric motility has not been studied. However, small intestinal bacterial overgrowth has been shown to be a common feature in patients with gastroparesis. pH may be an indirect measure to detect altered fermentation processes in the caecum. We therefore wanted to study whether there was any relation between pH throughout the gastrointestinal tract and gastric emptying time in patients with symptoms of gastroparesis as a surrogate marker of altered microbial fermentation. To test this, thirty-four (73.4% women) non-diabetic patients with symptoms compatible with gastroparesis were prospectively included. Twenty-three were idiopathic, four post-operative, three rheumatic, three neurologic and two were of miscellaneous origin. Patients were examined with a wireless motility capsule. We measured gastric emptying time and regional pH including stomach, post-pyloric, small bowel, colonic and caecal pH. Fifty-two percent of patients had delayed gastric emptying time. Patients with delayed gastric emptying time had a higher pH of the duodenum, small bowel and colon, and gastric emptying time correlated moderately with pH of

the duodenum (r = 0.505, $P < 0.01$), small bowel (r = 0.492, $P < 0.01$) and caecum (r = 0.475, $P < 0.05$). As we found significant associations between gastric emptying time and pH of the gastrointestinal tract in patients with symptoms of gastroparesis, these findings imply that altered intestinal fermentation may play a role on gastrointestinal motility in this patient group.

390 | Colonic propulsion of endogenous fecal pellets in diabetic and toxic neuropathy: Fluoroscopic study in conscious rats

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Diabetes and cancer chemotherapy trigger the development of peripheral neuropathy, which may also affect the enteric nervous system, leading to gastrointestinal dysmotility. Fluoroscopy allows direct evaluation of endogenous fecal pellet (EFP) colonic propulsion in experimental animals (1, 2). Our aim was to determine, using fluoroscopic recordings, whether colonic propulsion of EFP is uncoupled in rat models of diabetic and toxic neuropathy.

Male Wistar rats were used. Streptozotocin (STZ, 60 mg/kg) or cisplatin (2 mg/kg/wk, 5 weeks) were intraperitoneally administered to induce diabetic and toxic neuropathy, respectively (adequate vehicles were injected in the corresponding controls). Fluoroscopy was performed at various time points, including the neuropathic stage (28 days after STZ; 30 minutes after the 5th cisplatin injection, 2 mg/kg/wk). For this, rats received barium sulfate intragastrically, 20-22 hours before the fluoroscopy, so that stained EFP could be seen at the time of recording. Rats were conscious and placed within movement-restrainers during recording (120 seconds). Different parameters were blindly analyzed to evaluate EFP colonic propulsion.

Compared with their respective controls, EFP tended to accumulate in the colon of neuropathic rats. EFP diameter increased in STZ- but decreased in cisplatin-treated rats. However, specific parameters measuring EFP propulsion were not significantly different from controls in any of the models at the neuropathic stage, or afterwards in cisplatin-treated rats.

In contrast with results previously obtained for vincristine (2), colonic propulsion of EFP was not altered in diabetic or cisplatin-treated rats, suggesting that enteric neuropathy in these models might be relatively mild and attenuated by compensatory mechanisms functioning in vivo. This needs further investigation.

Acknowledgements: SAF2012-40075-C02-01. I. Pérez-García, for technical support.

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391 | Constipation in paediatric coeliac disease

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Background: Coeliac disease (CD) has a prevalence of almost 1% worldwide. The presentation of coeliac is variable and indications for performing coeliac testing are unclear. Many postulate that constipation can be a presenting complaint of CD in paediatric populations. Functional constipation (FC) has a prevalence of about 30%, thus the decision to perform coeliac testing in paediatric patients with constipation is challenging. We aimed to determine if paediatric patients with FC had increased prevalence of CD.

