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WILEY Neurogastroenterology & Motility NGM

ORAL PRESENTATIONS: DIETARY INTERVENTIONS INCLUDING PROBIOTICS, PREBIOTICS AND SYNBIOTICS

1 | Bifidobacterium Breve NCFB 2258 stimulates vagal nerve firing across an intact colonic barrier

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Objective: Mounting evidence implicates the vagus nerve in signalling between colonic bacteria and the central nervous system (CNS), in what has been termed the microbiome-gut-brain axis. However, the mechanism by which bacteria signal across an intact barrier to their eukaryotic hosts is not understood. Bifidobacterium Breve NCFB 2258 is a commensal bacterial strain which produces polyunsaturated fatty acids (PUFAs) with reported health-promoting effects. The study aim was to investigate if this bacterial strain could signal across the gut barrier to stimulate the host nervous system.

Methods: Using ex-vivo Sprague Dawley rat colonic tissue, immunofluorescent staining and calcium imaging were utilised to investigate activation of submucosal neurons in response to mucosal application of PUFA-producing probiotic secretions (supernatants). To determine if the effects were local to the enteric nervous system of if they also stimulated colonic afferents, extracellular recordings of vagal nerve activity were also undertaken.

Results: Mucosal exposure to the bacterial supernatants stimulated increased nuclear expression of cFos and peroxisome proliferator-activated receptor alpha (PPARα) in submucosal neurons. A robust increase in neuronal $[Ca^{2+}]i$ was also observed in response to mucosal application of supernatants. This response was reduced ($P<.001$) but not abolished by the PPARα antagonist, GW6471. Similarly, exposure of the colonic mucosa to supernatants ($P<.001$) stimulated increased firing in vagal afferents. The PPARα antagonist reduced ($P<.001$) but did not abolish this response.

Conclusions: These findings illustrate that PUFA secretions from Bifidobacterium breve NCFB 2258 signal across a healthy intact gut barrier to the intrinsic and extrinsic gut nerves. Supernatants induced activation of underlying submucosal neurons, which regulate absorption and secretion, but may also act as a relay for gut-to-brain signalling. Indeed, supernatants also increased vagal afferent firing, which was mediated in part by activation of PPARα. These findings begin to elucidate the molecular mechanisms underlying signalling by specific bacteria in the microbiome-gut-brain signalling axis.

Policy of full disclosure: None.

2 | Protease activity and tryptase expression is increased in a post-inflammatory rat model for visceral hypersensitivity

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Objective: Previously, we confirmed beneficial effects of the serine protease inhibitors nafamostat mesilate and the newly developed UAMC-0050 and UAMC-1162 on visceral hypersensitivity in an acute and post-colitis rat model [Ceuleers et al. Neurogastro 2015, DDW 2016, DDW 2017]. The objective of this study was to explore which serine proteases are specifically involved based on the profiles of the inhibitors used targeting tryptase, matriptase, uPA, cathepsin G and kallikrein 2, 4, 8.

Methods: As previously described, male Sprague-Dawley rats were intrarectally instilled with TNBS (colitis) or 0.9% NaCl (control). Successively, colonoscopies were performed to document the acute colitis on day 3 and the complete healing of the mucosa in the post-colitis phase (day 10-18). Colon sampling of control, acute colitis and post-colitis rats was performed to measure the mRNA expression of the serine proteases described above by qPCR, as well as to quantify mast cell tryptase by immunohistochemistry. Next, fecal samples were collected at day 0 (control), day 3 (acute colitis) and the day of sacrifice (post-colitis) to determine general protease activity using an azocasein assay.

Results (Table 1): All TNBS rats developed acute colitis on day 3, while the post-inflammatory status was confirmed on the day of sacrifice for post-colitis rats. The qPCR experiments showed a significant downregulation of matriptase in the colon of rats with acute colitis compared to controls. In post-colitis rats, tryptase was significantly upregulated,

while no significant differences were found for matriptase. No alterations could be observed for the other serine proteases studied (Table 1). Immunohistochemical staining for mast cell tryptase (# tryptase positive cells/mm^2) showed a significant increase from 86.4±8.8 in control to 119±24 in acute colitis and 168±30 in post-colitis animals (n=8; *P*<.05). The azocasein assay revealed a significantly increased protease activity (U trypsin/mg protein) in the feces of both acute colitis (647±63; n=12; *P*<.05) and post-colitis rats (613±75; n=12; *P*<.05) compared to controls (332±14; n=12).

Conclusions: These results confirm colonic serine proteases as potential therapeutic targets for visceral hypersensitivity. Interestingly, their colonic expression profiles differed in the acute inflammatory vs the post-inflammatory state. The source of tryptase needs further investigation focusing on cellular sources within the colonic wall and/or the microbiome.

Policy of full disclosure: None.

Table 1. Relative mRNA expression of serine proteases in colonic samples

Gene	Control	Acute colitis	Control	Post-colitis
Tryptase	1.25 ± 0.28	2.45 ± 1.03	1.29 ± 0.37	4.65 ± 1.07*
Matriptase	1.04 ± 0.10	0.58 ± 0.06*	1.06 ± 0.15	1.00 ± 0.16
uPA	1.05 ± 0.12	1.05 ± 0.17	1.12 ± 0.21	1.43 ± 0.31
Cathepsin G	LOD	LOD	LOD	LOD
KLK2	LOD	LOD	LOD	LOD
KLK4	LOD	LOD	LOD	LOD
KLK8	1.16 ± 0.27	1.05 ± 0.17	1.21 ± 0.24	1.18 ± 0.18
	n=8	n=8	n=8	n=8

Data are expressed as relative mRNA expression and presented as mean ± sem for n=8 per group. Independent samples T-test. *p<0.05 significant effect of the factor "group". uPA; urokinase plasminogen activator; KLK; kallikrein; LOD; below limit of detection.

3 | Influence of the herbal extract combination STW 5 on the human intestinal microbiota in vitro

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Objective: Several clinical studies have shown that STW5, a fixed herbal extract combination, is effective in treating functional dyspepsia and irritable bowel syndrome (IBS). As some of the preparation´s constituents are believed to reach the distal gut, we were interested, whether STW-5 might influence gut microbiome function and composition.

Methods: Lyophilized STW-5 dissolved in buffer was incubated with human fecal suspension under physiological conditions. Samples were taken 0.5 hours, 4 hours (t=4) and 24 hours (t=24) after STW 5 or vehicle (control) addition. The composition of the microbial community at the different time points was analyzed by amplicon-based next generation sequencing of 16S rRNA genes. Propidium-monoazide (PMA) treated samples were processed in parallel to reduce background noise from dead microbial cells. Focus was set on beta-diversity analyses (which microorganisms are supported by STW5, which microbes are potentially inhibited), and functional analyses at the different time points.

Results: Incubation with STW5 caused significant changes in microbial community composition (Figure 1, bubble plot). Major changes occurred already within the first 30 minutes. Most strongly enhanced bacterial taxa belonged to the genus Enterococcus, which are capable of glucose fermentation without gas production. The strongest decrease could be observed in gas-producing Clostridia representatives, in particular for Ruminococcaceae. Bacteroidetes species were similarly affected. Microbial functions involved in xenobiotics degradation were significantly enhanced in samples containing STW5.

Conclusions: In our in vitro study, STW5 was found to have a tremendous effect on intestinal microbial community composition and function, potentially reducing gas production and thus increasing well-being for the human host. Our findings indicate an additional explanation for the efficacy of STW5 in IBS, namely the impact on the human microbiota, which now needs to be confirmed in vivo.

Policy of full disclosure: I am fully employed by Steigerwald Arzneimittelwerk GmbH.

4 | Disruption of colonic microbiome and circulating metabolome in antibiotic-treated mice

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Objective: In many neurologic and psychiatric disorders the profile of the intestinal microbiota is altered. This led to the hypothesis that a distorted microbiota profile or dysbiosis constitutes an insufficiently comprehended pathogenic factor. Therefore we tried to explore pathways of communication from gut to brain by a detailed analysis of the effect of antibiotic-induced dysbiosis on gut microbiome and plasma metabolome.

Methods: Mice were administered the probiotic OMNi BiOTiC Stress Repair® (9 bacterial strains; Institut Allergosan, Graz, Austria) or placebo via the drinking water for 3 weeks prior to and during an 11-day treatment with an antibiotic mix (bacitracin, meropenem, neomycin, vancomycin). The microbiome in stool samples was analysed by 16S rDNA sequencing, and blood plasma was analysed by targeted LC-HRMS metabolomics.

Results: Antibiotic-induced microbial community disruption was associated with a markedly altered plasma metabolome. Specifically, the plasma levels of hippuric, tricosanoic, heneicosanoid, erucic, and pentacosanoic acid were decreased whilst various lysophosphatidylcholines (20:0 and 16:0) were enhanced in antibiotic-treated mice, relative to control animals. Probiotic administration in antibiotic-treated animals led to a significant increase of circulating aspartic acid levels. Microbiome analysis of stool samples revealed a clear separation of the placebo- and probiotic-treated groups before and after antibiotic exposure. Specifically, probiotic administration preserved the Lactobacillaceae population following antibiotic treatment.

Conclusion: These findings suggest that differential alterations in the gut microbiota profile due to antibiotic as well as probiotic plus antibiotic treatment are signalled to remote organs including the brain via changes in circulating metabolites. The alterations of aspartic acid levels in particular warrant further investigation in view of the implications of this amino acid in brain function. Supported by the Austrian Science Fund (P25912-B23 and W1241-B18).

Policy of full disclosure: None.

CHALLENGES IN SEVERE DIGESTIVE DISORDERS

5 | Novel mutations in neurogenic chronic intestinal pseudo-obstruction identified by high-throughput sequencing

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Objectives: Chronic intestinal pseudo-obstruction (CIPO) is a severe gut dysmotility with recurrent sub-occlusive episodes without demonstrable mechanical causes. This study was aimed to identify genes mutated in a subset of neurogenic CIPO cases, including those associated to small fiber neuropathy (SFN), a peripheral neuropathy involving the autonomic nervous system.

Methods: Whole exome sequencing (WES) was performed on genomic DNA of 6 patients with clinical, radiological and manometric evidence of neurogenic CIPO and neurologically characterized SFN (4F, 2M). Exome variants were annotated with Gemini (GEnome MINIng). Sporadic CIPO patients (n=104; 31 M/73 F; age: 7-70 years) were analyzed with a custom TruSeq Amplicon Low Input kit. Data analysis was performed with Variant Studio.

Results: WES analysis identified a novel de novo missense variant in B3GAT2, and a missense variant in SCN11A in another patient with CIPO, SFN and severe abdominal pain. Target sequencing showed a premature stop codon in B3GAT2, and different rare/novel missense variants in SCN5A, SCN9A, SCN10A, SCN11A, TRPA1 in the other 104 CIPO patients.

Conclusions: We identified novel gene defects in: B3GAT2, a glucuronyl transferase implicated in neuronal adhesion/migration; and genes encoding for sodium channel subunits involved in pain and gut motility control suggesting that a channelopathy may occur in a subset of CIPO. High-throughput sequencing technologies help deciphering genetic mechanisms contributing to severe gut dysmotility and visceral pain in CIPO.

Policy of full disclosure: None.

6 | Delivery of neural stem cells to the gut using mesenterial perfusion

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The enteric nervous system (ENS) is formed by the migration of enteric neural stem cells (NSPCs) into the gut. Deficits in the ENS development can lead to severe intestinal neuropathies. The innate absence of the enteric colonization of distal gut regions can lead to the most prominent ganglionic neuropathy, Hirschsprung´s disease, which is characterized by a segmental or total aganglionosis. While surgical treatments are the current gold standard, a cell replacement therapy constitutes an appropriate alternative, especially in cases of a total aganglionosis.

A chance to regain an intact innervation of the GIT is the translational approach of autologous stem cell transplantation. To achieve a large variety of NSPCs to be guided and differentiating into the affected aganglionic sections, a novel method was established: the mesenterial perfusion with the cells to be transplanted.

To do so, NSPCs were isolated from the ENS of postnatal mice and purified by fluorescence-associated cell sorting (FACS) based on neural crest cell (NCC) specific, surface markers like p75. After the expansion of these sorted and uniform progenitor cells, they were locally applied into murine gut segments ex vivo by cannulating their supporting blood vessel arcades. A homogenous distribution of the colonizing cells all over the perfused segment was achieved. The cells left the capillaries and formed network like structures within the muscle layer. Immunostainings of the previously transplanted and subsequently cultured intestinal segments against neuronal and glial cell markers showed both the successful migration from the vessel, the engraftment and the differentiation of introduced cells into their intended position during the post-transplantational days. This method allows the single transplantation of one cell type, but can also easily be adapted to transplant additional cell types, such as immune cells to improve the overall transplantation success.

In conclusion, the local application of enteric progenitor cells and their successful engraftment into diseased gut segments can be easily performed by consistent perfusion into blood vessel arcades.

Policy of full disclosure: None.

7 | Effects of intestinal alkaline phosphatase on intestinal permeability and bacterial translocation in an experimental model for sepsis

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Introduction: Sepsis is a severe condition characterized by a dysregulated inflammatory response, resulting in an activation of the intestinal inflammatory cascade, mucosal barrier dysfunction and bacterial translocation. Intestinal alkaline phosphatase (IAP) has shown nephroprotective effects during sepsis in clinical trials, yet its effects on the leaky gut and bacterial translocation in a caecal ligation and puncture model (CLP) remain generally unknown.

Objectives: This study aimed to investigate the effects of IAP on gastrointestinal permeability and bacterial translocation in a validated experimental model for sepsis, namely the CLP-model.

Methods: Male OF-1 mice were randomized into 4 groups (n=10/group): sham+vehicle, sham+IAP, CLP+vehicle, CLP+IAP. On the first day either a CLP-(50% ligation, single 21G puncture) or sham-(laparotomy) procedure was performed. Mice received an IP injection of vehicle (saline) or calf IAP (1 IU/g mouse, New England Biolabs) 5 minutes prior to the procedure and subsequently twice a day postoperatively, therefore combining preventive and curative treatment. Analgesia and fluid resuscitation were provided. The validated clinical disease score (Heylen et al.) and the body weight were monitored daily throughout the experiment. At day 2, the abdomen was reopened and 100 µL of 4kDa FITC-Dextran was injected directly in the distally ligated ileum to measure small intestinal permeability. One hour later, mice were sacrificed and blood was drawn for quantification by fluorescence-spectrophotometry. Cultures of mesenteric lymph nodes, liver tissue, blood and peritoneal lavage fluid were incubated and identified after enrichment with MALDI-TOF.

Results: Septic mice had significantly worse clinical disease scores, lost significantly more weight and had increased intestinal permeability in comparison with their sham-operated counterparts. IAP-treatment did not reduce the sepsis-associated weight loss nor did it result in better clinical outcomes in CLP mice (Table 1). On the other hand, IAP significantly reduced the observed disturbances in small intestinal permeability in septic animals (ANOVA and post-hoc analysis). Moreover, IAP-treatment decreased the amount of cultured enteropathogens up to 20%.

Conclusion: In mice, CLP-induced sepsis is associated with disturbed intestinal permeability and bacterial translocation. In our model, treatment of septic-mice with systemically administered IAP significantly lowered the intestinal permeability for FITC-dextran at the level of the small bowel.

Policy of full disclosure: None.

Table 1

	Sham + Vehicle	Sham + IAP	CLP + Vehicle	CLP + IAP
Clinical Disease score (n=10/group)				
Score at day 2; SEM	0.0 ±0.00	0.1 ±0.10	4.5 ±0.40*	3.7 ±0.45*
Postoperative weight (n=10/group)				
Weight loss (%) day 2; SEM	5.41 ±1.21	2.95 ±1.71	10.17 ±0.63*	11.51 ±0.95*
Permeability (n=8-9/group)				
FITC-Dextran blood concentration (ng/ml); SEM	341.83 ±90.38	266.57 ±49.56	2348.98 ±658.14*	1012.74 ±135.74*#
Enteropathogen-positive cultures (Descriptives)				
Mesenteric Lymph Nodes	1/10 (10.0%)	1/10 (10.0%)	10/10 (100.0%)	8/10 (80.0%)
Liver Tissue	1/10 (10.0%)	1/10 (10.0%)	8/10 (80.0%)	6/10 (60.0%)
Hemocultures	1/10 (10.0%)	0/10 (0.0%)	5/10 (50.0%)	2/8 (25.0%)
Peritoneal Lavage Fluid	1/10 (10.0%)	1/10 (10.0%)	9/10 (90.0%)	7/10 (70.0%)

Enteropathogens identified: E. coli, E. faecalis, E. cloacae, E. gallinarum, Citrobacter freundii.
SEM (Standard error of mean); CLP (Caecal ligation and puncture)
* Significant result compared to sham-procedure.
# Significant result compared to vehicle.
Statistics – univariate analysis with post-hoc (SNK) test, a p-value of 0.05 was considered statistically significant.

8 | Inflammatory state and phenotypic switch of human smooth muscle in diverticulosis and complicated diverticular disease

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Background and Aim: Colonic diverticulosis, as well as diverticular disease, is a multifactorial disorder characterized by neuro-muscular alterations consisting in impaired contraction, inflammation and fibrosis. Aim of this study was to determine, both in human uninvolved and involved tracts of asymptomatic diverticulosis (AD−, AD+) and in stenotic segments of complicated diverticular disease (CDD), the intrinsic alterations to smooth muscle.

Methods: Circular and longitudinal smooth muscle strips and cells (SMC) were isolated separately from surgical colon specimen of 12 patients (58<age<80 years) affected either by sigmoid AD (6) or CDD (6) and 6 patients (61<age<80 years) submitted to surgery for cancer as control (CTR). qPCR analysis, expressed as Relative Quantification, was performed for transcription of mRNA encoding for TNF-α, inflammasome components (NLRP3, ASC, CASP1, IL1β) and for SMC phenotypic switch molecules (Collagen I, α-SMA, TGF-β, PDGF-β, Trb3, Smad2/3). Contraction was tested in response to carbachol. qPCR data were normalized to β-actin mRNA and expressed as mean±SE.

Results: In both muscle layers, AD− and AD+ SMC, compared to CTR, showed an increase in inflammatory gene expression, with a trend of decrease from AD− to AD+. This inflammatory state was associated with a progressive inhibition of contraction to carbachol, in strips and SMC that was significantly reduced in AD+. Peculiarity of circular SMC was a progressive increase in Coll1 expression from AD to CDD compared to CTR (3 hundred fold increase) associated with about 50% decrease in the contractile protein α-SMA. Differently, longitudinal SMC, both in AD and CDD, presented a homogenous reduction of contraction, increased Coll1 expression and decrease in α-SMA. Furthermore in CDD, a phenotypic switch was observed, driven in circular layer, by a TGF-β-dependent pathway (increased expression for TGF-β: 2.88±0.6 and Smad3: 1.67±0.12 and reduced Smad2: 0.39 ±0.11) while in longitudinal driven by PDGF-β-dependent pathway (increase of PDGF-β: 2.27±0.44 and parallel decrease of Trb3: 0.58±0.13).

Conclusion: Intrinsic myogenic alterations are present in colonic asymptomatic diverticulosis and complicated diverticular disease, both in circular and longitudinal layers consisting in a myogenic pro-inflammatory state and impaired contractile activity that, in complicated diverticular disease, ended in a muscular pro-fibrotic switch.

Policy of full disclosure: None.