Methods: We enrolled 622 children from schools in Colombia, screened them for functional gastrointestinal disorders (FGID) including FC, per Rome III/IV criteria, and tested them for CD. Testing was initially done by TTG-IgA and total IgA screening. Of those testing positive for TTG-IgA, we performed HLA genotyping and endoscopy to obtain 4 duodenal biopsies for classification of CD using Marsh criteria.

Results: Two hundred and three patients were diagnosed with FC (32.6%) and 419 (67.3%) had no functional disorders. The overall prevalence of CD was 1.8%. Of those with FC, only 3 (1.5%) were diagnosed with CD. Additionally, 8/419 (1.9%) of the control patients without FGIDs were found to have CD.

Conclusion: The prevalence of CD in our cohort was 1.8%, similar to worldwide estimates (~1.4%). However, only 1.5% of patients with FC were diagnosed with CD. This was similar to the prevalence in those without FGIDs (1.9%). The prevalence of CD does not appear to be increased in paediatric patients with FC thus routine testing of patients with FC for CD is not indicated.

392 | Does diabetes mellitus impact recto-anal inhibitory reflex parameters? Comparison to published healthy control values

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Introduction: Patients with diabetes mellitus are commonly referred for high-resolution anorectal manometry (HR-ARM) to investigate refractory constipation or fecal incontinence to direct pelvic floor physical therapy recommendations. ARM measures of recto-anal inhibitory reflexes (RAIR) are abnormal with Hirschsprung's disease but also are blunted in some patients with rectal hyposensitivity. We aimed to (i) compare RAIR parameters in diabetic patients vs. published healthy control values (*Thiruppathy, K. Dig Dis Sci 2012*) and (ii) explore associations between RAIR parameters in diabetic patients and indications for HR-ARM testing.

Methods: Retrospective analyses were performed on HR-ARM findings from diabetic patients from January 2017 – September 2019 at a tertiary center. RAIR was tested by rapidly inflating a rectal balloon (50 mL) followed by immediate deflation. RAIR presence was defined by ≥25% reduction of internal anal sphincter (IAS) pressure. RAIR parameters included: resting IAS pressure (mmHg), excitation latency (seconds), duration of RAIR (seconds), and reduction in RAIR amplitude (%). Bivariate calculations and linear regression analyses compared diabetic RAIR parameters to published healthy control values.

Results: 22 diabetic patients underwent HR-ARM. Demographics included: 81.8% female, 81.8% Caucasian, mean age 58.6 years (SD = 15.1; Range: 20-82), and mean BMI of 32.8 kg/m^2 (SD = 6.9; Range: 20.5-45.5). Indications for HR-ARM testing: 77.3% constipation, 22.7% fecal incontinence. Diabetic patients exhibited significantly lower resting IAS pressure, reduced excitation latency, longer mean reflex duration, and lesser % amplitude relaxation compared to healthy controls (Table 1). Diabetic patients with chronic constipation showed longer mean reflex duration (17.6; SD = 6.2) vs. diabetics with incontinence (11.8; SD = 2.2) (P = 0.004). Other RAIR parameters were not different between constipation and incontinence.

Discussion: Diabetic patients referred for high-resolution anorectal manometry exhibit significant abnormalities in RAIR activity, which are similar in refractory constipation and fecal incontinence except for RAIR duration. These findings likely reflect underlying sensory neuropathy that may be a target for sensory retraining on physical therapy.

393 | Patients with heartburn express transient receptor potential vanilloid subfamily member-1 (TRPV1) on superficial afferent oesophageal nerve fibres

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Background/Objectives: Capsaicin infusion to the oesophagus induces chest pain and heartburn. Protein localisation studies in non-erosive and erosive reflux disease patients have shown increased oesophageal TRPV1 mRNA and protein levels suggesting increased TRPV1 expression may mediate hypersensitivity in patients with heartburn symptoms [1-2]. Our aim was to characterise TRPV1 expression on human oesophageal sensory neurons innervating the oesophageal mucosa in patients with heartburn.