NEUROGASTROENTEROLOGY: ACROSS THE LIFESPAN

9 | Brain-microbiome-behaviour associations following early adversity: A proof of concept study across development

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The microbiome is increasingly recognized as important for brain function. In particular, rodent models have shown that microorganisms residing in the gut influence emotional behaviour and are linked to altered brain states, especially in emotion-related regions (eg, prefrontal cortex and hippocampus). Rodent models also demonstrate that both brain and the microbiome are regulated by environmental events, and are maximally responsive to those events in early life. We performed this proof-of-concept study in humans to establish the existence of brain-microbiome associations in children, and examine their influence by early stress exposure. Community composition of the gastrointestinal microbiome and brain activity during an emotional faces task (performed in a magnetic resonance imaging MRI scanner) were examined in mid childhood. These measures were collected in N=16 children (5-13 years of age) purposefully selected to differ in early adversity exposure (in order to increase the chance of meaningful microbiome variability). The adversity exposed group experienced extreme parental deprivation in early life as a result of rearing in institutional settings abroad, before adoption into families in the United States—previously institutionalized (PI). Comparison (COMP) children were not deprived of parental care. Across taxonomic levels, bacterial richness estimates were lower in the previously deprived children than in the typically developing sample. As has been reported in adults, all samples were dominated by either genus Bacteroides or Prevotella. However, Prevotella dominated samples were more commonly observed in adversity exposed children, which was not associated with diet. Due to this stratification, we chose to characterize the associations between Bacteroides or Prevotella and whole brain reactivity during the emotional faces task (fear>baseline contrast) across all subjects (controlling for adversity exposure). Interestingly, the neural patterns predicted by these two bacteria were largely overlapping (medial and left lateral prefrontal cortex), but were opposite valences (ie, Bacteroides predicted increases, whereas Prevotella predicted decreases, in reactivity). Functional connectivity analyses of these prefrontal clusters revealed different connectivity patterns, with functional consequences for anxiety. These data provide firm proof of concept for the importance of adversity-associated microbes in brain function and anxiety across development.

Policy of full disclosure: None.

10 | Development of coordinated electrical activity in the human foetal enteric nervous system

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Introduction: The enteric nervous system (ENS) is the largest branch of the peripheral nervous system consisting of complex networks of neurons and glia. These networks act in conjunction with other cell types to regulate many of the crucial functions of the intestine including motility. Significantly, recent work has demonstrated the early development and maturation of electrical activity within the murine ENS raising the possibility that neural activity influences the wiring of the developing ENS. Although studies in human samples have clearly demonstrated the spatiotemporal development of the ENS, in terms of gross morphology, little is known regarding the development of electrical activity within the human foetal ENS.

Objective: To assess the emergence of evoked electrical activity within the developing human ENS.

Methods: Human foetal gut samples were obtaining via the MRC-Wellcome Trust Human Developmental Biology Resource (HDBR). Characterisation of the developing ENS was performed by immunohistochemistry and confocal microscopy. Functionality, at the tissue level, was assessed using calcium imaging in conjunction with electrical point stimulation.

Results: Human foetal gut samples (terminal ileum or colon) displayed robust Tuj1 immunohistochemistry by embryonic week (EW) 12 with a dense neural network at the level of the myenteric plexus. Co-labelling with the synaptic marker SNAP-25 demonstrated the development of synaptic contacts by EW12. However, electrical train stimulation (40 V, 2 s, 20 Hz of 300 μs electrical pulses) did not evoke activity within the myenteric plexus of EW12 foetal gut (n=0/3). At EW14, Tuj1+ neurons were similarly apparent within the myenteric plexus with nerve fibres also present within the submucosal region. The majority of EW14 gut tissues (80%), assessed by calcium imaging, demonstrated no response to electrical stimulation (n=1/5). In contrast, at EW16 Tuj1+ expression, in the myenteric plexus, was observed within discrete ganglia and was combined with the emergence of evoked calcium transients (F/F0=1.273±0.024; n=3), upon electrical stimulation (n=3/3), which were abolished after the addition of 1 μmol/L TTX (n=3/3). RNA sequencing analysis is currently being performed to determine temporal transcriptional changes within developing foetal gut samples.

Conclusion: Here we demonstrate, for the first time, the emergence of coordinated electrical activity within the human foetal ENS at approximately EW16.

Policy of full disclosure: None.

11 | Infant faecal microbiome diversity and behavioural outcomes at age two

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This study aimed to test associations between infant faecal microbiota composition diversity (at age 12 months) and parent-reported behavioural problems (at age 2).

Faecal samples were collected from 944 infants at 12 months of age. In a random subset of n=256, bacterial DNA was extracted and the V4 region of 16S rRNA gene sequenced using the Illumina MiSeq platform. Bacterial alpha diversity was then measured using the Shannon Index. When children were 2 years old, parents completed the Child Behaviour Checklist, a validated 99-item questionnaire of problem items with Internalising, Externalising and Total Problems subscales. Children were classified as having 'elevated' behavioural problems on one or more of these three subscales (above the 85th percentile). We developed a directed acyclic graph a priori which identified the following minimum adjustment set of potential confounding factors: duration of breastfeeding, maternal depression during pregnancy, maternal microbiota diversity, parental education, socioeconomic disadvantage and pet ownership.

Twenty-two of the 204 participants for whom all relevant data were available were classified as having 'elevated' behavioural problems. On average the Shannon Index was higher in the 'elevated' problem group than the 'normal' behaviour group (Mean diff: 0.23, 95%CI: 0.04-0.43; Welch's *t*-test, *P*=.02, see Figure 1). Each unit increase in Shannon Index approximately doubled the odds of having behaviour problems, however the 95% confidence intervals were wide (OR: 2.21, 95%CI: 0.96-5.47, *P*=.073; age and gender included in this model). Further individual adjustment for the factors listed above did not alter this estimate by 10% or more. There was no apparent association between Shannon Index and Total Problems subscale of the CBCL as a continuous measure (*r*=.01, *P*=.86).

With a categorical, but not continuous measure of behaviour problems, we observed weak evidence of an association between increased infant faecal microbiota diversity and subsequent child behavioural problems. An exploratory analysis is underway to investigate relationships between other covariates that may be relevant to the observed association.

Policy of full disclosure: None.

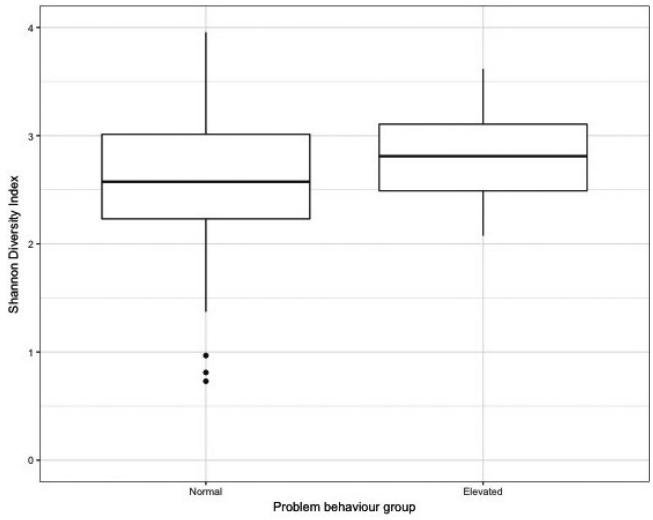

FIGURE 1 12 month Shannon Diversity Index by Problem behaviour status at 2 years

12 | Development of functional innervation of the gastrointestinal mucosa

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Objective: The wall of the gastrointestinal tract is made up of many concentric layers of different cell types, innervated by enteric neurons whose cell bodies are located in the myenteric and submucous plexus. Communication with target cells is vital for gastrointestinal function, however, there is currently little known about the development of neuronal projections to their target cells.

In this study, we investigated the development of innervation of the mucosal villi during embryonic development using live calcium imaging performed on embryonic gut from Wnt1-Cre;R26R-GCaMP3 mice, where all enteric neurons express the genetically-encoded calcium indicator, GCaMP3.

Results: Tuj1-immunoreactive neurites were first observed projecting out of the myenteric plexus to the epithelial cell layer lining the gut lumen at embryonic day (E)14.5, even prior to villi formation. To examine whether these neurites are capable of transmitting information to the plexus, the tips of individual villi were stimulated by through a small focal electrode (50 µm diameter). Electrical stimulation triggered increases in intracellular calcium in myenteric neurons from E15.5 onwards. These responses were sensitive to the voltage-dependent sodium channel inhibitor, tetrodotoxin. The basolateral release of 5-HT from enteroendocrine cells was mimicked by applying local spritz injections of 5-HT (10 µmol/L) into the tips of individual villi directly onto nerve terminals using glass micropipettes (tip diameter=10-20 µmol/L). 5-HT injections triggered calcium transients in myenteric neurons from P0 onwards, with significant postnatal maturation.

Conclusions: Enteric neurons project out of the myenteric plexus early during ENS development, innervating the gastrointestinal mucosa even prior to villus formation. Neurites are already able to conduct electrical information at E15.5 and responses to 5-HT develop postnatally.

Policy of full disclosure: None.

ENTERIC PLASTICITY

13 | Role of semaphorin 3A in the postnatal development of the enteric nervous system

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Objective: Critical developmental stages of the enteric nervous system (ENS) occur during the postnatal period leading to the formation of a mature neuroglial network characterized by the assembly of enteric neurons into ganglia and the formation of a highly organized pattern of neuronal connectivity. However, the mechanisms underlying

these maturation processes are poorly understood. Semaphorin 3A (SEMA3A) is a secreted protein playing key roles in the neuronal circuitry formation of the central nervous system. Here, we studied the expression, the cellular distribution and the role of SEMA3A and its receptor neuropilin 1 (NRP1) in the maturation of enteric neurons.

Method: Gene and protein expression of SEMA3A and its receptor NRP1 were analyzed in rat distal colon from postnatal day 1 (PN1) to adulthood by qRT-PCR and Western blot respectively. The cellular distribution of SEMA3A and NRP1 was performed at PN7 and PN36 in whole mount distal colon tissue by double immunofluorescence for SEMA3A or NRP1 with specific markers of glia (S100B), neurons (Hu, Tuj-1), and muscle cells (a-SMA). The impact of SEMA3A on neuronal outgrowth was assessed in cultures of enteric neurons cocultured with SEMA3A-transfected COS-7 cells.

Results: A peak of mRNA expression for SEMA3A and NRP1 was observed in distal colon at P7, corresponding to a stage of intense neural circuit remodelling. At the protein level, NRP1 was also found to be predominantly expressed during the early postnatal period. Immunohistofluorescence of colon tissue indicated that SEMA3A immunoreactivity was not associated with any specific cellular profile, but was distributed in small clusters disseminated throughout the tissue, a pattern consistent for a secreted protein. NRP1 was found in neurons, mainly associated with axonal processes, and was not detected in glial or muscle cells. Enteric neurons cultured in the presence of SEMA3A-expressing COS cells showed a strong reduction in axon length and complexity, while the ganglion size was unaffected.

Conclusion: This study shows the expression of SEMA3A and its receptor NRP1 in the ENS during early postnatal period. By controlling axonal outgrowth, SEMA3A might be an important factor to restrict the axonal trajectories in the appropriate paths between ganglia.

Policy of full disclosure: None.

14 | Small intestine neuromuscular dysfunctions in Toll-like receptor 4-null mice: Role of enteric glia

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Objective: Toll-like receptor 4 (TLR4) signalling regulates gut motility and myenteric neuronal survival. Since we have recently determined an impact of TLR4 in enteric glial cells (EGCs) homoeostasis, this study aimed to evaluate the role of EGCs in ileal neuromuscular dysfunctions of TLR4-/-mice.

Methods: Male TLR4 knockout (TLR4-/-, 9±1 weeks old) and age-matched wild-type (WT) C57BL/6J mice were used. In ileal longitudinal muscle-myenteric plexus (LMMPs), the distribution of neuronal HuC/D and glial GFAP and S100β markers together with inducible NOS (iNOS) immunofluorescence was analyzed by confocal microscopy. In ileum segments mounted longitudinally in organ baths, changes in muscle tension were isometrically recorded following: (i) electric field stimulation (EFS, 0-40 Hz), (ii) 10 Hz-EFS in non-adrenergic non-cholinergic (NANC) condition (1 μmol/L guanethidine+1 μmol/L atropine) with or without 10 μmol/L 1400W (iNOS inhibitor) or 100 μmol/L L-NAME (pan-NOS inhibitor). To assess the impact of glia, ileal segments were subjected to the same experimental studies after 2 hour-incubation with 10 μmol/L fluorocitrate (FC), an inhibitor of glia function.

Results: In TLR4-/- myenteric plexus the number of HuC/D+ neurons was significantly reduced (by 33±2%) whereas GFAP, S100β and iNOS immunoreactivity significantly increased compared to WT mice. Treatment with FC determined a glial phenotype comparable between WT and TLR4-/- mice with no effects on neural network. In TLR4-/- ileal segments an impaired cholinergic neuromuscular response was found together with a higher NANC relaxation, partially mediated by iNOS-derived NO signalling. Inhibition of glial activity by FC abolished NO-mediated relaxation of TLR4-/- ileal segments. In TLR4-/- myenteric plexus the increase of iNOS fluorescence intensity in EGCs was attenuated by FC treatment.

Conclusion: Our findings show that alterations in myenteric plexus architecture and anomalies in ileal neuromuscular contractility are mainly dependent on ECGs activity in TLR4-/- mice. Absent or defective TLR4 signalling could disrupt the bidirectional dialogue between enteric neurons and glia potentially leading to gut functional disorders.

Policy of full disclosure: None.

15 | ANO1 knockdown causes disrupted antral pacemaker activity, discordinated popagating antral contractions and delayed gastric emptying

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Interstitial cells of Cajal (ICC) are specialized pacemaker cells within the gastrointestinal tract (GI) that organize and coordinate GI smooth muscle contractile behaviour. ICCs control smooth muscle contraction through active generation of spontaneous electrical events called slow waves. Electrophysiology studies have shown that slow waves are generated by a Ca^{2+}-activated Cl− conductance (CaCC) that are blocked using membrane-permeable Ca^{2+} buffers or Cl− channel inhibitors. Recently it was shown that CaCC mediated by Ano1 is required for slow waves in mouse GI smooth muscles. Ano1 mutant mice do not exhibit slow waves in gastric antrum and small intestine. Since Ano1 null mice die prematurely, the importance of Ano1 in adult GI motility is currently unknown. Also unclear is whether reduced Ano1 disruption of slow waves impairs gastric emptying and/or decreases intestinal transit in adults. Objectives: Determine the role of Ano1 in gastric emptying and gastrointestinal transit of adults. Methods: An inducible Cre-recombinase allele that expresses

within ICCs (c-KitCreERT2) has enabled investigation of the effects of targeted deletion of genes within ICC populations. We crossed the c-KitCreERT2 allele with Ano1fx (Ano1fx) to determine the importance of Ano1 in ICC in the GI tract of adult tissues. We characterized recombination and Ano1 knockdown efficiency of the c-KitCreERT2 allele within ICCs using an eGFP reporter and confocal immunohistochemistry. We assessed whether knock down of Ano1 disrupted electrical slow waves within the gastric antrum and small intestine and the consequences of Ano1 knock-down on in vivo gastric propagating motility patterns, gastric emptying and gastrointestinal transit.
Results: Reduction of Ano1 leads to a loss of gastric but not intestinal slow waves. Spike complexes were often observed when slow waves were absent. Loss of slow waves leads to discordinated propagating antral contractions and delayed gastric emptying.
Conclusions: Our findings show for the first time, that disrupting Ano1 expression in ICC populations in adult tissues abolishes gastric slow waves, disrupts propagating antral contractions, delays gastric emptying and total GI transit time. Thus reduction, of Ano1 in ICC, rather than loss of c-Kit and disrupted ICC networks could underlie delayed gastric emptying in a sub-population of patients with gastroparesis.
Policy of full disclosure: None.

16 | Single cell photo-stimulation elicits neuron-to-glia communication in the enteric nervous system

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The enteric nervous system is a network of neurons and glia within the wall of the gastrointestinal tract that is able to control many aspects of digestive function independently from the brain. Enteric glial cells are closely associated with enteric neurons and their processes within and outside enteric ganglia respectively. Similar to other parts of the nervous system, there is communication between enteric neurons and glia. To unravel the crosstalk between enteric neurons and glia we use wide-field and spinning-disk confocal microscopy in combination with conditional transgenic reporter mice that express the genetically-encoded Ca^{2+} indicator GCaMP3 in enteric glial cells selectively. In freshly dissected gut preparations isolated from adult mice, activation of enteric neurons induced by trains of electric pulses transmitted via a focal electrode positioned on interganglionic nerve strands induced transient increases in GCaMP3 fluorescence spreading throughout the ganglionic glial network. Our system of glia-restricted Ca^{2+} reporter expression confirms previous reports using synthetic Ca^{2+} indicator dyes and clearly reveals the potential importance of Ca^{2+} transients isolated in enteric glial cell bodies or processes. Because of the intimate association between enteric neuronal cell bodies and ganglionic enteric glia we further investigated their functional relationship by stimulating single enteric neurons through UV-mediated photolysis of the Ca^{2+} chelator nitrophenyl EGTA-AM. Neuronal photo-stimulation induced increases in intracellular Ca^{2+} in glial cells enwrapping the stimulated neuronal cell body. On average, stimulating a single neuron elicited responses in 3 to 4 enteric glial cells. Pharmacological treatment showed that these Ca^{2+} responses involve purinergic signalling mechanisms but don't depend on synaptic activity. Thus, our experiments indicate that in addition to monitoring synaptic neuronal activity, ganglionic enteric glial cells also respond to non-synaptic neuronal activity. Our future studies aim to identify the mediators and mechanisms involved.
Policy of full disclosure: None.

MIXED TOPIC FREE PAPER SESSION I

17 | Proton pump inhibitor therapy improves esophageal symptoms by restoring a normal esophageal peristalsis in patients with proton pump inhibitor-response esophageal eosinophilia

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Objective: Introduction: Proton Pump Inhibitor-response esophageal eosinophilia (PPI-REE) is a condition characterised by symptoms of esophageal dysfunction in the setting of eosinophilic inflammation on esophageal biopsies responding to PPI therapy. Recent data collected by using esophageal high resolution manometry (HRM) documented that patients with PPI-REE present frequently motility abnormalities. Data on the effect of PPIs in improving these motor abnormalities are lacking Aim: We aimed to prospectively compare HRM features of patients with PPI-REE before and after a course of PPI therapy.
Methods: Consecutive patients with symptoms suggestive of EoE underwent upper endoscopy to assess the presence of at least 15 eos/hpf on oesophageal biopsies at mid/proximal esophagus and, then, were treated with twice-daily PPIs for at least 8 weeks. Thereafter, patients repeated upper endoscopy and PPI-REE was identified in case of less than 15 eos/hpf and a 50% decrease from baseline. Patients with PPI-REE underwent HRM at the time of the diagnosis (off-PPI) and after the course of PPIs (on-PPI). Patients with achalasia and absent peristalsis were excluded (Chicago Classification v.3).
Results: Twenty-eight patients [23M/5F; mean age 35] reporting dysphagia (93%), bolus impaction (68%) and chest pain (25%) were diagnosed with PPI-REE. After anti-secretory therapy, most of the patients reported complete resolution of esophageal symptoms ($P<.001$). Compared to HRM features at baseline, HRM after PPI therapy showed that patients with PPI-REE had higher median EGJ resting pressure [baseline 11 (1-34) vs post-PPI 17 (1-34); $P<.05$], greater mean distal contraction integral [1094 (483-5281) vs 2634 (495-6450); $P<.01$], and less frequent panesophageal pressurization [6 (21%) vs 0 (0%); $P=.02$)]. As to the manometric diagnoses, after PPI therapy patients with PPI-REE showed a reduced rate of ineffective

motility or fragmented peristalsis [16 (57%) vs 7 (25%), *P*=.02] and increased frequency of normal peristalsis [9 (32%) vs 18 (64%), *P*=.03].

Conclusions: In most PPI-REE patients, PPI therapy restores to a normal pattern the impairment of esophageal motility as expressed by ineffective or fragmented peristalsis. This finding, paralleled with symptoms improvement, seems to emphasize the important role of inflammation of the esophageal wall and its effect in inducing motor dysfunction and symptoms.

Policy of full disclosure: None.

18 | Actions of bis-(p-hydroxyphenyl)-pyridyl-2-methane—The active metabolite of the laxative bisacodyl—On human intestine in vitro

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Objective: The laxative bisacodyl is a prodrug which is enzymatically converted in the gut lumen into the active metabolite bis-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM). We aimed to describe the mode of action of BHPM on muscle and epithelial functions in isolated human intestinal preparations.

Methods: We used non-afflicted surgical resections from small (n=75) and large (n=84) intestine to record muscle activity with force transducers as well as epithelial secretion by recording short circuit current (ISC) with the Ussing chamber technique.

Results: BHPM dose dependently (0.5-5 µmol/L) increased the tone of circular and longitudinal muscle in small and large intestinal preparations. At the highest concentration the tone significantly increased by 37%-52% depending on intestinal region and muscle layer. The response was significantly larger in large compared to small intestinal muscle strips. The enhanced muscle activity was unaffected by tetrodotoxin (1 µmol/L) but abolished by nifedipine (1-10 µmol/L). Mucosal application of BHPM caused a decrease while serosal application evoked an increase in ISC. The mucosal effect was abolished by the BKCa channel blocker iberiotoxin (250 nmol/L) while the serosal effect was significantly reduced by tetrodotoxin. Mucosal application of BHPM did not effect nerve evoked secretion induced by electrical field stimulation.

Conclusions: BHPM has prokinetic and prosecretory effects in human small and large intestine. The prosecretory action consists of K+ flux into the lumen when BHPM acts luminally. Once it is absorbed and has access to deeper layers BHPM activates submucous nerves which in turn stimulate Cl−/HCO3− secretion. The prokinetic effect of BHPM is independent of nerves and involves activation of L-type Ca++ channels on muscles.

Policy of full disclosure: The study was supported by a grant in aid by Boehringer. The company had no influence on data collection or interpretation.

19 | Low-dose penicillin exposure in adolescent mice has long-term, sex-dependent consequences on behaviour and physiology

K.-A. McVey Neufeld[1]; A. Stanisz[2]; P. Forsythe[2]; J. Bienenstock[2]

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Low-dose Penicillin Exposure in Adolescent Mice Has Long-term, Sex-Dependent Consequences on Behaviour and Physiology KA McVey Neufeld1,2, AM Stanisz1, P Forsythe1,3, J Bienenstock1,2 1Brain-Body Institute at St Joseph's Healthcare, Hamilton, CANADA 2Dept of Pathology, McMaster University, Hamilton , CANADA 3Firestone Institute for Respiratory Health and Dept of Medicine, McMaster University, Hamilton, CANADA Recent work has demonstrated that low-dose penicillin treatment in dams during the pre- and early post-natal period has long-term effects on offspring metabolism, behaviour, and brain neurochemistry (Cox et al., 2014; LeClercq et al., 2017), some of which are prevented by concurrent treatment with probiotic. To date, no work has examined the potential impact of low-dose antibiotic treatment during later critical windows of development. Adolescence is a vulnerable period during which the brain is highly plastic and psychiatric illness often first manifests. Examining low-dose penicillin administration during this developmental period, and its effects on the microbiota-gut-brain axis is of high relevance. 3-week-old Balb/c mice were weaned and randomized into treatment groups of water (control), low-dose penicillin (AB), or AB/Lactobacillus rhamnosus JB-1TM (AB/JB-1). AB treated animals received 31 mg/kg penicillin V in drinking water from 18:00-9:00/day. AB/JB-1 treated animals received the same but with the addition of JB-1 (109 c.f.u/day) in drinking water from 9:00-18:00. Treatments continued from 3w to 6w of age. At 8w of age animals underwent behavioural testing, examining locomotor activity, anxiety-like behaviour and stress reactivity. Brain, gut, blood and stool were collected post-mortem. Low-dose penicillin treatment affected anxiety-like behaviour of mice in a sex-dependent manner. AB treated males showed reductions in movements and risk-assessment in the open field test, while females showed increased anxiety-like behaviour. In the light/dark apparatus, male mice demonstrated increased anxiety-like behaviour. Co-treating with AB/JB-1 resulted in no differences as compared to controls. AB treatment resulted in stress hyperreactivity following 30 minutes restraint stress, with significant increases observed in plasma corticosterone. This was ameliorated by co-treatment with JB-1. Low-dose penicillin treatment during adolescence has long-term, sex-dependent effects on anxiety-like behaviour and stress reactivity in mice. Continued work examining mRNA expression of relevant genes in brain tissue, gut motility, and microbiome is ongoing.

Policy of full disclosure: None.

20 | Corticotrophin-releasing factor in activated mucosal eosinophils is associated with clinical severity in diarrhea-prone Irritable Bowel Syndrome (IBS)

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Introduction: Corticotropin-releasing factor (CRF) has been identified in mucosal eosinophils and associated with psychological stress. Irritable bowel syndrome (IBS) is characterized by high stress level, mucosal micro-inflammation and significant substance P nerve positivity. However, the contribution of eosinophils to IBS pathophysiology remains unknown.

Aims: To identify the role of mucosal eosinophils in diarrhoea-predominant IBS-D, and to evaluate the mechanisms underlying the stress response.

Methods: Jejunal biopsies were obtained from 19 healthy controls (HC) and 35 IBS-D patients fulfilling Rome III criteria. Mucosal eosinophil activation, secretory activity, and, CRF content were evaluated by transmission electron microscopy or gene expression (qPCR). Clinical severity and psychosocial stress were recorded in all participants. The eosinophil cell line 15HL60 was stimulated with SP, carbachol (CCh), and lipopolysaccharide (LPS) to evaluate the secretory activity and the release of CRF by qPCR, immunofluorescence, or flow cytometry.

Results: IBS-D patients showed decreased mucosal expression of eotaxin, EDN and ECP, but increased SNAP23 ($P<.05$), and an increment in eosinophil degranulation compared with HC participants ($P<.0001$). CRF was identified only in eosinophil granules, and its content was higher in IBS-D ($P<.0001$). Notably, the amount of CRF correlated with eosinophil degranulation and with clinical activity. In vitro, eosinophils responded to stimulation with SP, CCh and LPS by relocating SNAP23 and VAMP2 from the cytoplasm to the plasma membrane, without changes in the expression of pro-inflammatory genes. In addition, CRF was also relocated to the plasma membrane together with a decrease in its amount after stimulation with SP and CCh.

Conclusion: Mucosal eosinophil content of CRF is associated with clinical severity in IBS-D. These results and the in vitro response to neuro-mediators suggest a potential contribution of eosinophils to IBS-D pathophysiology.

Policy of full disclosure: None.

TREATMENT OF VISCERAL PAIN

21 | Faecal supernatants from diarrhoea predominant Irritable Bowel Syndrome (IBS) patients disrupt colonic epithelial barrier function and directly activate colo-rectal afferent nerves

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Objective/Introduction: Luminal contents are altered in diarrhoea-predominant Irritable Bowel Syndrome (IBS-D), with evidence of altered inflammatory mediators (Hughes et al. Am. J. Gastro. 2013). However, it remains unclear if luminal mediators actively participate in symptom generation or are simply epiphenomena. Objectives: To determine whether faecal supernatants (FSN) from IBS-D patients affect colonic epithelial barrier and sensory afferent nerve function.

Methods: FSNs were prepared from 10 subjects with IBS-D (ROME III) and 8 sex/age-matched healthy controls (HC). 0.3 g of faecal sample was homogenised in 1 mL of Ringers solution before being filtered (100 μm). Proteolytic activity and cytokine concentrations were quantified using colorimetric protease activity assay and multiplexing, respectively. The effects of IBS-D-FSN, HC-FSN or vehicle±protease inhibitor cocktail and IL-6 (5 nmol/L) on epithelial permeability were assessed using Ussing Chambers. Colonic mRNA expression of Protease Activated Receptors (PARs) and tight junction proteins were assessed using quantitative RT-PCR. IBS-D-FSN and HC-FSN were applied to high-threshold putatively nociceptive pelvic colonic extrinsic afferents from healthy mice for 5 minutes and changes in mechanosensitivity determined.

Results: Proteolytic activity and IL-6 concentrations were increased in IBS-D-FSN relative to HC-FSN. IBS-D-FSN decreased trans-epithelial electrical resistance (RTE) and increased conductance, which was not observed with HC-FSN or vehicle. IL-6 caused comparable changes in RTE and conductance to IBS-D-FSN. Protease inhibition partially rescued IBS-D-FSN-induced changes in conductance ($P=.02$). JAM-A, ZO-1 and occludin mRNA expression were decreased following IBS-D-FSN exposure, but PAR-1, 2, 3 or 4 were not altered. IBS-D-FSN directly activated colonic afferents (100% response) and sensitised them to mechanical stimuli, which was not observed with HC-FSN.

Discussion/Conclusion: IBS-D-FSN impairs epithelial barrier integrity and activates high-threshold putatively nociceptive colonic sensory afferents. These effects are partially mediated by proteases and are similar to effect caused by the cytokine IL-6. These data indicate that luminal contents are potentially involved in generating symptoms of pain and diarrhoea in IBS-D. This research was supported by the National Health and Medical Research Foundation.

Policy of full disclosure: None.

22 | Abdominal pain in hypermobile Ehlers Danlos Syndrome (hEDS) may be associated with proliferation of colonic nociceptive nerve endings

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Objectives: It is well documented that patients with hEDS experience recurrent abdominal pain [1]. Phenotypically hEDS patients are similar to the classic type of EDS (classical-like EDS) due to mutations in the TNXB gene that encodes tenascin X (TNX)-an extracellular matrix glycoprotein. Previously, we have shown that TNX is absent from CGRP-immunoreactive (-IR) colonic fibres in both mouse and human tissue (a marker for nociceptive sensory nerves) despite the increased pain in hEDS [2]. Our aim was to (i) establish if abdominal pain is in fact reported in patients with TNXB deficiency in a cohort of classical-like EDS compared to controls, and (ii) determine if there are adaptive changes in nociceptive sensory fibres in TNX knockout (TNX-KO) mouse colon.

Methods: 11 patients with genetically confirmed TNXB deficiency [3] were recruited and compared with a control Swedish reference group. Participants were given the validated Gastrointestinal Symptoms Rating Scale (GSRS) questionnaire which assesses 5 domains including abdominal pain. Fluorescence immunohistochemistry was performed on colonic sections in wild type (WT, N=4) and TNX KO (N=4) mice. PGP-9.5 (pan neuronal marker) and CGRP expression was measured in colonic nerve fibres in each group.

Results: TNX deficient patients showed significantly increased abdominal pain scores compared to healthy controls (2.57±0.39 vs 1.56±0.21, *P*<.0001). There was a threefold proliferation of CGRP-IR nerve endings in the colonic mucosa of TNX-KO mice (TNX-KO 9350±1018 pixels/field of view, WT 3411±508, *P*<.0001), and a smaller increase in PGP-IR endings (TNX-KO 8615±1132, WT 5152±832, *P*=.0224). No changes were seen in either marker in deeper endings around the myenteric plexus (CGRP: TNX-KO 1065±198, WT 1333±242), (PGP-9.5: TNX-KO 2133±266, WT 3154±675).

Conclusion: Akin to hEDS patients, classical-like EDS patients due to TNX deficiency experience significantly more abdominal pain compared to healthy controls. This could be associated with proliferation of nociceptive nerve fibres like that we observed in colonic mucosa in TNX-KO mice. These results suggest that although TNX is not directly expressed by nociceptors, it plays a role in determining the density of these fibres. The functional phenotype of the hypertrophied endings is currently under investigation.

Policy of full disclosure: None.

23 | The histamine receptor H4 is functionally expressed on murine colonic sensory neurons and contributes to chronic visceral hypersensitivity

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Objective: It has been shown that inhibition of histamine receptors H4 (Hrh4) reduces visceral pain in a rat model of chronic visceral hypersensitivity (CVH)(1). However, the underlying mechanism of action is unclear. We aimed to explore the functional expression of Hrh4 on (i) colonic sensory neurons within the dorsal root ganglia (DRG) and (ii) their terminal afferent endings in the colonic wall. We also studied how Hrh4 function is altered during CVH.

Methods: We investigated healthy C57BL/6 mice and mice with CVH, 28 days post-TNBS administration, when inflammation had resolved and nociceptors were mechanically hypersensitive(2). Using calcium imaging we assessed the ability of 4-methylhistamine (selective Hrh4 agonist; 100 nmol/L-10 µmol/L), histamine (100 µmol/L), capsaicin (TRPV1 agonist; 50 nmol/L) and allyl isothiocyanate (AITC; TRPA1 agonist; 100 µmol/L) to induce calcium influxes in retrogradely traced colonic DRG neurons from thoracolumbar (T10-L1) and lumbosacral (L6-S1) regions. In an ex vivo gut-nerve preparation, we evaluated the effect of JNJ7777120 (selective Hrh4 antagonist; 1 nmol/L-10 µmol/L) on colonic afferent firing to phasic distension (0-20-60 mmHg).

Results: In healthy mice, calcium influxes to 4-methylhistamine were observed in 4%-17% of colonic DRG neurons compared to <1% of non-colon innervating neurons (***P<.001). In CVH mice, significantly more thoracolumbar colonic neurons responded to 100 nmol/L (27%, *P<.05) and 1 µmol/L (33%, **P<.01) 4-methylhistamine. All neurons responding to 4-methylhistamine were responsive to histamine. The majority also responded to capsaicin (healthy:TL 100%, LS 83%; CVH:TL 85%, LS 100%), AITC (healthy:TL 83%, LS 100%; CVH:TL 85%, LS 100%) or both (healthy:TL 83%, LS 83%; CVH:TL 73%, LS 100%). Splanchnic afferent firing in response to colorectal distension was enhanced in CVH relative to healthy mice (*P<.05). JNJ7777120 dose-dependently reduced afferent firing in CVH mice only.

Conclusions: Our data indicates functional expression of Hrh4 receptors on colonic DRG neurons, with up-regulation in CVH states, especially within thoracolumbar DRG neurons. Hrh4 was highly functionally co-expressed with TRPV1 and TRPA1, indicating potential key coupling mechanisms underlying afferent hypersensitivity. As inhibition of Hrh4 dose-dependently reduced enhanced afferent firing in CVH mice only, Hrh4 antagonists could be an interesting new treatment strategy for visceral pain. (1)Gut 2014;63(12):1873-82 (2) Gastroenterology 2013;145(6):1334-1346 Supported by NHMRC Australia and nEUROgastro TANDEM (ESNM).

Policy of full disclosure: None.

24 | Involvement of the serotonin pathway in ileal neuromotor dysfunction associated with TLR2 and TLR4 inhibition in juvenile mice

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Objective: Oxidized phospholipids (OxPAPC) inhibit TLR2- and TLR4-dependent signalling recognized to be involved in ensuring enteric nervous system (ENS) integrity. This study aimed to evaluate the effects of in vivo OxPAPC administration on ileal contractility and serotonin pathway in juvenile mice.

Methods: Male mice C57BL/6J (3±1 weeks old) were treated intraperitoneally with OxPAPC (1.5 µg/g body weight) or vehicle (CNTR), twice daily for 3 days. Distribution of neuronal HuC/D and glial GFAP markers was determined by confocal immunofluorescence in longitudinal muscle-myenteric plexus preparations (LMMPs). Serotonin receptors (5-HTR) and SERT expression was evaluated by qRT-PCR and confocal microscopy in LMMPs. Plasma levels of 5-HT, tryptophan, 5-hydroxytryptophan and kynurenine were measured by HPLC-fluorescence detection. Contractile activity of ileum segments longitudinally mounted in organ baths was evaluated as changes in isometric muscle tension following electric field stimulation (EFS, 0-40 Hz) or 5-HT addition (0.01-10 µmol/L) with or without 0.1 µmol/L ketanserin (5-HT2AR antagonist) or 1 µmol/L ondansetron (5-HT3R antagonist).

Results: In OxPAPC myenteric plexus a significant reduction of HuC/D+ neurons and an increase of GFAP immunofluorescence were observed together with an altered 5-HTRs and SERT immunoreactivity. OxPAPC treatment increased 5-HT-mediated response of ileal segments by 1.4-fold and augmented mRNA levels of 5-HT2AR and SERT in LMMPs. EFS-evoked response was significantly higher in OxPAPC mice (Emax=+39±5.5%) and resulted differently affected by 5-HT2AR or 5-HT3R inhibition compared to CNTR mice. Interestingly, 5-HT plasma concentrations were below detection limit in OxPAPC mice. No differences in tryptophan and 5-hydroxytryptophan plasma levels were found whereas kynurenine and kynurenine/tryptophan ratio were significantly higher in OxPAPC compared to CNTR mice.

Conclusion: Our study demonstrated that OxPAPC-mediated TLR2 and TLR4 inhibition affects gut neuromuscular function and serotonin pathway during adolescence, suggesting an involvement of innate immunity in tryptophan metabolism and potentially in the microbiota-gut-brain axis.

Policy of full disclosure: None.

NEW TECHNOLOGIES IN CLINICAL NEUROGASTROENTEROLOGY

25 | Optogenetic induction of propagating colonic motor complexes and propulsion of fecal content induced by light

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Optogenetics is an exciting technique that has been shown to selectively control neural pathways in the central nervous system (CNS), but not in the enteric nervous system (ENS), leading to changes in gastrointestinal (GI) transit. The ability to selectively control GI-motility and GI-transit without using non-specific agonists or antagonists (that act all throughout the body) offers great hope for patients with impaired GI-transit, but without having to endure the side effects of non-specific drugs. Our aims were two fold. Firstly, to generate transgenic mice expressing channelrhodopsin in specific excitatory neurons of the enteric nervous system (ENS). Secondly, to demonstrate that specific wavelengths of light can modify colonic motility and the propulsion of content, without using any agonists or antagonists. To do this, we generated a novel transgenic mouse using Cre-driven expression of the light-gated cation channel, channelrhodopsin-H134R (ChR2-H134R) in excitatory enteric neurons expressing calretinin (CAL). Immunohistochemical analysis of colonic myenteric neurons revealed 97% of the cholinergic CAL-immunoreactive neurons were selectively expressing ChR2(H134R)-eYFP+. Mechanical recordings were made from intact whole colons in vitro (n=7). Both CAL-ChR2(H134R) and wild-type mice generated ongoing propagating neurogenic colonic motor complexes (CMCs), with a mean interval 280±37 seconds (n=7). Focal illumination (1-5 Hz, 10-60 seconds) of blue light to the proximal, mid or distal colon evoked a significantly premature CMC in CAL-ChR2(H134R) mice (P=.006, N=7; Figure 1), but not in the wild-type littermates. Also, green light had no effect in CAL-ChR2(H134R) mice. Video imaging of colonic wall movements revealed that light-evoked CMCs caused propulsion of fecal pellets over significant lengths along the isolated whole mouse colon of CAL-ChR2(H134R) mice. Tetrodotoxin prevented optogenetic activation of CMCs (7/7 times tested, n=3). We provide the first demonstration that focal illumination of light to specific regions along the large intestine can evoke propagating neurogenic contractions (CMCs), leading to the propulsion of fecal content. Optogenetics offers great potential for controlling the excitability of the ENS and improving GI-transit, without using drugs that are well known to act on receptors all throughout the body; and without inducing numerous unwanted side effects, in many organ systems. Funded by NH&MRC #1067335 N.J.S and an NIH RO1 to H.Hu.

Policy of full disclosure: None.