Methods: Distal oesophageal biopsies of 48 patients presenting with heartburn were co-stained with CGRP (Thermo Fisher Scientific, PA1-30927, 1:400) and TRPV1 (Alomone, ACC-050, 1:400) antibodies and were evaluated with immunofluorescence-immunohistochemistry. Efficacy of TRPV1 antibody staining was confirmed using positive (IBD colon biopsies and skin tissue) and negative controls.

Results: In 21/48 patients studied (6/21 NERD patients; 15/21 awaiting physiology testing), we identified superficial nerve fibres that co-expressed CGRP and TRPV1. In 27/48 biopsies (4/27 Barrett's oesophagus patients, 9/27 erosive reflux disease (ERD), 1/27 NERD, 3 functional heartburn (FH), and 10/27 awaiting definitive physiology testing) where CGRP-immunoreactive nerve fibres were identified in the deeper layers of the oesophageal mucosa, TRPV1 was not co-expressed but some TRPV1 expression was detected in epithelial cells (5/27 patients).

Conclusion: Superficial afferent fibres of the oesophageal mucosa express TRPV1 in NERD patients. We previously demonstrated that patients with NERD have significantly more superficial nerve fibres compared to healthy volunteers, FH, ERD and Barrett's and therefore TRPV1 expression on sensory fibre endings in the mucosa suggest a mechanism for heightened acid sensitivity in NERD patients [3].

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394 | Efficacy and safety of fexuprazan, a novel potassium-competitive acid blocker, compared to esomeprazole in patients with erosive esophagitis: Phase 3, non-inferiority randomized controlled trial

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Background/Objectives: Fexuprazan is a novel potassium-competitive acid blocker which can provide clinical benefit in acid-related disorders. This study aims to establish non-inferiority of

fexuprazan compared to esomeprazole and to confirm safety in patients with erosive esophagitis.

Methods: In this multicentre, randomized, double-blind, parallel-group trial, Korean adult patients with endoscopically confirmed erosive esophagitis (LA Grades A-D) were randomly allocated to receive fexuprazan 40 mg or esomeprazole 40 mg once daily. The primary endpoint was the proportion of patients with endoscopically confirmed healed erosive esophagitis up to week 8.

Results: Of the 260 subjects randomized, 218 completed the study. Fexuprazan was non-inferior to esomeprazole in week 8 healing rate (99.1% (106/107) vs 99.1% (110/111)) with a difference of 0.89% (95% confidence interval, −0.86 to 2.64). The cumulative healing rates at week 4 were 90.3% (93/103) for fexuprazan and 88.5% (92/104) for esomeprazole. Fexuprazan showed comparable symptom relief with esomeprazole. No significant differences in gastrin concentration and incidence of adverse events were observed.

Conclusions: Fexuprazan is non-inferior to esomeprazole in healing erosive esophagitis. Fexuprazan was well-tolerated and showed better symptom relief for heartburn and atypical symptom, which require further investigation.

395 | Fast forward bioprospecting of the gut microbiota for novel live biotherapeutics and anti-inflammatory bioactives

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Microba has identified over 800 numerically abundant and prevalent gut bacteria associated with human health, which are underrepresented or absent in several disease states including inflammatory bowel disease (IBD), asthma, gastroesophageal reflux disease (GERD), irritable bowel syndrome (IBS), hypertension, anxiety and depression. These comprise a heterogenous group of diseases however several feature inflammation as a major pathology. Notably, the nuclear factor-κB (NF-κB) driven inflammatory response plays a central role in the pathogenesis of IBD, asthma and GERD, and it also plays a role in other diseases including metabolic disease, colorectal cancer (CRC), rheumatoid arthritis, multiple sclerosis, chronic obstructive pulmonary disorder (COPD) and atherosclerosis. Inflammation is the primary pathology in IBD and Microba researchers have demonstrated gut bacteria produce potent NF-κB suppressive bioactives. Moreover, we have established methodologies relevant to IBD that could expedite the identification of precision Live Biotherapeutics (LBPs) and NF-κB suppressive bioactives. These LBPs and/or their bioactives could potentially be exploited to prevent or treat IBD.