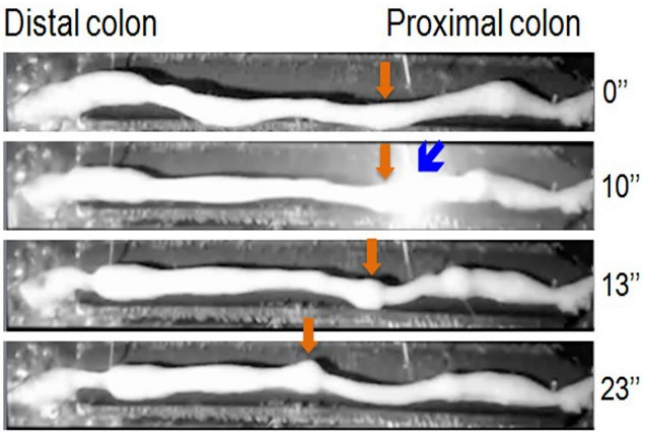

FIGURE 1 Focal illumination of blue light to the proximal, mid or distal colon evoked a significantly premature CMC (arrows)

26 | Assessment of duodeno/jejunal baseline impedance as a surrogate of evaluation of mucosal integrity in patients with functional dyspepsia: Importance of timing of measurement relative to Phase III of the MMC

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Background: Recent in vitro studies with biopsies from patients with functional dyspepsia have reported microscopic mucosal damage in the proximal duodenum, that can cause impaired mucosal barrier function and potentially underlie dyspepsia symptoms. Measurements of baseline impedance (BI) in the esophagus provide a surrogate for assessment of esophageal mucosal integrity. Unlike the esophagus, the intestinal mucosa is almost always covered by fluids making difficult the assessment of mucosal impedance. We hypothesized that immediately after passage of a phaseIII (pIII) of the MMC, the intestinal segment is devoid of fluids and we could measure BI. We aimed to evaluate duodenal BI by performing duodeno-jejunal high resolution manometry/impedance (HRM/Z) in patients with functional dyspepsia.
Methods: Ten patients (9 females; mean age 35.4±8.4 year) with functional dyspepsia (Rome IV criteria) underwent 12-24/h ambulatory duodeno-jejunal HRM/Z (MMS ambulatory system B.V. Netherlands). The HRM/Z- catheter (UniSensor, Switzerland) comprises 20 pressure sensors (2 cm apart) and 9 impedance channels. HRM data was analyzed to identify MMC pIIIs. We measured BI in the proximal duodenum (DI) and in the jejunum (J I) 24 cm more distally, immediately before and immediately after the passage of MMC pIIIs (Figure).
Result: A total of 33 nocturnal MMC pIIIs (3.3±2.5/per patient) were recorded. The mean duration of MMC cycle, pIIIs amplitude and pIIIs duration were 94.2±70.1 minutes, 34.2±13.1 mmHg and 6.02±2.1 minutes, respectively. When we compared BI before the passage of pIII, there were no significant differences between the duodenum (151.0±73.7 Ω) and the jejunum (169.7±70.1 Ω, N.S.). In contrast, when we analyzed BI after the passage of the pIII, mean BI was significantly lower in duodenum (163.6±41.9 Ω) compared to the jejunum (261.2±44.9 Ω, *P*<.001).
Conclusion: This pilot study aimed to determine the best timing to measure duodenal BI. Our results suggest that measurement of BI after passage of nocturnal MMC pIII (when intestinal fluids are minimal) might provide a good surrogate to evaluate duodenal mucosal integrity. Lower BI in the duodenum compared to jejunum might be a marker of functional dyspepsia. Further studies in healthy subjects and other patients group are undergoing to test this hypothesis.
Policy of full disclosure: None.

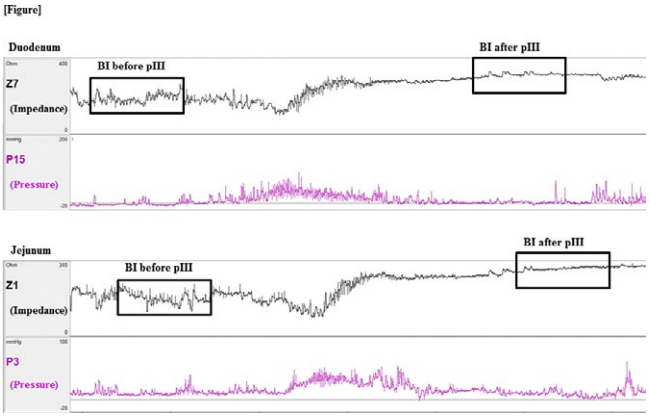

FIGURE 1 Impedance in the proximal duodenum and jejunum before and after the passage of pill

BASELINE IMPEDANCE AFTER MMC PHASEIII

27 | A comparison of the efficacy and safety of two dosing regimens, 2 and 5 times per week, of an intraluminal vibrating capsule in the management of chronic idiopathic constipation

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Objective: The intraluminal vibrating capsule (VC) (Vibrant Ltd., Yokneam, Israel) is the first chemical-free treatment for chronic idiopathic constipation (CIC). Orally administrated, it stimulates bowel movements by mechanically inducing vibrations, which promote mass movements within the colon.
Methods: Data from 2 studies were aggregated to compare two VC dosing regimens; both enrolled subjects with Rome III CIC and had comparable inclusion and exclusion criteria. Study 1 was an 8-week double-blind, sham-controlled study (USA & Israel, 2014-2016) that enrolled 163 subjects who ingested 2 capsules per week at the site.

This analysis was confined to 77 subjects who had an average of 1.1-2.5 spontaneous bowel movements (SBMs) per week at base line. Study 2 was a 6-week single-arm study (USA, 2016) that enrolled 25 subjects who ingested 5 capsules per week for 6 weeks at home. They had on average >1 and <3 SBMs)/week at baseline. The endpoint was an increase of at least 1 complete SBM (CSBM)/week.

Results: Compared to baseline, the number of CSBMs increased in both studies: by an average of 1.1 in study 1 ~3.5 CSBMs above baseline in study 2. The endpoint was met by 35.1% and 15% in the active arms, respectively, in study 1 (Δ=20.1%, *P*=.0307); in study 2, it was met by 66.7%. Using a more conservative endpoint of at least 2 CSBM/week, the responder rate for study 2 was 50%. Improvements in various CIC symptoms were recorded: stool consistency (study 1: Δ=0.5, *P*=.0428; study 2: mean change 2.08), straining (Δ=−1.6, *P*=.0072, study 2: mean change −2.69), bloating (Δ=−1.5, *P*=.0031, study 2: mean change −0.77) (table). Safety analysis confirmed an excellent profile; no serious adverse events (AEs) related to the device reported in either study. In study 1 (N=163), 13 moderate GI AEs were reported; with the exception of a sensation of vibration, the frequency of all AEs was similar in active and sham groups. Only one subject in either study discontinued because of an AE.

Conclusion: In subjects with moderate to severe chronic idiopathic constipation, a vibrating intraluminal capsule provides dose-dependent relief without incurring serious adverse side effects.

Policy of full disclosure: EMMQ has served as a consultant to Vibrant Inc.

28 | MRI detection and histological localization of transplanted neural crest derived stem cells (NCSCs) labelled with superparamagnetic nanoparticles in future perspectives for cell therapy of Hirschsprung's disease

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The repopulation with neurons, neuronal progenitor or stem cells is a central issue for the therapy of Hirschsprung's disease, where patients suffer from symptoms mainly caused by the lacking intrinsic innervation of gut segments. Transplanted stem cells can be tracked by magnetic-resonance-imaging (MRI) after incorporation of superparamagnetic nanoparticles prior to cell implantation. Until now in vivo tracking of transplanted neural crest derived stem cells for the treatment of Hirschsprung's disease have not been performed. Here we propose a new method to follow the transplanted cells and their integration via MRI. To do so, enteric neurospheres were generated from postnatal myenteric plexus and labelled with superparamagnetic iron oxide nanoparticles coupled GFP (Green Fluorescence Protein). These allow a combined MRI detection and fluorescence control of successful incorporation of nanoparticles (NP's). Prior to implantation, the possibility of following the labelled cells was proofed in vitro. Here we observed the differentiation of successful labelled neurospheres using fluorescence microscopy and MRI. We could quantify the MRI measurements regarding signal density and status of neutrospheres differentiation. The signal density of nanoparticles in MRI gets lower and the signal area increases, while the background value decreases. After implanting NP-labelled neurospheres in ex vivo gut segments, we could locate them in MRI-images and verify these data with histological sections after imaging. Frontal sections of the gut segments did confirm the MRI data. The incorporation of magnetic nanobeads thus allows to follow the transplantation success and migration of the cells within a short time window following injection. Future experiments will show whether we might even guide or spread the cells within the tissue using electromagnetic fields.

Policy of full disclosure: None.

MIXED TOPIC FREE PAPER SESSION II

29 | Satiety is modulated by the intraluminal colonic volume in healthy subjects

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Introduction: Previous studies have shown that interaction of gut stimuli may modulate perception and reflex responses of the gut. Different studies have shown that overlap between symptoms referred to different segments of the intestinal tract is common in patients with functional gut disorders. However, the role of interaction between stimuli at different segments of the digestive tract on symptom overlap is not completely understood.

Aim: To determine the effect of increased volumes of colonic contents on gastric sensory/motor responses and satiety in healthy subjects. Methods: In ten healthy subjects (4 women and 6 men, age range 20-36 years) gastric sensitivity was studied by stepwise distensions of the stomach using an electronic tensostat. In 2 days and at random order, gastric sensitivity was studied before and after: (i) colonic gas filling with a non-absorbable gas mixture infused at 24 mL/min for 45 minutes, (ii) sham infusion of gas for 45 minutes. During colonic infusion, gastric tone was continuously recorded using an electronic barostat, and epigastric and abdominal perception were registered using specific questionnaires. Following the gastric sensitivity test, and before colonic emptying was allowed, a satiety test was performed by ingestion of Nutridrink 100 mL/min up to maximal tolerance.

Results: During real colonic gas infusion, gastric tone increased progressively (−72±37 mL) whereas it remained unchanged during sham infusion (−14±30 mL; *P*<.05 vs real infusion). Colonic infusion was associated to a progressive increment in abdominal perception (1.9±0.6 score increment; *P*<.05 vs 0.3±0.3 score increment during sham infusion), whereas epigastric perception remained similar whether real of sham infusion was applied (score increment 1.4±0.4 and 1.1±0.4. real

vs sham gas infusion; NS). Gastric sensitivity to distension increased after real gas infusion (by 63±29%; *P*=.05 vs before infusion), this increment was less pronounced after sham infusion (41±28%; *P*=.29 vs before infusion). These sensory/motor changes observed during real gas infusion were associated to a decrease in the maximum volume tolerated during the nutridrink test (830±96 mL after real gas infusion and 970±73 mL after sham infusion; *P*<.05).

Conclusion: Increments in the volume of the colonic contents produce sensory and motor responses in the stomach that are associated to a reduction in the tolerance to ingestion.

Policy of full disclosure: None.

30 | Duodenal acidification impairs duodenal integrity and activates the duodenogastric reflex, independently from mast cell activation

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Objectives: We recently reported that functional dyspepsia patients show impaired duodenal integrity, associated with low-grade inflammation (Vanheel, Gut 2014). A potential cause may be the increased duodenal acid exposure present in some patients. Our aim was to evaluate the effect of duodenal acidification on duodenal integrity in healthy volunteers and to investigate whether mast cell activation is required for acid-induced impairment of mucosal integrity. We also assessed activation of the duodenogastric reflex by measuring intragastric pressure (IGP).

Methods: This study consisted of 2 parts, each including 10 healthy volunteers. (i) HCl 0.1 N or saline was infused in the duodenum during 30 minutes (5 mL/min). IGP was measured using a high resolution manometry probe. Duodenal biopsy specimens were obtained after infusion to measure transepithelial electrical resistance (TEER) and paracellular passage (fluorescein-labeled dextran, 4kDa) in Ussing chambers. Expression of cell-to-cell adhesion proteins (claudin 1-4, occludin, zonula occludens 1-3, β-catenin, E-cadherin, desmocollin-2, desmoglein-2) in biopsies was evaluated by PCR, western blot and/or immunofluorescence. The number of mast cells and eosinophils was counted using immunohistochemistry for tryptase and eosinophilic major basic protein respectively, and by evaluating the expression of these proteins using PCR and western blot. (ii) The participants were orally treated with placebo or with the mast cell stabilizer disodium-cromoglycate (DSCG) 200mg qid for 2 weeks. After treatment, the study design was as described above (only acid perfusion).

Results: Compared with saline, acidification resulted in lower IGP (*P*=.003), decreased TEER (*P*=.005), increased passage (*P*=.007) and lower protein expression of claudin 3 (*P*=.0006). No difference in mast cell (*P*=.34) and eosinophil (*P*=.34) counts were detected, but an increased protein expression of tryptase (*P*=.0008) was found after acid perfusion. Acidification induced a similar drop in IGP (*P*=.68) in the placebo and the DSCG group. There was also no difference in TEER (*P*=.70) and passage (*P*=.21) after acid perfusion between both pretreatments.

Conclusion: Duodenal acidification in healthy volunteers disrupts epithelial integrity and activates an inhibitory duodenogastric reflex. Although this effect seems to be independent from mast cell activation, increased duodenal acid exposure in functional dyspepsia is a potential pathophysiological mechanism contributing to altered permeability and gastric sensorimotor dysfunction.

Policy of full disclosure: None.

31 | Dopamine transporter genetic reduction affects small-bowel neuromuscular contractility in mice

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Objective: Antidopaminergic gastrointestinal prokinetics are commonly used to treat gastrointestinal motility disorders, although the precise role of dopaminergic transmission in the gut is still unclear. This study evaluated the impact of dopamine transporter (DAT) genetic reduction on the integrity of ileal enteric nervous system (ENS).

Methods: Female DAT heterozygous (DAT+/−) and wild-type (DAT+/+) mice (14±2 weeks) were genotyped by PCR on tail DNAs. Changes in muscle tension of ileal segments, longitudinally mounted in organ baths, were isometrically recorded following: (i) electric field stimulation (EFS, 10 Hz) in presence or absence of 1 µmol/L guanethidine+1 µmol/L atropine (NANC conditions), 100 µmol/L L-NAME (pan-NOS inhibitor) or 10 µmol/L L732138 (NK1 receptor antagonist); (ii) concentration-response curves to dopamine (0.1-300 µmol/L); (iii) 30 µmol/L dopamine with or without SCH-23390 (10 µmol/L; D1 antagonist) or sulpiride (10 µmol/L; D2 antagonist). In ileal longitudinal muscle-myenteric plexus whole-mount preparations, the distribution of glial (GFAP and S100β) and neuronal (HuC/D, nNOS, ChAT, SP) markers was determined by confocal immunofluorescence.

Results: In DAT+/− mice, in vitro electrically-induced cholinergic and NK1R-mediated tachykininergic contractions significantly increased (+24±7% and 45±5%, respectively). Changes in EFS-induced NANC relaxant responses were altered in DAT+/− mice and partially abolished by L-NAME. DAT+/− mice showed changes in inhibitory concentration-response curves to dopamine compared to wild-type, which were significantly reduced by blockade of D1 and D2-receptors. The number of HuC/D+ and nNOS+ neurons and SP immunoreactivity were unaltered in ileal myenteric plexus of DAT+/− mice. However, a significant reduction in ChAT immunoreactivity (−19±5%) and an altered density of GFAP+ processes were found in DAT+/− compared to wild-type mice.

Conclusion: Our study provides evidence that genetic-driven DAT defective activity determines anomalies in ENS architecture and neurochemical coding together with ileal dysmotility, highlighting the essential physiological role of dopaminergic system in the gut.

Policy of full disclosure: None.

32 | Altered expression of the homeobox transcription factor Phox2b in the myenteric plexus of patients with diverticular disease

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Background and aims: Diverticular disease (DD) is associated with intestinal motility dysfunctions, enteric neuropathy and altered expression of the neurotrophic factor GDNF and its receptor Ret. The transcription factor Paired-like homeobox 2b (Phox2b) is crucial for Ret expression during enteric nervous system (ENS) development, and is associated with major gastro-intestinal (GI) disorders, such as Hirschsprung's and Crohn's diseases in humans. However, a detailed analysis of the expression of Phox2b in DD has not been performed yet.

Material and methods: Site-specific expression of Phox2b was assessed in human colonic samples by quantitative qPCR analysis on mRNA samples extracted from the mucosa, submucosa, muscularis propria and myenteric ganglia harvested by laser microdissection. Expression of Phox2b was further analyzed by immunohistochemistry and qPCR in the myenteric ganglia of patients with DD and controls.

Results: Under physiological conditions, Phox2b mRNA expression was highest in isolated myenteric ganglia than in other intestinal compartments. Nuclear expression of Phox2b was observed in most myenteric neurons but also in a subset of cells expressing the glial marker S100ß. Phox2b mRNA expression was increased in isolated myenteric ganglia of patients with DD. The percentage of neurons displaying specific nuclear Phox2b staining was decreased, whereas the proportion of Phox2b-positive enteric glial cells was increased in these patients.

Conclusion: Our data represents the first detailed characterization of the expression of Phox2b in patients with DD and suggest that the altered expression of Phox2b may contribute to the dysregulation of Ret and to the associated GI-disorders observed in these patients. Altogether, these data indicates that Phox2b may play important functions in the regulation of ENS functions in adults and may be involved in the pathogenesis of DD and other GI-disorders.

Policy of full disclosure: None.

ENTEROCHROMAFFIN CELLS, ENDOCRINE CELLS AND BRUSH BORDER CELLS: ROLE IN SIGNALLING FROM THE LUMEN

33 | Intrauterine growth retardation in rats alters palmitoleate sensing by duodenal entero-endocrine cells, leading to increased-intestinal permeability

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Objective: Intrauterine growth retardation (IUGR) predisposes to gastrointestinal and metabolic disorders at adulthood. Entero-endocrine cells (EEC) in response to duodenal nutrients such as long chain fatty acids (LCFA) secrete peptides like cholecystokinin (CCK) which relay nutrients actions upon gut and brain functions. In particular, EEC derived peptides can regulate gastrointestinal functions via their paracrine action upon the enteric nervous system (ENS). We therefore hypothesized that in adults, IUGR alters GI response to nutrients via pathways involving EEC and the ENS.

We analyzed, in 2 months-old IUGR rats (R; obtained by perinatal protein restriction of dams) and in controls rats (C; non restricted dams), the impact of the LCFA palmitoleate gavage (210 mg/kg) on CCK-secretion in vivo and intestinal permeability to fluorescein sulfonic acid (FSA) both in vivo and ex vivo. We also characterized ex vivo the direct impact of CCK upon paracellular permeability.

Palmitoleate increased intestinal permeability to FSA in vivo and ex vivo in the duodenum in R rats but not in C. Interestingly, duodenal claudin-2 mRNA expression, a tight-junction protein which favors permeability, was increased in response to palmitoleate in R rats as compared to C. The palmitoleate-induced increase in permeability in R rats was concomitant with an increased concentration of plasma CCK and duodenal expression of the LCFA receptor GPR120. Finally, CCK induced an increase in duodenal permeability to FSA ex vivo at a concentration of 200 but not 20 ng/mL.

Our data show that in IUGR rats, LCFA increase duodenal paracellular permeability and CCK secretion as compared to control. Our study further suggests that CCK could mediate LCFA effects upon permeability, in part, via ENS activation pathways which remain to be identified. These modifications could contribute to the alterations of the gut brain axis observed in IUGR. This work was supported to grant Region Pays de la Loire and LCL.

Policy of full disclosure: None.

34 | L-cells are key to cross-barrier signalling to the host peripheral nervous system by a GLP-1-secreting putative probiotic

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Objectives: Recent studies demonstrated that modification of the colonic microbiome altered behaviour via signals sent through the vagus nerve. However, the molecular mechanisms of how microbiota signal across an intact mucosal barrier to the host nervous system remains unclear. Intestinal L-cells are electrically-excitable biosensors embedded in the epithelium which secrete Glucagon-like peptide (GLP)-1 and can stimulate action potential firing in colonic extrinsic nerves. This study aimed to elucidate the cellular and molecular mechanisms

involved in cross-barrier signalling by a GLP-1 secreting Lactobacillus paracasei NFBC338 probiotic strain.

Methods: Immunofluorescent labelling and calcium imaging were used to investigate if mucosal exposure to GLP-1 secreting probiotics stimulated activation of underlying submucosal neurons in colonic tissue preparations from male Sprague Dawley rats. c-Fos immunostaining in GLP-1 receptor expressing neurons was compared between probiotic-treated samples and controls. Intracellular calcium ($[Ca^{2+}]i$) in submucosal neurons was observed when probiotic secretions were applied to the mucosal layer. Using extracellular electrophysiology, vagal nerve activity was recorded with a bipolar electrode in response to mucosal stimulation of intact colonic mucosal sections.

Results: Lactobacillus paracasei supernatants increased nuclear c-Fos expression in GLP-1 receptor expressing submucosal neurons as compared to controls (n=3, P<.05). Neuronal $[Ca^{2+}]i$ was also increased by mucosally applied supernatants (P<.01), a response that was attenuated by the GLP-1 receptor antagonist, exendin-3 (9-39) amide (n=38 neurons, P<.05). Vagal nerve firing was also stimulated by supernatants applied to the mucosa and this was similarly reduced by the GLP-1 receptor antagonist (n=4, P<.001).

Conclusions: For the first time, we have provided mechanistic evidence implicating L-cells as a lynch-pin in the communication pathway between luminal GLP-1 secreting bacterial strains and the host gastrointestinal intrinsic and extrinsic nervous system. These findings may represent a cellular and molecular mechanism underlying microbiome-gut-brain signalling.

Policy of full disclosure: None.

35 | Long-term effects of Roux-en-Y gastric bypass surgery on colonic nutrient-sensing receptors and enteroendocrine cells

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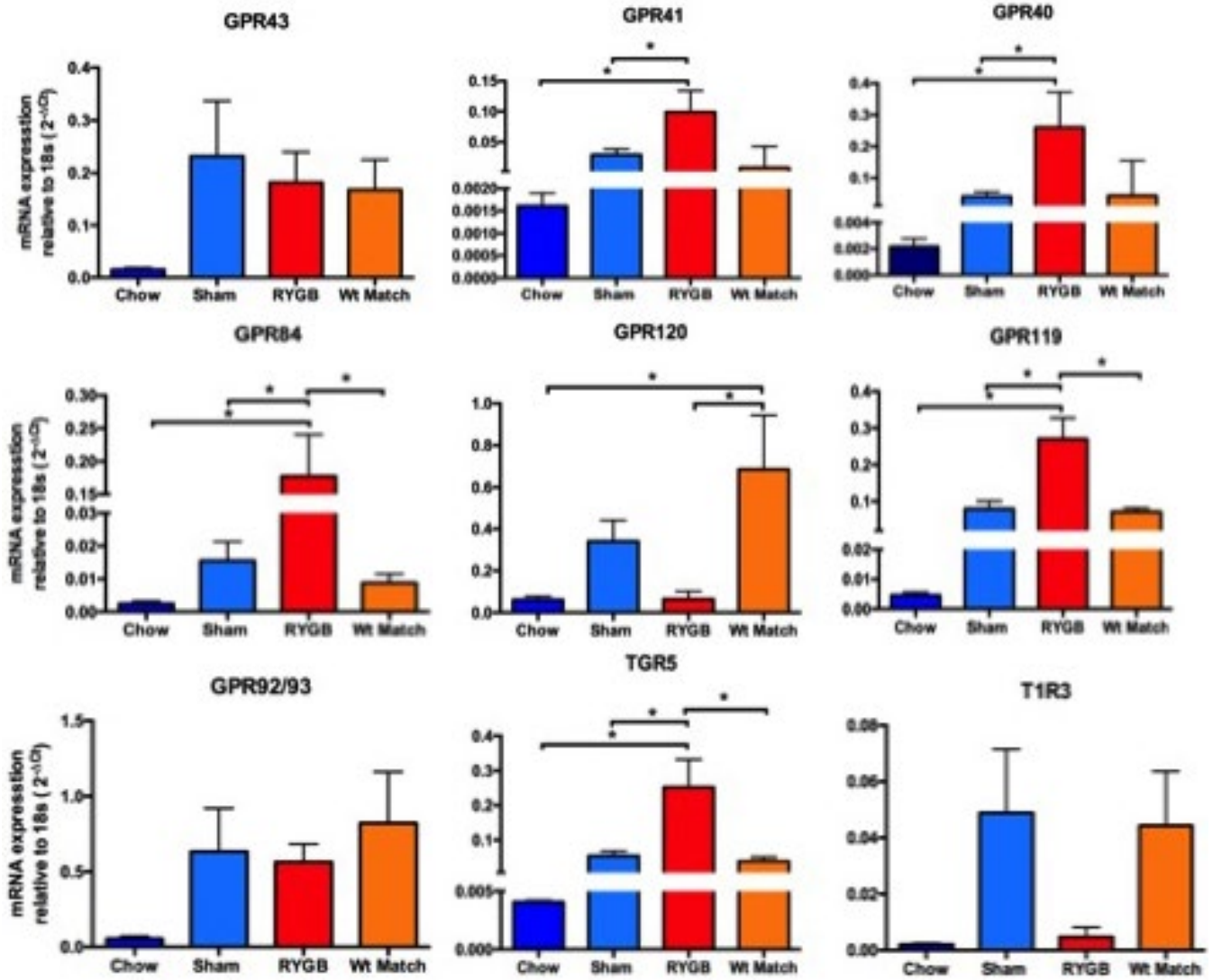

FIGURE 1 mRNA Expression relative to 18 seconds (2−ΔCt)

Introduction & Aims: Enteroendocrine cells are critical in the control of appetite and glucose homeostasis [1]. We and others have shown that both L-cells and enterochromaffin (EC) cells express a large range of nutrient sensing receptors [2]. Roux-en-Y gastric bypass (RYGB) effectively treats obesity and type 2 diabetes (T2D) by increasing gut hormone release of hormones GLP-1 and PYY, via 'shunting' of undigested meals to the distal gut [3]. We hypothesized that RYGB may also change the cellular identity of this region subsequent to a new luminal environment. The aim of this study was therefore to identify long-term cellular and molecular changes occurring in colonic nutrient sensing after RYGB.

Method: Colon was collected from 4 groups: one chow-fed lean control group and three high-fat diet-induced obese (DIO) groups that underwent either sham-surgery, RYGB, or calorie restriction induced weight loss (weight match control). Mucosal gene expression was calculated relative to 18 seconds ($2^{-\Delta Ct}$) in 4-6 mice. For immunohistochemistry, positive cells were counted over 5 fields of view/mouse. All values are mean±SEM.

Results: Twelve weeks post-surgery, RYGB mice had significantly increased mRNA expression of fatty acid receptors (FAR) GPR40, GPR84, GPR41, the oleoyl ethanolamide receptor GPR119 and the bile acid receptor TGR5 compared to sham and chow groups (Figure 1). However, GPR92 (protein receptor) and GPR43 (FAR) did not change and GPR120 (FAR) and T1R3 (amino acid receptor) expression decreased (Figure 1). Numbers of colonic L-cells increased after RYGB (chow: 0.44±0.12 vs sham: 0.68±0.08 vs RYGB: 1.07±0.11 cells/crypt), as did enterochromaffin cells (EC) (chow: 1.75±0.17 vs sham: 2.54±0.4 vs RYGB: 2.85±0.15 cells/crypt).

Conclusions: Here we demonstrate that a specific group of nutrient receptors are up-regulated in response to RYGB, potentially increasing the activation of hormone release by enteroendocrine cells, which are also up-regulated in numbers. It is likely that these two components of the mucosa play a major role in weight loss via RYGB.

References:
[1] Gribble & Reimann, Annu Rev Physiol, 2016.
[2] Symonds, Peiris et al, Gut, 2015.
[3] le Roux et al, Ann Surg, 1996.

Policy of full disclosure: Takeda Pharmaceuticals.

36 | Multiple nutrient stimulation enhances enteroendocrine cell responses in human and mouse colon

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Objective Introduction & Aims: We have previously shown that (i) enteroendocrine cells can be activated and induced to release anorexigenic hormones GLP-1 and PYY by stimulating with nutrients [1] and (ii) the presence of multiple nutrient-sensing GPCRs on the surface of these cells [2]. We have focused on characterising the cellular expression of GPCRs particularly in the proximal colon as it is an important site of appetite regulation when exposed to undigested nutrients such as Roux-en-Y gastric bypass (RYGB). Our aim was to determine if there is convergence and/or synergism of multiple nutrient signals on colonic enteroendocrine cells, such as that recently shown in mouse small intestine [3].

Methods: Undiseased human colonic tissue was obtained from cancer resection patients consented on the day of surgery. Proximal colon tissue from human and C57BL/6 mice were exposed to nutrients as previously described [1]. Hormone release was measured by multiplex assay (Merck Millipore), and cell activation assessed by immunohistochemical labelling for pCamKII and pERK.

Results: The GPR84 agonist lauric acid (LA) induced pERK activation but not pCamKII activation in these cells. Conversely, the GPR120

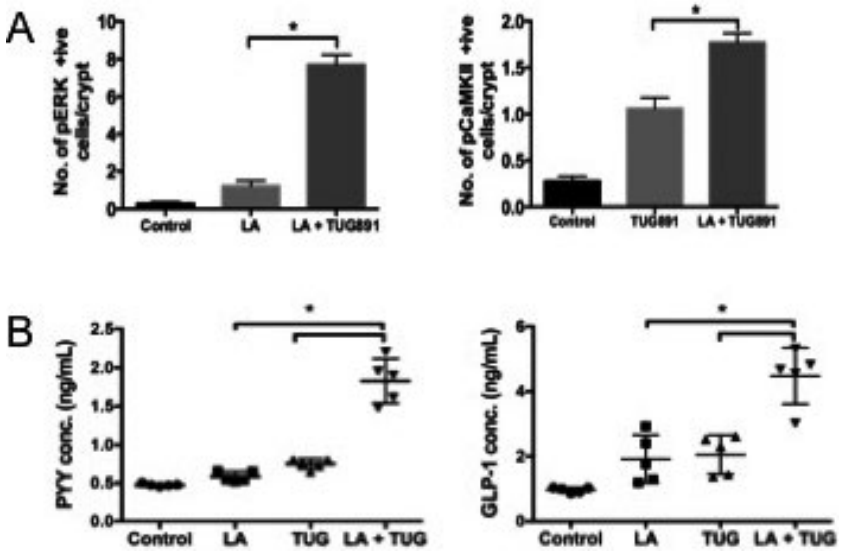

FIGURE 1 Combined stimulation of mucosa by GPR84 and GPR120 agonist synergistically increase (A) cell activation as measured by phosphorylated ERK and CaMKII and (B) release of anorectic hormones PYY and GLP-1

agonist TUG891 induced pCamKII activation but not pERK activation. When tissue was stimulated with both agonists, there was a super-additive increase in the expression of both pERK and pCamKII (Figure 1A). In mouse, stimulation with each individual nutrient did not result in activation of specific pathways as it did in human, however, combination nutrient treatment also enhanced pERK (TUG891: 1.2±0.22 vs LA+TUG891: 1.6±0.17 cells/crypt, $P<.001$) and pCamKII (TUG891:0.55±0.06 vs LA+TUG891: 0.8±0.04 cells/crypt, $P<.001$) labelling. Importantly, combination treatment of human mucosa also induced super-additive release of both GLP-1 and PYY (Figure 1B).

Conclusion: Specific activation of GPR84 and GR120 together optimally enhances activation of enteroendocrine cells and promotes super-additive release of key appetite regulating hormones. This shows a key mechanism that may be exploited to reduce appetite by targeting peripheral anorexigenic pathways.

References:
[1] Symonds et al., Gut, 2015.
[2] Peiris & Blackshaw, DDW 2015.
[3] Ekberg et al., Endocrinology, 2016.

Policy of full disclosure: Takeda Pharmaceuticals.

BIOMARKERS IN IRRITABLE BOWEL SYNDROME

37 | Granins are linked to bacterial richness, innate immunity, markers for intestinal permeability and symptom severity IBS patients

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Objective: Altered levels of chromogranins (Cg) and secretogranins (Sg) have been demonstrated in IBS patients, but little is known about the link to IBS pathophysiology. Therefore, this study aimed to determine if granins are associated with bacterial diversity, markers of intestinal permeability, immune activation and symptom severity.

Method: IBS patients meeting Rome III criteria (n=143) were included and subgrouped according to IBS Severity Scoring System (IBS-SSS) (mild/moderate symptoms <300, severe symptoms ≥300). Fecal levels of CgA, CgB, SgII and SgIII were analyzed with immunoassays. Fecal microbiota was analyzed by 16S rRNA targeted pyrosequencing (n=119). Mucosal expression of CgA, CgB, SgII, SgIII, interleukin (IL)-10, tumor necrosis factor (TNF), toll like receptor (TLR) 2, TLR6, TLR9 and Occludin (OCLN) were evaluated with RT-qPCR.

Results: In the full study cohort, fecal bacterial richness (specific OTUs) was negatively associated with fecal levels of CgA, CgB, SgII and SgIII ($r=-.28$, $r=-.30$, $r=-.26$ and $r=-.34$, all $P<.01$). IBS patients with severe symptoms (n=86) had higher fecal levels of CgA, CgB, SgII and SgIII (all $P<.05$) compared to patients with mild/moderate symptoms (n=57). In patients with severe symptoms OCLN was positively associated, while TLR2 were negatively associated, with all determined fecal granins (Table 1). Mucosal mRNA expression of CgA, CgB, SgII and SgIII were linked to OCLN, TLR2, TLR6 and TLR9 in patients with severe symptoms. Patients with mild/moderate symptoms demonstrated no associations between fecal or mucosal granins and the determined immune or permeability targets. There were no differences in fecal levels or mucosal expression of granins between Rome III subgroups based on predominant bowel habits.

Conclusion: Fecal granins are linked to symptom severity and an altered fecal microbiota composition in IBS patients. Moreover, associations between granins and innate-immune and permeability markers, respectively, were found to be dependent on IBS symptom severity.

Policy of full disclosure: None.

Table 1. Significant non-parametric Spearman correlations between fecal levels and mucosal expression of CgA, CgB, SgII and SgIII with immune- and permeability factors in IBS patients with severe symptoms (IBS-SSS > 300). No associations to target immune or permeability factors were detected in IBS patients with mild/moderate symptoms (IBS-SSS < 300).

IBS-SSS	Host factor	Immune/permeability factor	* r-value	p-value
Severe				
	CgA	mRNA TLR2	-0,3026	0.007
	CgB	mRNA TLR2	-0,22377	0.047
	SgII	mRNA TLR2	-0,30503	0.006
	SgIII	mRNA OCLN	0,252588	0.050
	mRNA CgA	mRNA OCLN	-0,26629	0.038
	mRNA CgA	mRNA TLR6	0,403084	< 0.001
	mRNA CgA	mRNA TLR9	0,447328	< 0.001
	mRNA CgA	mRNA TLR2	0,487287	< 0.001
	mRNA CgB	mRNA OCLN	-0,28102	0.028
	mRNA CgB	mRNA TLR9	0,346706	0.002
	mRNA CgB	mRNA TLR2	0,423046	< 0.001
	mRNA CgB	mRNA TLR6	0,439039	< 0.001
	mRNA SgII	mRNA TJP1	0,316922	0.013
	mRNA SgII	mRNA OCLN	0,492068	< 0.001
	mRNA SgIII	mRNA OCLN	-0,26832	0.037
	mRNA SgIII	mRNA TLR9	0,256598	0.022
	mRNA SgIII	mRNA TLR6	0,295072	0.008
	mRNA SgIII	mRNA TLR2	0,532111	< 0.001

Cg = Chromogranin
Sg = Secretogranin
TLR = Toll-like receptor
TJP = Tight junction protein
OCLN = Occludine

* non-parametric Spearman correlations

38 | Insular brain metabolites are related to somatic symptom burden and cognitive coping in Irritable Bowel Syndrome (IBS)

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Objective: As a key brain region of interoceptive awareness involved in the integration of visceral sensory input and top-down regulation, the anterior insula (AI) is crucially involved in the communication

along the brain-gut-axis. Functional alterations of AI are consistently observed in Irritable Bowel Syndrome (IBS), an exemplary disorder of disturbed brain-gut-interaction. However, whether excitatory and inhibitory neurotransmission within the AI is altered in IBS remains unknown. Using quantitative magnetic resonance spectroscopy (qMRS), we compared IBS patients and healthy controls regarding concentrations of γ-aminobutyric acid (GABA+) and glutamate-glutamine (Glx) in bilateral AI. We further addressed associations between biochemical measures and disease-related and psychological factors.

Methods: Metabolite concentrations for group comparisons of 35 female IBS patients with 20 age-matched healthy women were measured using a 3 T scanner and a MEGA-PRESS sequence with a 4.5×2×3 cm^3 voxel placed in the AI, localized based on individual T1-weighted images (Figure 1A). IBS-severity, somatic symptom burden, pain intensity and interference, gastrointestinal-specific and general anxiety, depression and coping strategies were assessed for correlational analyses.

Results: IBS patients exhibited significantly lower levels of bilateral AI Glx with more pronounced differences in the right hemisphere (left: *P*=.007; right: *P*<.001; Figure 1B). No differences were observed for GABA+ concentrations. In IBS, bilateral AI Glx levels negatively correlated with somatic symptom burden (left: *P*=.017; right: *P*=.004). In left AI, lower Glx concentrations were further related to less use of adaptive pain-coping (divert attention: *P*=.004; self-talk: *P*=.014; Figure 1C). Glx levels were not associated with other disease-related or psychological measures.

Conclusion: This is the first study to show reduced AI excitatory neurotransmission in IBS. Observed alterations were associated with symptom burden and less adaptive pain-coping. Disturbed neurotransmission in AI may therefore play a key role in pain processing and its cognitive regulation in IBS.

Policy of full disclosure: None.

39 | Magnetic resonance imaging assessed small bowel dysmotility and its relationship with patient reported symptoms: An exploration of automated vs subjective assessment techniques

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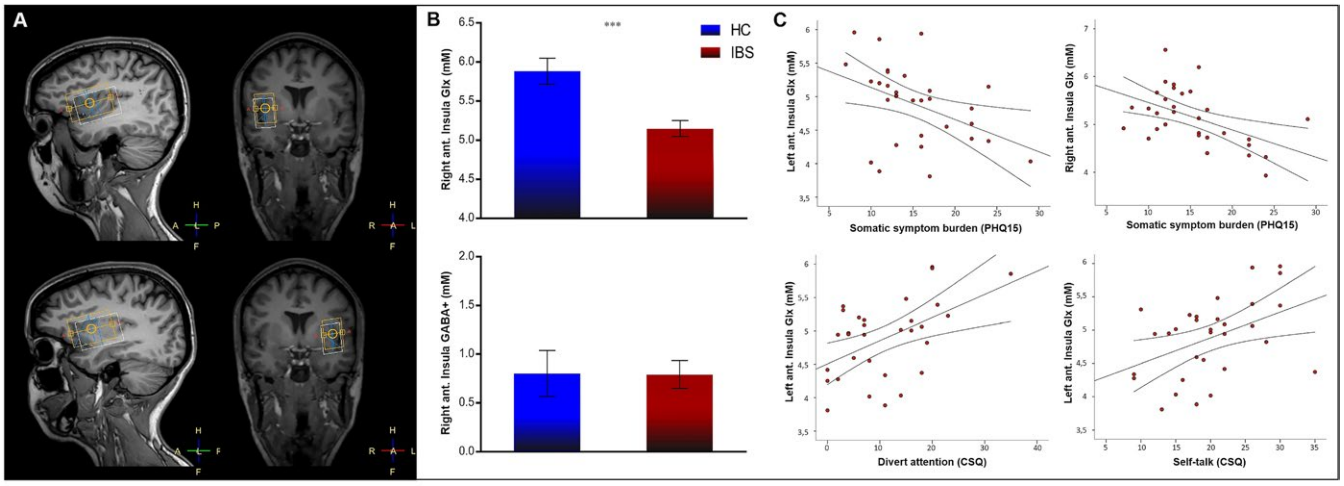
Objective: Previously, an association between intestinal motility patterns and patient reported abdominal symptoms has been reported in Crohn's disease patients undergoing small bowel Magnetic Resonance Enterography (MRE) (Menys et al., IBD, 2016). Quantitative motility measures can now be derived from routine clinical MRE protocols, which may provide novel insights into the pathophysiology driving patient reported symptoms. Here we examine the relationship between either radiologist-based grading of motility patterns, or automated image metrics, to the scores from a symptom-based questionnaire.