396 | Utility of digital rectal examination in identifying patients with dyssynergic defecation

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Background & Aims: Dyssynergic defecation (DD) is a common cause of chronic constipation; its diagnosis requires anorectal physiological tests that are not widely available. It has been suggested that digital rectal examination (DRE) is a useful test to identify dyssynergia. We examined the diagnostic yield of DRE in patients with DD.

Methods: Consecutive patients with chronic constipation who underwent DREs, anorectal manometry (ARM) analyses including balloon expulsion test were included in the study. In DRE, dyssynergia was identified by 2 or more of the following features: impaired perineal descent, paradoxical anal contraction, or impaired push effort. On ARM, a diagnosed of DD was based on standard criteria. Diagnostic yields of DRE were compared with ARM.

Results: 51 consecutive patients were included in the study, 43 (84.3%) had DD based on ARM. Mean age was 48 years; 64.7% (33/51) were male. The sensitivity and specificity of DRE for identifying DD was 88.4% and 62.5%, respectively; the positive predictive value was 92.7% and accuracy was 84.3%. Paradoxical anal contraction on DRE had a sensitivity of 81.4%, specificity of 62.5%, PPV 92% and accuracy of 78.5%.

Conclusions: DRE is a reliable bedside tool for identifying DD in patients with chronic constipation. DRE could facilitate the selection of appropriate patients for further physiologic testing and treatment.

397 | Effects of M1 receptor positive allosteric modulators on colorectal function

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Background and Objectives: Defective colorectal propulsion, encountered in patients with spinal cord injury, Parkinson's disease, multiple sclerosis or having no identified cause, is difficult to treat. Because M1 muscarinic receptors are expressed by enteric excitatory motor neurons and interneurons, we hypothesized that M1 positive allosteric modulators (M1PAM), which facilitate physiological actions of acetylcholine, could be used as colokinetics to facilitate colorectal propulsion.

Methods: The potencies of three M1PAMs, T440, T662 and T523, were investigated. The effectiveness of M1PAMs on defecation was investigated by oral administration in dogs, mice and rats, by recording propulsive contractions in anaesthetized rats and by recording

high amplitude propagating contractions in dogs. M1PAM effectiveness was tested in loperamide-induced constipation and the constipation of aging.

Results: The M1PAMs, tested in transfected cells, enhanced the action of acetylcholine, but were 1000-fold less potent in direct action at M1 receptors. M1PAMs stimulated defecation in conscious dogs, mice and rats, with no adverse effects. In anesthetized rats, intravenous and intraduodenal administration of M1PAMs elicited highly propulsive colorectal contractions. In dogs instrumented with tension transducers on the bowel, M1PAMs evoked high amplitude propagated contractions (HAPCs). Denervation and isolated organ experiments from mice and rats indicated that the M1PAMs exert their effects at the level of the colon, not on central defecation pathways. Ussing chamber experiments showed that M1PAMs increased fluid secretion in isolated rat colon, but did not cause bowel leakiness. Feces expelled under the influence of M1PAMs was moistened. The effectiveness of M1PAMs was tested in two models of constipation in mice, opiate (loperamide)-induced and age-related constipation. Loperamide reduced fecal output to a novel environment test by 80%. This was reversed by T523 at a dose of 1 mg/kg. Eighteen month old mice produced 20% of the fecal pellets of 2 month mice. Fecal output in 18 month mice was returned to 2 month values with 0.3-1 mg/kg of T523.

Conclusions: Positive allosteric modulators of muscarinic M1 receptors have potential to treat constipation because of their effective enhancement of colorectal propulsive activity, stool softening abilities and lack of adverse side-effects.