Methods: 114 Crohn's disease subjects (55 male, 59 female, 16-68 years old, median 31) underwent MRE at two sites (UCL, UK and AMC, Netherlands) after ingesting up to 2500 mL of 2% mannitol solution over the 3 hours prior. Each subject completed a Harvey-Bradshaw Index (HBI) to assess current abdominal symptoms before undergoing MRE including a 20 second breath hold cine motility sequence. Motility data was processed with a validated image registration algorithm (Odille et al., MRM, 2012). A total of 5 motility analysis metrics were developed to assess (i) mean motility, (ii) spatial motility variability, (iii) temporal motility variability, (iv) area of activity and (v) distension (Figure 1B). For comparison, a Radiologist (10 years



(A) T1-weighted image depicting voxel-placement in right (top) and left (bottom) AI of a representative participant
(B) Reduced right AI Glx concentrations in IBS patients compared to healthy controls (HC; top) and comparable levels of right AI GABA+ (bottom)
(C) Correlation of AI Glx concentrations with somatic symptom burden (top) and of left AI Glx concentrations with divert attention (bottom left) and self-talk (bottom right) coping skills in IBS patients

FIGURE 1

experience) visually graded the same small bowel motility metrics. Multivariable linear regression was performed for (i) the automated motility analysis data and (ii) the radiologist graded data, against the total HBI score.

Results: For the automated method both metric 2 (P=.002) and metric 4 (P=.017) were associated with patient HBI with this automated method model (Figure 1A), achieving a correlation of 0.29 (P=.0018) against the HBI score. None of the radiologist graded metrics demonstrated a significant relationship against HBI score.

Conclusions: In this initial study, the correlation between software derived motility metrics and patient symptoms has again been demonstrated. However no such relationship was found for radiologist grading of motility patterns, suggesting subjective assessments cannot capture the complexity of aberrant small bowel motility potentially underpinning patient symptomatology. This presents a simple initial step towards automated analysis of motility patterns and an objective biomarker for aberrant physiology potentially underlying functional aspects of bowel disease.

Policy of full disclosure: None.

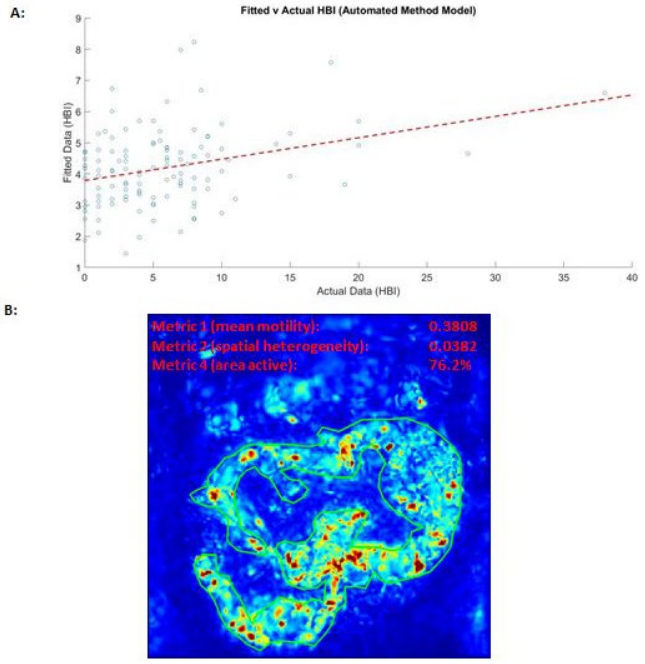


FIGURE 1 Fitted HBI model data based on quantitative measures vs Actual HBI data (A). Motility map with associated algorithm derived metrics for mean motility (metric 1), spatial motility variability (metric 2) and area of activity (metric 4) displayed (B)

40 | *Lactobacillus rhamnosus* GG soluble mediators ameliorate visceral hypersensitivity and changes in spinal cord gene expression induced by early life stress

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Visceral hypersensitivity is associated with functional gastrointestinal disorders with gut microbiota potentially playing a role. We have shown that early life stress-induced visceral sensitivity is reduced by soluble mediators from the probiotic *Lactobacillus rhamnosus* GG (LGG). Here, we explored if such intervention can modulate the response to acute stress in adulthood and if it mediates changes in pain signalling through spinal cord gene expression. Rats were separated from their mothers for 3 h/day from postnatal day (PND) 2 to 12. From PND 21 both non-separated (NS) and maternally separated (MS) offspring were provided drinking water with or without supplementation of standardized preparations of LGG soluble mediators until PND 85. Visceral hypersensitivity was assessed using colorectal distension on PND 79. Corticosterone blood levels were determined 30 minutes after an acute restraint stress on PND 83. Genome Sequencer Illumina HiSeq was used to analyse spinal cords for whole genome RNA sequencing. Maternal separation led to exaggerated corticosterone blood levels following restraint stress compared to NS (P<.05), and administration of LGG soluble mediators reduced corticosterone levels (P<.05). RNA sequencing highlighted that 63 genes were upregulated and 23 downregulated in the spinal cord of MS compared to NS rats. In MS rats, 66 genes were upregulated and 199 genes downregulated by the LGG soluble mediators. Of the genes that were changed by MS, 41 were reversed by supplementation with LGG soluble mediators with some being associated with pain and stress mechanisms. Administration of LGG soluble mediators in rats reversed visceral pain sensitivity, response to acute restraint stress and gene expression changes in the spinal cord induced by MS. Further functional analyses of the specific genes and pathways implicated are being carried out to elucidate specific mechanisms of action.

Policy of full disclosure: S Manurung and G Gross are employees of Mead Johnson Nutrition.

MIXED TOPIC FREE PAPER SESSIONS III

41 | Serum from patients with Crohn´s Disease activates guinea pig enteric neurons

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Objective Background: Neuronal activity in the enteric nervous system (ENS) is modulated by mediators released from tissue resident non-neuronal cells. Ganglia of the enteric nervous system are close to blood vessels and thus, there is a potential that blood borne factors influence neural excitability.

Methods: We applied serum from patients with Crohn´s disease (CD) (n=12 CD-remission, n=20 CD-active) and healthy control (HC) (n=20) on guinea pig submucous neurons and analysed their spike discharge with voltage-sensitive dye (Di-8-ANEPPS) recording in 44 submucous plexus preparations (50 ganglia, 574 neurons). We further tested in a pairwise manner the serum from CD patients before and after they went into remission (n=8), as well as their response to pre-incubation with the TNF-α antibody Adalimumab.

Results: Sera from CD-active or CD-remission patients caused a fast onset spike discharge of 2.9+0.3 Hz or 3.6+0.3 Hz, respectively, which was significantly higher than the one observed after HC-serum (0.6+0.2 Hz, *P*=.026, *P*=.003). Moreover, the proportion of neurons responding to CD-active or CD-remission sera was 37.5%+20 or 37%+27, respectively, and thereby, significantly higher than the proportion sensitive to HC-serum (11+9%, *P*=.003). The neuroindex (percentage of responding neurons×their spike frequency) as a measure of overall neuronal activity was 124.0+106.5, 127.8+114.3 and 39.6+49.9%×Hz for CD-active, CD-remission and HC, respectively, and significantly different between the three groups (*P*<.05). Strikingly, sera from CD-active and CD-remission patients activated neurons to a similar degree. Pre- incubation of both CD-active and CD-remission serum with adalimumab showed a tendency towards reduced neuronal activity (neuroindex: *P*<.05) except of one patient.

Conclusions: We demonstrated for the first time that sera from CD patients activate enteric neurons suggesting that neuronal hyperactivity is a feature in active and inactive Crohn. TNF-α seems involved in ongoing neuronal activation. This emphasizes the importance of serum factors for the excitability of enteric neurons and the functional relevance of a blood-ENS axis.

Policy of full disclosure: None.

42 | Colorectal cancer cells induce neurogenesis in the enteric nervous system of the tumor microenvironment via a NGF-dependent pathway

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Background: Several studies have demonstrated that colorectal cancer (CRC) microenvironment harbors an increased neurite density as compared to healthy areas. Moreover recent findings indicate that specific stimuli activate neurogenesis in the enteric nervous system (ENS), in adult mice. However whether tumor epithelial cells (TEC) induce neuritogenesis and/or neurogenesis in the ENS during CRC remains unknown.

Methods: Human colon specimens from patients with CRC and from AOM/DSS treated mice were whole-mount dissected in order to isolate the myenteric and submucosal plexus from healthy area and tumor margin. The number of enteric neurons per ganglia was compared between healthy area and tumor margin after anti-Hu immunostaining in both plexus. Human CRC supernatants, TEC (Caco-2 cells)-conditioned medium or NGF were added to primary culture of rat enteric nervous system and primary culture of rat enteric neurons. In fixed primary cultures, the number of enteric neurons per ganglia and the length of neurites were calculated after anti-Hu and anti-tubulin-III immunostaining respectively. In order to determine the role of NGF in CRC supernatants and TEC-conditioned medium effects, anti-NGF blocking antibodies were added to primary cultures.

Results: Immunofluorescence studies on whole-mount dissected ENS plexus demonstrate that the densities of neuronal bodies were significantly increased in the margins of both human CRC and murine AOM/DSS-induced dysplasia compared to healthy areas (n=7 and n=5 respectively; *P*<.05). The increase in neuronal density was similar in low-grade and high-grade dysplasia as well as carcinoma (n=5). In vitro human CRC supernatants and TEC-conditioned medium enhanced the densities of neurites and neuronal cell bodies in primary cultures of ENS (pcENS) as compared to healthy mucosa supernatants and control medium, respectively (n=6; *P*<.05). Finally, nerve growth factor (NGF) induced an increase in the density of neuronal bodies in pcENS (n=4; *P*<.05), and our preliminary data show that the addition of an anti-NGF blocking antibody to human CRC supernatant and TEC-conditioned medium inhibits their impact on neurogenesis and neuritogenesis (n=6; *P*<.05).

Conclusion: Altogether these results indicate that the increased neuronal density in CRC margin may result from the activation of neurogenesis in the ENS induced by TEC via a NGF-dependent pathway.

Policy of full disclosure: None.

43 | Neurodegeneration of the ENS might be prevented by treatment with nanomodified antioxidants

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With increasing life expectancy the number of neurodegenerative disorders such as Parkinson's and Alzheimer's disease rise. While the loss of neurons, mainly derived from oxidative stress, in clinically apparent diseases is irreversible, preventive measures are needed. Based on the theory that the pathology in neurodegenerative disorders might start in the enteric nervous system (ENS) before it spreads to the brain, the gastrointestinal tract might be used for either staging and treatment of the diseases. During the onset of the disease oral applications of herbal nutritional supplements with high antioxidant capacity have a promising neuroprotective potential. However, these natural compounds have low solubility in water and consequently very poor resorption in the gut. In order to enhance

the solubility, we applied specific top-down technologies to produce rutin nanocrystals. The particles were characterized using light and electron microscopy. Measuring reactive oxygen species, showed a much higher radical scavenger properties of nano- than microparticles when hydrogen peroxide was used. The impact of nanocrystals in vitro was evaluated in a Parkinson's disease culture model. Cells from both central (LUHMES) and enteric nervous system cells (adult primary enteric neurons) were exposed to conditioned media containing α-synuclein. Cell surviving, oxidative stress, optical density and number of TH positive neurons were evaluated. Cells from the CNS and ENS were affected by α-synuclein, resulting in significant decrease of cell number and increased oxidative stress. Neurite outgrowth and number of TH positive cells were reduced. The addition of rutin nanocrystals (50 μg/mL) together with the α-synuclein challenge inhibited the fatal synuclein effect significantly. More neurons survived, the oxidative stress decreases and the neurons grew comparable to the control in a very dense network. Interestingly, the nanoparticles always provided a more beneficial effect when compare to particles with micro dimensions. In conclusion, in vitro studies clearly demonstrated that the application of antioxidative nanocrystals protected both central and enteric neurons from cell death by oxidative stress.

Policy of full disclosure: None.

44 | Electrophysiological changes and mucosal permeability in phenotypes of gastroesophageal reflux disease

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Objective: Three different phenotypes of gastroesophageal reflux disease (GERD) such as erosive reflux (ERD), nonerosive reflux (NERD), esophageal hypersensitivity (EH) and functional heartburn (FH) might have different pathophysiological changes within the esophageal epithelium and the data is limited. We aim to investigate the electrophysiological differences and diffusion characteristics as a reflection of tissue integrity using Ussing chamber system.

Methods: Distal esophageal mucosal biopsies from 14 healthy controls (5 men, 40.6±11.2 years) and 62 patients with GERD (40 men, 42.9±12.3 years, n=26 LA grade A/B, n=8 LA grade C/D, n=22 NERD, n=6 EH) and 11 patients with FH were studied from November 2015 until March 2017. GERD and quality of life questionnaires, high-resolution esophageal manometry, 24 hours impedance-pH monitoring, upper gastrointestinal endoscopy with esophageal biopsies were performed in all patients. Biopsies were put into the chambers to measure the transepithelial resistance (TEER), potential difference (PD) and tissue permeability via fluorescein diffusion within 2 hours as well as evaluation of dilated intercellular spaces with light microscopy.

Results: Esophageal biopsies of healthy volunteers (163.6±41.1 ohms) had significantly higher TEER when compared to total GERD patients (132.5±38.7 ohms). Although the TEER results of whole GERD subtypes were decreased compared to healthy controls, only ERD groups were significantly lower (123.3±29.8 ohms) (Table 1). There was also no significant difference in any parameters between NERD, FH and EH groups. The mucosal permeability of GERD subtypes was significantly higher than the healthy controls. The PPI-unresponsive subjects (n=10, 94.8±36.5 pmols) were much more permeable to fluorescein compared to PPI-responsive subjects (n=52, 56.0±32.4 pmols) within all GERD patients (*P*=.009).

Conclusions: The TEER and permeability results imply that ERD and NERD groups showed a barrier disruption. However, epithelial permeability was not different in EH and FH groups. The dilatation of intercellular spaces may contribute to increased mucosal permeability in true-NERD and ERD patients. EH and FH patients might have different pathophysiology than others.

Policy of full disclosure: None.

SUBJECTS	TEER (Ohms)	PD (V)	PERMEABILITY (pmols)
Healthy Controls	163.6 ± 41.1	2.2 ± 0.9	43.9 ± 16.6
GERD (total)	**132.5 ± 38.7****	2.6 ± 1.5	**62.2 ± 35.8***
Esophageal Hypersensitivity	150.6 ± 23.9	2.2 ± 1.0	71.8 ± 34.5
NERD	139.6 ± 50.2	2.2 ± 1.3	**65.6 ± 39.2***
ERD (total)	**123.3 ± 29.8*[ø]**	**3.0 ± 1.6*[ø]**	**58.3 ± 34.7***
ERD grade A/B	**130.5 ± 27.8***	2.9 ± 1.6	54.0 ± 30.5
ERD grade C/D	**105.8 ± 32.7***	3.2 ± 1.6	72.6 ± 43.6
Functional Heartburn	145.3 ± 42.7	1.9 ± 0.9	67.0 ± 35.2

*; p< 0.01 (vs. healthy controls); [ø]; p<0.05 (vs. EH); [#]; p<0.01 (vs. FH) (All results are mean.)

POSTER SESSIONS: DIETARY INTERVENTIONS INCLUDING PROBIOTICS, PREBIOTICS AND SYNBIOTICS

45 | Obesity-induced alterations in colonic transit are normalized by dietary prebiotic supplementation: Role of gut microbiota

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Obesity is associated with significant alterations in colonic motility and intestinal microbiome. Gut bacteria can modulate intestinal transit through the production of short-chain fatty acids (SCFAs) and activation of serotonin metabolism in the host. Prebiotics are non-digestible dietary fibers which promote the growth of beneficial microbes in the gut. To this end, we investigated whether dietary supplementation of a prebiotic fructooligosaccharides and galactooligosaccharides mixture (FOS/GOS) can change microbiota composition and improve impaired colonic transit in high fat diet-fed obese mice. C57/BL6 mice were fed either a high fat diet (HFD, 45% kcal from fat) or a low fat diet (LFD, 10% kcal) for 6 weeks, after which half of the animals were co-supplemented with 6.5% FOS/GOS in

drinking water for further 6 weeks. To analyse colonic transit, spontaneous propagation of artificial pellet along the isolated colon was recorded ex vivo. Caecal content was harvested for the analysis of microbiota composition and SCFAs concentration. Colonic tissue was collected to measure serotonin and gene expression levels. Here we show that colonic transit was substantially delayed in HFD group, while FOS/GOS co-supplementation significantly improved the propulsive activity of the colon in HFD fed mice. However, this effect was not associated with an increase in either SCFAs production or serotonin tissue levels in the gut. FOS/GOS altered microbiota composition in both HFD and LFD fed mice, decreasing bacterial species diversity, and supporting the growth of Bacteroidetes at the expense of Firmicutes. Interestingly, a few bacterial taxa were affected by prebiotic in a diet-specific manner. To conclude, dietary enrichment with FOS/GOS can attenuate the deficit in colonic transit associated with the ingestion of high fat diet. These data suggest that prebiotics may be a useful dietary adjunct for co-morbid gastrointestinal symptoms in obesity.

Policy of full disclosure: None.

46 | Sheep and cow milk and yogurt drinks influence gastrointestinal transit in a rat model

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Background: Fermentation of milk is thought to improve digestion but few studies have investigated alterations in gastrointestinal (GI) transit due to consuming fermented milk products. Because of differences in protein composition between species we hypothesized that sheep milk would produce different GI motility-modulating peptides compared to cow milk, and that there would also be differences following fermentation. We compared the effect of cow and sheep milk, and fermented drinks derived from these, on GI transit in a rat model.

Methods: Male Sprague Dawley rats (n=10-12 per group) were fed one of four reconstituted skim dairy drinks (3% protein; sheep and cow milk, pre- and post-fermentation) for 2 weeks. Transit of metallic beads was assessed by X-ray, and bead location was rated in vivo. This was used to compare stomach emptying (4 hours), and transit through the small (9 hours) and large intestine (12 hours). Peptides in the drinks were analysed and sequenced by LC-MS/MS followed by searching of cow and sheep protein databases. Bioactive sequences were then identified by matching against a database of known bioactive peptides.

Results: Stomach emptying in animals treated with sheep yogurt was 2.7-fold greater than that for cow yogurt. Transit to the large intestine was 18% faster for sheep milk-treated animals than for cow milk ($P<.05$), and 2.3 times more beads had moved from the caecum to the colon over 3 hours. Similarly, 1.4 times more beads had moved from the caecum to the colon following consumption of the sheep yogurt drink compared with the cow yogurt drink.

Conclusions: Our study did not detect a fermentation effect but demonstrates prominent species differences between the dairy drinks in their effects on GI transit of solids, and differences between their bioactive peptide content. The faster colonic transit for sheep milk compared with cow milk may be as a result of these peptide differences.

Policy of full disclosure: None.

47 | Diurnal regulation of colonic motility by short-chain fatty acids

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Objective: The gastrointestinal tract has a powerful circadian clock that participates in the process of food digestion. In addition, intestinal microbiota, which are at the interface between ingested nutrients and the gut epithelium, undergo diurnal oscillations that are influenced by feeding rhythms. Perturbations of the circadian clock lead to dysbiosis and favour metabolic syndrome.

Aim: We aimed to investigate whether microbial production of short-chain fatty acids (SCFAs) shows a diurnal rhythm of 24 hours and regulates the rhythm of SCFA receptors and SCFA-induced gut contractility.

Methods: C57Bl6/5 mice were housed under a 12-h/12-h light/dark-cycle (zeitgeber (ZT) 0 lights-on) and were sacrificed over the course of 24 hours at 4-hour intervals. The effect of increasing concentrations of a SCFA mix (1-100 mmol/L, molar ratio 3 acetate: 1 propionate: 1 butyrate) on electrical field (EFS)-induced excitatory neural responses in proximal colonic smooth muscle strips was measured. The expression of SCFA receptors FFAR2/3, OLFR78 and GPR109a was determined by qPCR and caecal SCFA concentrations were analyzed by gas chromatograph-mass spectrometry.

Results: The SCFA mix concentration-dependently (EC50=57 mmol/L) inhibited EFS-induced neural contractions and showed a diurnal rhythm ($P<.01$) with a peak amplitude reached at ZT 4.5. In contrast, neither excitatory neural responses nor acetylcholine-induced smooth muscle contractions showed a diurnal rhythm. FFAR2/3 and OLFR78 but not GPR109a were expressed in the smooth muscle layer. The mRNA expression of FFAR2 was not diurnal, while FFAR3 and OLFR78 showed diurnal rhythmicity ($P<.01$). The peak of FFAR3 expression (ZT 3.6) coincided with the peak of the inhibitory effect of the SCFA mix on neural responses (ZT 4.5) but not with OLFR78 mRNA expression which was 11 hours out of phase. Caecal concentrations of propionate showed no diurnal rhythm, while acetate and butyrate concentrations were diurnal ($P<.01$), and peaked at ZT 19.8.

Conclusion: Caecal SCFAs show a diurnal rhythm that precedes diurnal variations in FFAR3 mRNA expression in the proximal colon, which in turn trigger rhythms in SCFA-induced neural inhibitory responses.

Policy of full disclosure: None.

48 | Effects of different bacterial strains in the form of the Probiotics Symbioflor® on the enteric nervous system and gut motility

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The enteric nervous system (ENS) plays a major role in physiological functions of the gut. One of the major roles of the myenteric plexus (MP), one out of two plexus of the ENS, is controlling the motility of the gut. The spatial proximity of the ENS with the gut microbiota leads to an outstanding interest of influences of the ENS by intrinsic microbiome, respectively the impact of specific strains of bacteria. In this study we investigate the influence of the probiotics Symbioflor®1 (Enterococcus faecalis) and Symbioflor®2 (*Escherichia coli*) on isolated cells of the myenteric plexus. For this purpose the cells are cultured together with bacteria, spatially divided by a non-permeable membrane. Additional experiments were performed with culture media that was conditioned with the bacteria for several hours. Changes in motility induced by distinct bacterial strains are monitored by an ex-vivo perfusion approach using an organ bath, combined with video recording. The activity of the ENS is directly stimulated by single bacterial strains. In co-cultures the bacteria alter the neuronal and glial outgrowth of the ENS cells as well as the neuronal/glial ratio in culture. It could be shown, that all bacterial strains tested so far decrease the density of both, neuronal and glial outgrowth. Moreover, there is evidence, that individual bacteria impact on the neural and glial activity. The motility experiments demonstrated also an increase of gut motility with all bacteria used, although individual strains show stronger effects. Thus we could clearly demonstrate that individual strains from the human enteric microbiome influence gastrointestinal motility, which might at least partially mediated by the ENS.

Policy of full disclosure: None.

49 | High fat diet-induced depression-like behaviour in mice: Roles of intestinal microbiome, neuropeptide Y, and brain metabolome

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Objective: Epidemiological studies suggest a link between obesity and depression but the biological mechanisms of this link remain unclear. To this end we assessed the effect of obesity induced by high fat diet (HFD) on emotional-affective and cognitive behaviour of mice and parallel changes in intestinal microbiome, brain neuropeptide Y (NPY) expression, and brain metabolome.

Methods: Male C57Bl/6J mice were fed a HFD (60 kJ% from fat) or control diet (12 kJ% from fat) for 8 weeks, followed by behavioural phenotyping. The caecal microbiome was determined by 16S rDNA sequencing, the brain metabolome by ^{1}H 1D and ^{1}H-^{1}H 2D nuclear magnetic resonance, NPY expression by PCR and immunoassay, and dipeptidyl peptidase-4 (DPP-4) activity by an enzymatic assay.

Results: HFD increased body weight and led to the development of a depression-like phenotype as revealed by reduced sociability, sucrose preference and self-care, whereas anxiety and cognition were not affected. Additionally, HFD significantly altered the composition of the intestinal microbiome with a significant reduction of the relative abundance of the phylum Bacteroidetes, an increase in the relative abundance of the phyla Firmicutes and Cyanobacteria, and further changes at lower taxonomic levels. In the brain, HFD caused a region-specific reduction of NPY expression, whereas the plasma levels of both NPY and its catabolizing enzyme DPP-4 were increased. The metabolic profile of distinct brain regions indicated that HFD induced a metabolic shift, with lactate and creatine phosphate being increased in the prefrontal cortex.

Conclusion: The current data show that obesity induced by HFD is associated with depression-like behaviour in mice along with distinct alterations in intestinal microbiome, NPY and DPP-4 activity, and brain metabolome. These microbial and molecular factors are candidate biomarkers of the pathophysiological link between obesity and depression.

Policy of full disclosure: None.

ENTERIC PLASTICITY

50 | Expression and neurochemical identity of sensory fibres is altered in Inflammatory Bowel Disease (IBD)

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Introduction and Aims: Calcitonin gene related peptide (CGRP), is a neurotransmitter often expressed in sensory neurones and associated with pain pathways. Ion channels expressed on sensory neurones such as P2X3, an ATP gated ion channel, and Nav1.8, a voltage-gated ion channel, are both implicated in sensitizing neurons in animal models of visceral hypersensitivity. Our aims were to (i) characterise CGRP expression and (ii) determine whether CGRP +ive neurons expressed P2X3 and Nv1.8, in inflamed and uninflamed human colonic tissue.

Methods: Normal, uninflamed human colonic tissue was obtained from cancer resection patients (N=4). Crohn's disease (CD) N=4 and Ulcerative Colitis (UC) N=3, tissue was from patients undergoing resection to remove diseased tissue. All patients were consented on day of surgery. Indirect immunohistochemistry with double labeling

was carried out on all tissues. Microscopy images were analysed on ImageJ for pixel counts and JACOP plug-in used to determine % co-expression. One-way ANOVA was used to determine statistical significance ($P<.05$) with SEM.

Results: CGRP was expressed in fibres of the mucosa and myenteric plexus (MP) of normal, CD and UC patients. However, mucosal expression in UC patients (115.6±12.3 +ve pixels) was significantly decreased compared to normal (245.8±24.6 +ve pixels) and CD (187.4±17.8 +ve pixels). While P2X3 co-localised with CGRP in both normal and IBD tissue, UC patients had significantly higher % co-localisation (70.4%±6.2) compared to normal (41.8%±1.4) and CD (59.4%±6.8) at the mucosal level but no change in MP. Meanwhile, co-localisation of Nav1.8 with CGRP +ve fibres was unchanged in mucosa, but increased expression was found in MP of CD patients (51.8%±4.6) compared to normal (10.5%±2.5) and UC (6.1%±2.1).

Conclusions: Decreased CGRP expression in mucosa of UC patients concurs with neuronal apoptosis [1]. However, we find that remaining CGRP fibres have increased P2X3 expression suggesting they are more likely to be chemosensitive, and thus involved in conducting pain. Finally, increased co-labelling of CGRP with Nav1.8 in MP of CD patients provides a possible molecular mechanism behind increased pain responses in these patients to colorectal distension [2].

References:
1. Gulbransen et al, Nat Med, 2012.
2. Bernstein et al, Pain, 1996.

Policy of full disclosure: Takeda Pharmaceuticals.

51 | nELAV mRNA-binding protein, HuC/D alteration in adolescent mice small intestine after antibiotic treatment-induced dysbiosis

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Objective: nELAV mRNA-binding protein, HuC/D, alterations in the small intestine and brain of adolescent mice were evaluated after antibiotic treatment-induced dysbiosis. In neurons, nELAV are fundamental proteins modulating all the steps of transcript fate. Changes in cerebral and intestinal expression of possible HuC/D targets involved in neuronal plasticity, such as BDNF and its receptor TrKB, were also investigated.

Methods: Dysbiosis was induced in male C57Bl/6 mice (4±1 weeks old) by orally administering a mixture of broad-spectrum antibiotics (50 mg/kg vancomycin, 100 mg/kg neomycin, 100 mg/kg metronidazol and 100 mg/kg ampicillin) every 12 hours for 14 days. HuC/D, BDNF and TrKB mRNA, and HuC/D and BDNF protein levels were evaluated in small intestine mucosa-deprived segments by qRT-PCR and western blotting, respectively. HuC/D immunostaining was performed in small intestine longitudinal muscle myenteric plexus preparations (LMMPs) obtained from antibiotic-treated (ABX) and control (CTR) animals by confocal immunofluorescence.

Results: In small intestine segments from ABX-treated animals, HuC/D, BDNF and TrKB mRNA levels significantly increased ($2^{-\Delta\Delta ct}$: 5.49±1.33; 6.52±1.99; 5.26±0.99, respectively). In the ABX group, levels of HuC/D monomer and dimer as well as BDNF protein levels increased to 56.1±9.89%, 109±22.03%, and 147±17.53%, respectively, with respect to CTR. In LMMPs from ABX mice, HuC/D immunostaining was altered. Preliminary data suggest that in the prefrontal cortex from ABX-treated mice nELAV protein levels are also altered.

Conclusion: Our data suggest that during adolescence, intestinal microflora alterations may influence nELAV proteins (ie, HuC/D) levels in both the intestine and brain. The HuC/D potential target, BDNF is also modulated. Overall, we provide hints to envision HuC/D-mediated mRNA stabilization as a relevant mechanism underlying both brain and gastrointestinal disorders in a neurodevelopmental critical phase of life such as adolescence.

Policy of full disclosure: None.

52 | Functional compensation of gastro intestinal motility indicates enteric plasticity in an early onset model of Alzheimer's Disease

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One of the major problems in the diagnostic of Alzheimer's Disease (AD) is the late development of perceivable symptoms due to the brain compensating functions of affected and degrading neuronal tissue. But AD patients also suffer from symptoms affecting the gut, eg, constipation and other motility malfunctions. Also amyloid was found in structures of the enteric nervous system in AD mice as well as in AD patients. So far, in different mouse models of AD reduced neuronal tissue in the myenteric and the submucous plexus has been reported. Even more interesting is the fact that in 5xFAD (Familial Alzheimer's Disease) mice no functional differences in motility compared to the wildtype could be observed under physiological conditions ex vivo. Only when luminally perfused with nicotine, which is not present under physiological conditions, differences between the transgenic and wildtype mice can be observed at different stages of the disease. These findings lead to the hypothesis that the enteric nervous system (ENS) is also compensating the reduction of neuronal tissue in some way. Therefore a more detailed analysis of the distribution of nicotinic acetylcholine receptor subtypes in neurons of the myenteric plexus is of major interest. In relation to the hypothetical compensation ability of the ENS the neural and glial ratio as well as the expression of the stem cell marker nestin in cultures of the myenteric plexus are topics of our current research and should provide insights into the potential in differentiation of those cells in vitro.

Policy of full disclosure: None.

53 | Enteric glial cells reaction to inflammation is lost in Crohn's Disease

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Enteric glial cells (EGC) are essential to intestinal epithelial barrier (IEB) homeostasis. In healthy intestines, EGC reduce IEB permeability and promote mucosal healing. In inflammatory bowel disease (IBD) such as Crohn's Disease (CD) and Ulcerative Colitis (UC), both EGC phenotype and IEB functions are altered, but putative involvement of EGC in IBD pathogenesis remains unknown. If the astrocyte reactivity is well studied, the reaction of EGC to chronic inflammation is not well documented, We investigated whether EGC impact on IEB permeability was altered in an inflammatory environment and in EGC from IBD patients. Rat EGC as well as human EGC from control, CD and UC patients were stimulated with the cytomix TI (TNFalpha+IL1beta; 1 to 100 ng/mL) or LPS for 2 or 4 days. Reactive EGC phenotype where characterized and reactive EGC functional impact on IEB permeability was studied (i) in vitro using human intestinal epithelial cells (IEC) in a non-contact co-culture model, or (ii) in vivo by grafting the treated rat EGC in colon wall of Sprague Dawley rats. Rat and human control EGC induced a significant reduction of IEB paracellular permeability after TI treatment when compared with untreated or LPS treated EGC. LPS or TI treatment had no significant effects on IEC alone. In vivo colon wall grafting with control EGC did not modified the permeability whereas colon wall grafting with EGC preconditioned by TI significantly reduced the permeability when compared to control animals. Human EGC from control or UC patients treated with TI induced a decrease in IEB permeability too, but EGC from CD patients did not. This work is not only the first evidence showing that reactive EGC can have beneficial effects upon IEB permeability, but also shows that EGC from CD but not UC patients have lost these reactivity. This could define EGC as active players in CD pathogenesis.

Policy of full disclosure: None.

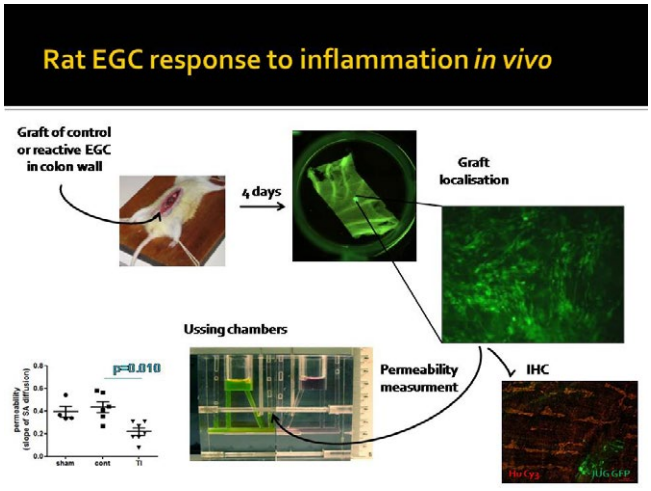

FIGURE 1 Experimental set-up and procedures

54 | Glial PGE2 production induced by inflammation regulates glial response to ATP

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The enteric nervous system (ENS), composed of enteric neurons and enteric glial cells (EGC) regulates gut homeostasis. In inflammatory bowel disease (IBD) such as Crohn's Disease (CD) and Ulcerative Colitis (UC), ENS phenotype is modified but the causes and consequences of this remodelling remain unclear. If reactive astrocytes is well studied, the reaction of EGC to chronic inflammation is not well documented. We have analyzed here the production and role of one inflammatory mediator produced by EGC, the prostaglandin E2 (PGE2), in the IBD context. Rat EGC as well as human EGC from control, CD and UC patients were stimulated with the cytomix TI (TNFalpha+IL1beta; 1 ng/mL) for 1 or 4 days. PGE2 production was measured by mass spectrometry in EGC conditioned media but also in biopsy supernatant from control CD and UC patients, in healthy and inflamed areas. We have measured the impact of TI or PGE2 treatment on EGC phenotype (glial marker expression and proliferation) and response to ATP (calcium intracellular concentration). PGE2 concentration was significantly increased in UC biopsies supernatant but not in CD patients. No differences were detected between healthy and inflamed areas. PGE2 production by rat and human control EGC was significantly increased after TI treatment, but EGC from CD patients produced significantly less PGE2 than control or UC EGC. Whereas expression of GFAP was not affected, S100b and Sox10 expressions were increased after 4 days of TI treatment. GFAP, S100b and Sox10 expressions were not modified by PGE2. TI as well as PGE2 reduced EGC proliferation. In addition, PGE2 treatment also increased calcium flux (amplitude and duration) induced by ATP stimulation. Previous works have shown that glial PGE2 sensitize neuronal response to bradykinin. Our work suggests that PGE2 glial production induced by inflammation can have autocrine effects that encompass EGC number control and an increased reactivity. In addition our work suggests that PGE2 glial production could participate in the differences observed between CD and UC pathological features.

Policy of full disclosure: None.

55 | Pitfalls in the interpretation of high-resolution anorectal manometry

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Background: Recto-anal inhibitory reflex (RAIR) is an integrated part of normal defecation, induced by increased distension in the rectum. The amplitude and duration of the reflex are dependent of the volume and

the frequency of rectal distension. High-resolution anorectal manometry (HR-ARM) is now standard for the physiological measurement of anorectic function, through gradual balloon filling up to 250 mL in the rectoanal canal. Absence of RAIR is seen in Hirschsprung's disease, where a congenital defect in the myentic plexis is present. In HR-ARM, RAIR is considered present if the anal relaxation is 25% or more. The purpose of the study was to investigate whether there were any other factors than Hirschsprung's disease which gave a negative outcome of RAIR in HR-ARM.

Method: Performed examinations HR-ARM at Karolinska University Hospital during April 2012 till November 2015, with negative RAIR, were compared with gender and age matched controls performed at the same time that had positive RAIR. Quantitative manometry measures were compared using unpaired Student's *t*-test whereas qualitative variables were compared by the Chi-squared test. Main Results 2 of 72 with no sign of RAIR, had Hirschsprung's disease, 59 surveys where the RAIR interpreted to be present although the pressure reduction didn't reached 25%, except for 4 examinations that turned positive after adjustment in the software program. In the rest of 11 patients with visually doubtful RAIR we found patients with systemic sclerosis, extremely low pressure at rest, reduced sensitivity for rectal volume (threshold>60 mL), dyssynergic defecation. No measurements were correlated to the absence of RAIR whether in the larger group of 59 or the minor of 11 patients.

Conclusion: The results of this study indicate the presence of false negative RAIR, why, where appropriate, closer examination of the colour contour chart and catheter mode in the software program should be made. We could visually detect a clear RAIR in the spatiotemporal plot, although pressure drop does not reach 25%. However, in order to recommend a change in the cut-off for RAIR we would need to examine a much larger number of patients with Hirschsprung disease.

Policy of full disclosure: None.

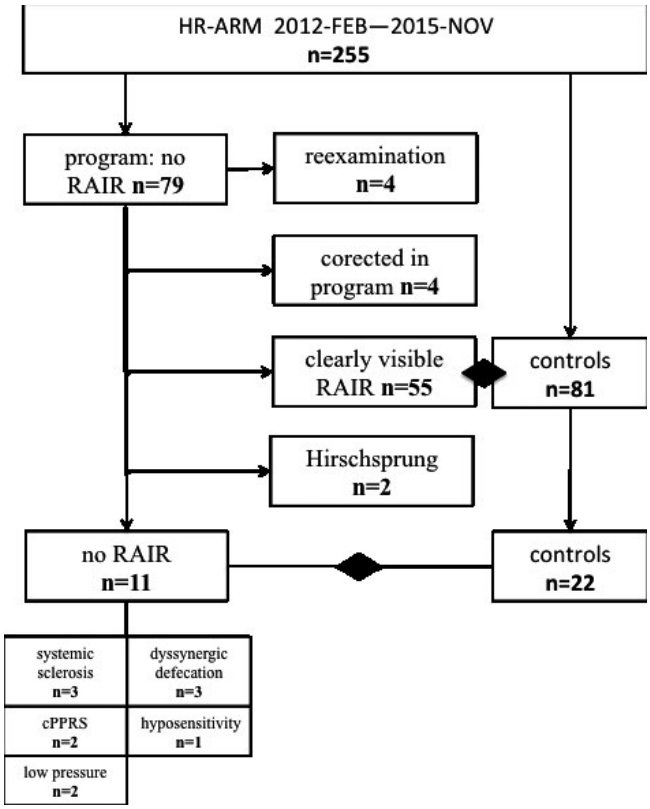

FIGURE 1 Dispensation

NEUROGASTROENTEROLOGY: ACROSS THE LIFESPAN

56 | Patients with all types of congenital anorectal malformation seem to have fecal continence reflexes

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Objective: There are different mechanisms that regulate fecal continence. Recently, we found that fecal continence is regulated by the Anal-External Sphincter continence reflex (AESCR) and the puborectal continence reflex. The aim of this study was to determine whether patients with congenital anorectal malformations (CARM) have these continence reflexes as well.

Methods: We retrospectively reviewed clinical records of 41 patients with CARM, who underwent anorectal function tests in the Anorectal Physiology Laboratory over a 5-year period. Of these patients, 38 were surgically corrected at the University Medical Center Groningen, and three were not operated. We distinguished the different forms of CARMs according to the Krickenbeck classification. We used data obtained from the anorectal pressure test and the balloon retention test to investigate the voluntary and involuntary contraction, respectively, for both the external anal sphincter and the puborectal muscle.

Results: Out of 41 patients, 39 (95%) could voluntary contract their external anal sphincter and 38 (93%) patients could voluntary contract their puborectal muscle. We found the AESCR in 35 (85%) and the puborectal continence reflex in 39 (95%) of the patients. The AESCR was present in patients with different CARM forms, except for patients with recto-bladderneck fistula. All patients who were not operated (n=3), who underwent ASARP (n=4), anoplasty (n=1) or mini-PSARP (n=2) possessed both reflexes. In 5 out of 28 (17,9%) patients who underwent PSARP, the AESCR was absent, while 1 (4%) lacked the puborectal continence reflex.

Conclusions: Patients with different forms of CARM seem to have fecal continence reflexes, ie, the AESCR and the puborectal continence reflex. However, patients who underwent more rigorous operations, such as PSARP, often lack AESCR, while still have the puborectal continence reflex.

Policy of full disclosure: None.

57 | Role of NO-GC on long distance contractions in the murine colon

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Objective: Regulation of gastrointestinal motility is complex and involves both excitatory and inhibitory neurotransmission. An important inhibitory neurotransmitter is nitric oxide (NO). In the GI tract, NO-sensitive guanylyl cyclase (NO-GC), is the main receptor for NO. It is expressed in several cell types such as smooth muscle cells (SMC) and interstitial cells of Cajal (ICC). Cell-specific knockout mice for NO-GC revealed NO-GC as an intricate modulator of spontaneous contractions in the colon: organ bath studies point to the involvement of NO-GC in ICC pacemaker activity and proved the regulation of muscle tone via NO-GC in SMC. Moreover, lack of NO-GC influenced whole gut transit time.

Methods: To fill the gap between organ bath and whole gut transit time measurements, this study focuses on the analysis of whole colon preparations of WT, GCKO, SMC-GCKO and ICC-GCKO. Therefore, video recordings of colon preparations were used to generate spatiotemporal maps in order to evaluate the contraction pattern and propagation characteristics. Outflow measurements gave detailed information about the efficiency of the propulsive contractions.

Results: Spatiotemporal maps enable the analysis of long distance contractions (LDC), the strongest propulsive motor pattern in the mouse colon. In all KO colon preparations, LDC were recorded. Blockade of neuronal NO synthase in WT colon increased the frequency of LDC and, simultaneously, decreased the efficiency of each contraction. Moreover, the topography of each single LDC was altered. In ICC-GCKO, colon frequency of LDC was not altered, however, efficiency was decreased and topography was altered.

Conclusion: NO is an important component of the neuronal circuitry that orchestrates the LDC, which is most likely the mouse equivalent of the 'High Amplitude Propagating Contraction' of the human colon. Without the NO component, the LDC becomes less effective as shown by an altered LDC topography. NO-GC in ICC does not appear to modulate the pacemaker that underlies LDC frequency.

Policy of full disclosure: None.

58 | Effects of nitric oxide on small intestinal motility

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Objective: Gastrointestinal (GI) motility and peristalsis originate from coordinated movements of circular and longitudinal smooth muscle layers. GI diseases affecting motility are often associated with impaired nitrergic signalling. In the enteric nervous systems, NO is released from nitrergic neurons as a major inhibitory neurotransmitter. The specific role of nitrergic inhibitory signalling on the circular and longitudinal muscle layers in the small intestine has not been clearly determined yet. Therefore, in the present study, we investigated the NO-mediated influence on these two muscle layers in murine ileum.

Methods: As NO-sensitive guanylyl cyclase (NO-GC) is the main receptor for NO in the GI tract, we first looked for NO-GC expression in murine ileum via immunohistochemistry. For functional analyses, we measured smooth muscle tone in ileal CM and spontaneous contractions in both ileal muscle layers from mice lacking NO-GC globally (GCKO) and specifically in smooth muscle cells (SMC-GCKO).

Results: In contrast to findings from other parts of the GI tract, the immunohistochemical stainings showed NO-GC expression in platelet-derived growth factor receptor α (PDGFRα)-positive cells but not in interstitial cells of Cajal (ICC). Organ bath experiments revealed NO-GC in SMC to be involved in the maintenance of tone of circular smooth muscle: Addition of an NO-GC inhibitor led to an increase and addition of an NO donor to a decrease in tissue tone. The amplitude of spontaneous contractions in CM was increased in the absence of NO-GC. In contrast, contractile activity in LM was not different between WT and knockout strains. When activated by NO, NO-GC led to suppression of spontaneous contractions in WT longitudinal smooth muscle whereas GCKO tissue was unaffected. To our surprise, NO suppressed spontaneous contractions in longitudinal strips from SMC-GCKO ileum indicating participation of other cell type(s).

Conclusions: NO-GC in SMC is involved in the regulation of tone and amplitude of spontaneous contractions in ileal CM. In LM, NO induces suppression of spontaneous contractions via NO-GC in a non-SMC type.

Policy of full disclosure: None.

59 | Degree of colonic cholinergic innervation determines bacterial translocation in pediatric Morbus Hirschsprung patients

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Objective: Hirschsprung`s disease (HD) is diagnosed shortly after birth and is characterized by the lack of enteric ganglions. Either before or after surgical correction between 20% and 50% of patients suffer from life-threatening HD associated enterocolitis, often due to opportunistic pathogen overgrowth. The lack of regulating enteric ganglia leads to a hyper-innervation of the distal colon region by parasympathetic cholinergic nerve fibers, secreting acetylcholine. Acetylcholine is able to control inflammation by dampening macrophage response against bacterial- or damage-derived stimuli. In the colon, activation of nicotinic acetylcholine receptors results in increased epithelial permeability for luminal bacteria and stimulates phagocytic activity of crypt-residing macrophages. This mechanism is described to be important in controlling intestinal inflammation during postoperative ileus and

bowel disease. However, under homeostatic conditions, strong cholinergic signals may influence the intestinal permeability and favor an immune cell response towards bacterial non-reactivity and persistence possibly linked to colitis manifestation.

Methods: The degree of cholinergic innervation was scored by immunohistochemistry. Mucosal lymphocytes were isolated from resected colon specimen and their phenotype was assessed by fluorescence-activated cell sorting and quantitative reverse transcription PCR. Further, we quantified bacterial translocation by bacterial 16S rRNA fluorescence in situ hybridization. Finally, underlying mechanisms were examined in vitro using blood-derived immune cells from healthy donors.

Results: Along with the increase in cholinergic fiber density and colonic acetylcholine expression, HD patients show an elevation of crypt macrophages as well as regulatory T cells. Cholinergic signals shift macrophages towards an anti-inflammatory phenotype and favor the differentiation of regulatory T cells. High cholinergic innervation correlates with increased bacterial translocation into lamina propria and persistence in crypt macrophages.

Conclusion: Our data show that increased parasympathetic/cholinergic activity leads to accumulation of anti-inflammatory regulatory T cells and macrophages favoring bacterial persistence in the distal colon. Understanding the molecular mechanisms of how nerve fibers interact with and bias the immune system may provide new therapeutic intervention possibilities for inflammatory enterocolitis and would essentially contribute to the understanding of pediatric mucosal immunology.

Policy of full disclosure: None.

60 | Distribution of RAD21 immunoreactivity in mouse and human gut neurons

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Objective: RAD21 is a double-strand-break repair protein and a critical component of the cohesin complex with key roles in several cellular functions including transcriptional regulation. A mutation in RAD21 is associated with chronic intestinal pseudobstruction (CIPO). This study investigated the distribution of RAD21 immunoreactivity (IR) in enteric and sensory neurons in order to investigate how RAD21 mutations might contribute to gut sensory-motor dysfunction.

Methods: Colocalization of RAD21-IR with markers for subsets of neurons was examined in mouse and human small intestine and mouse sensory dorsal root ganglia (DRG).

Results: RAD21-IR was found in a subset of neuronal cell bodies and nerve fibers in myenteric and submucosal plexuses of mouse and human small intestine, as labeled by PGP9.5 and HuC/D. Within the myenteric plexus this pattern was found in 61.56±0.9 and 144.7±4.1 neurons/mm^2 for RAD21 and HuC/D, respectively (n=4, P<.05, t-test). The percentage of neurons expressing cytoplasmic RAD21 was not different between adult and 10 day old mice. RAD21-IR did not colocalize with neuronal nitric oxide synthase (nNOS). A subset of choline acetyl transferase (ChAT) positive neurons were RAD21-IR. RAD21-IR was also present in neurons that were ChAT and nNOS negative. RAD21-IR was not detected in the cytoplasm of interstitial cells of Cajal or fibroblast-like cells which were positive for Kit and PDGFRa respectively. Many neuronal cell bodies and nerve processes in mouse DRG displayed RAD21-IR.

Conclusions: In mouse and human small intestine, RAD21 was detected in subsets of ChAT+/nNOS− and ChAT−/nNOS− enteric neurons and in sensory neurons of mouse DRG. Cytoplasmic localization of RAD21 in subsets of peripheral neurons may contribute to the enteric neuropathy found in patients with mutations in RAD21 that are linked familial CIPO.

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Policy of full disclosure: None.

61 | The influence of calorie restriction on gut microbiota in long-living Ames dwarf mice

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Growth hormone (GH) deficient Ames dwarf (df/df) mice live significantly longer and healthier lives than their normal (N) littermates. Calorie Restriction (CR) is also known to extend lifespan, reduce body weight and improve insulin sensitivity in laboratory animals. Ames dwarf mice exhibit several characteristics of CR mice, however, previous studies showed that CR further extends longevity and enhances insulin sensitivity in df/df animals indicating that the mechanism of action is not identical. In the present study, N and df/df male mice were subjected to six or twelve months of 30% CR or ad libitum (AL) feeding (n=10/group). We analyzed the effects of CR on the expression levels of insulin signalling genes in skeletal muscle and adipose tissue, and the effects of CR on the intestinal microbiome. Our results indicated that skeletal muscle adiponectin levels were increased in df/df when compared with N mice (P<.0212), and twelve months CR increases adiponectin in skeletal muscle of N mice (P<.0001). Analysis of the microbiome displays significant differences in the gastrointestinal location (P<.001), genotype (P<.001) and the diet (P<.004). LefSe Analysis showed distinct microbiome distribution between CR and AL feeding regimen in N animals, with lack of similar and only minor changes after diet in df/df mice. In summary our results showed that df/df mice are characterized by elevated levels of adiponectin in skeletal muscle when comparing with N mice, and long term CR is necessary to mimic increased levels of adiponectin in N animals. Importantly, we also found that CR provides divergent effects on gut microbiota in N when comparing with df/df mice. Overall, our findings suggest that GH deficiency in long-living df/df mice might play a significant part in regulating gut microbiota.

Policy of full disclosure: None.

62 | Motor function of digestive tract at children with the obesity

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Background/aims: Studying of a motility of digestive tract at children with an obesity by method of a peripheral electric gastrointestinal miography. It is supposed that acceleration of evacuation from a stomach and acceleration of transit in proximal departments of intestines leads to loss of control over appetite, and delay of transit in distal departments of a small bowel and in a colon—to change of a microbiocenosis and increase in absorption of nutrients.

Methods: 20 teenagers (12 boys and 8 girls) aged from 13 up to 17 years (an average age −15.7±1.8) having IMT from 26 to 40 are examined. For an electric gastrointestinal miography used Gastroskan of GEM (Russia)—the computer device, defining motility of a stomach, duodenum, lean, ileal and thick guts by filing of electric signals from these bodies from three cutaneous electrodes located on skin of the right forearm and the lower extremities of the patient. The research was conducted in the morning in two phases: 1—research on an empty stomach—after 10-12 hour hungers (the night period), and also 2—after reception of a reference breakfast. In total 6 researches with an interval of 10 minutes were conducted (2—in the morning on an empty stomach, 4—after a reference trial breakfast).

Results: High frequency of constipations at teenagers with an obesity is revealed that is caused by decrease in electric activity of a colon on an empty stomach and after stimulation by food, dismotility of ileal and thick guts. Along with it, the increased body height of electric activity of a stomach with a poor growth of electric signals from a duodenum is noted in response on food stimulation, it increased a gastrointestinal dismotility.

Conclusions: The method of a peripheral electric gastrointestinal miography can be used in examination of children with an obesity.

Policy of full disclosure: None.

63 | The cells and conductance mediating cholinergic neurotransmission in the stomach

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Objective: Enteric motor neurotransmission is essential for regulation of gastrointestinal (GI) motility. Controversy exists about the cells and ionic conductance(s) that mediate post-junctional responses to motor neurotransmitters.

We utilized gastric fundus muscles and specific cell types to study ionic conductances activated by cholinergic stimulation.

Methods: Isolated intramuscular ICC (ICC-IM) and smooth muscle cells (SMCs) from fundus muscles were studied using patch clamp protocols to determine the conductances activated by carbachol (CCh) in each specific cell-type. Intracellular microelectrodes and isometric force measurements were used to study neural post-junctional responses in intact tissues in Ano1 congenital knockout and cell-specific conditional Ano1 knockdown mutant animals. Molecular approaches and confocal immunohistochemistry revealed cell-specific expression of Cre recombinase via eGFP, and Ano1reduction following induction of Cre using the Cre-Lox recombination approach.

Results: We found Ano1 protein is expressed by ICC-IM in the stomach but not resolved in SMC. CCh activated a Cl- conductance in ICC and a non-selective cation conductance (NSCC) in SMC. In wildtype mice nerve stimulation activated excitatory junction potentials (EJPs). EJPs were absent in animals with congenital knockout of Ano1 and greatly reduced in animals in which Ano1 was knocked down using the Cre/loxP method. We also found that several 2nd. generation inhibitors of Ano1 channels blocked EJPs. Contractions to cholinergic nerve stimulation were reduced in Ano1 knockouts and by Ano1 blockers. SMCs cells also have receptors and ion channels activated by muscarinic agonists. Blocking acetylcholine esterase with neostigmine revealed a slow depolarization that developed after EJPs in wildtype mice. This depolarization was apparent in mice with genetic deactivation of Ano1 or in the presence of Ano1 blockers.

Conclusions: Our data are consistent with the hypothesis that ACh released from cholinergic motor nerves binds to muscarinic receptors on ICC-IM with preference. For the first time we show that Ano1 and not NSCC is the conductance activated by ACh in ICC-IM. If metabolism of ACh is blocked, the neurotransmitter can overflow to reach extrajunctional receptors on SMCs and activates a NSCC in these cells.

Policy of full disclosure: None.

64 | Investigation of the brain gut axis in the rAAV-alpha-synuclein PD model: Reveals enteric nervous system pathology and alterations in the gut microbiome

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Objective: Gastrointestinal pathophysiology in Parkinson's disease (PD) is a primary non-motor defect preceding motor symptoms of PD by many years, and gut microbiome alterations occur in PD patients. Research has focused on how PD α-synuclein pathology may originate in the enteric nervous system (ENS) and spread via vagal nerves to the brain. Limited information is available on whether CNS accumulation of α-synuclein in PD impacts gut pathology, the objective of this work.

Methods: The recombinant adenoviral vector overexpression human-α-synuclein (rAAV-α-syn) rat model of PD was employed. ENS pathology was assessed by immunofluorescent microscopy of enteric

wholemounts. Gut microbiota composition was analysed via 16s rRNA, bile acid composition was analysed using UPLC mass spectrometry.

Results: Results demonstrate alterations in ENS pathology in rAAV-α-synuclein rats compared to controls, including enteric neuronal loss specific to the submucosal plexus, increased tyrosine hydroxylase positive swollen varicosities along sympathetic axons and alterations in enteric glia. Beta diversity analysis of faecal microbiota composition shows separation and clustering of rAAV-α-synuclein samples compared to controls. Analysis of faecal bile acid composition show significant alterations including changes caused by microbial modifications. Voluntary running had a significant beneficial effect on gut pathology in the rAAV-α-synuclein model.

Conclusion: This CNS-initiated PD model demonstrates alterations in ENS pathology and gut microbiome composition, indicating a bidirectional relationship between brain and gut in PD.

Policy of full disclosure: None.

65 | Extracellular matrix composition in the enteric nervous system of mice

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Objectives: Extracellular matrix (ECM) molecules in the central nervous system (CNS) form highly organised ECM structures around neurons. One such structure is the perineuronal net (PNN) which provides synaptic integration and controls the functional wiring between neurons (1). It is unknown whether a PNN like structure similarly exists in the enteric nervous system (ENS). Therefore, our objective was to verify the presence of ECM molecules in the gastrointestinal tract (GIT) and the association of these with enteric neurons in mice.

Methods: We investigated the presence and localization of hyaluronan synthase 1 (HAS1), aggrecan (ACAN) and phosphacan (PTPζ) in stomach, small and large intestine of C57BL/6 mice. The GIT wall was divided into two parts: (i) mucosa+submucosa and (ii) external muscle for analysis of quantitative real-time (RT-PCR). Frozen cross sections (10 μm) from each GIT region were submitted to immunofluorescence using anti-HAS1, anti-ACAN, anti-PTPζ, and anti-PGP-9.5.

Results: Our results demonstrated the presence of HAS1, ACAN, and PTPζ in the stomach, small intestine and large intestine. The relative expression of ACAN was similar between the layers of the GIT ($P>.05$). However, the relative expressions of HAS1 and PTPζ were higher in muscle compared to mucosa in all analysed GIT regions ($P<.001–.01$). Additionally, immunofluorescence analysis showed that HAS1, ACAN and PTPζ are associated with myenteric and submucosal neurons.

Conclusion: The presence of HAS1, ACAN, and PTPζ in the ENS may contribute to the adequate functioning of the GIT similar to the PNN found in the CNS. Further physiological tests in knockout mice for these ECM molecules will provide evidence of this possible interaction. 1. De Luca C, Papa M. Looking Inside the Matrix: Perineuronal Nets in Plasticity, Maladaptive Plasticity and Neurological Disorders. Neurochem Res [Internet]. 2016 Jul 2;41(7):1507-15. Available from: http://link.springer.com/10.1007/s11064-016-1876-2 2. Calegari VC, Abrantes JL, Silveira LR, Paula FM, Costa JM, Rafacho A, et al. Endurance training stimulates growth and survival pathways and the redox balance in rat pancreatic islets. J Appl Physiol [Internet]. 2012 Mar 1;112(5):711-8. Available from: https://doi.org/jap.physiology.org/cgi/doi/10.1152/japplphysiol.00318.2011.

Policy of full disclosure: None.

66 | Depressive symptoms during pregnancy disrupt gut microbiome dynamics during critical prenatal and postnatal time windows

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Objectives: The experience of depressive symptomology during pregnancy is associated with adverse obstetric and infant outcomes. Inappropriate remodelling of the microbiome during pregnancy and subsequent vertical transmission of a suboptimal microbiome at birth may be a potential mechanism underpinning these associations. This study aimed to assess this theory.

Methods: Nulliparous pregnant women enrolled in the IMPROvED study completed the Edinburgh Postnatal Depression Scale (EPDS) and provided fecal samples in their second (N=46) and third trimesters (N=33). Vaginal swabs were collected prior to delivery. Fecal samples were acquired from infants at 1, 2 and 3 weeks, 3 and 5 months old. EPDS≤8 and EPDS≥9 indicated low and high depressive symptoms respectively. Microbial community structure was analysed by 16S rRNA gene sequencing.

Results: Maternal depressive symptoms in the second trimester were associated with significant alterations in the maternal microbiome. Notably, reduced phylogenetic diversity ($P=.024$) and species richness (chao1; $P=.040$) was observed among the higher depressive group. These depressive-associated alterations were no longer apparent in the third trimester gut or vaginal microbiome. There was a reduction in Actinobacteria (49.4% vs 28.5%) and an expansion of Proteobacteria (2.5% vs 15.1%) among infants at 1 week old whose mothers reported depressive symptomology in the second trimester. These changes were no longer evident at later ages.

Conclusion: Maternal depressive symptoms in mid pregnancy influences the composition of the maternal and infant microbiome. Alterations in the infant gut microbiome due to prenatal maternal depressive symptoms may be independent of direct vertical transmission of a depression-associated microbiome during parturition. The consequence of these changes, if any, to infant health needs further investigation.

Policy of full disclosure: None.

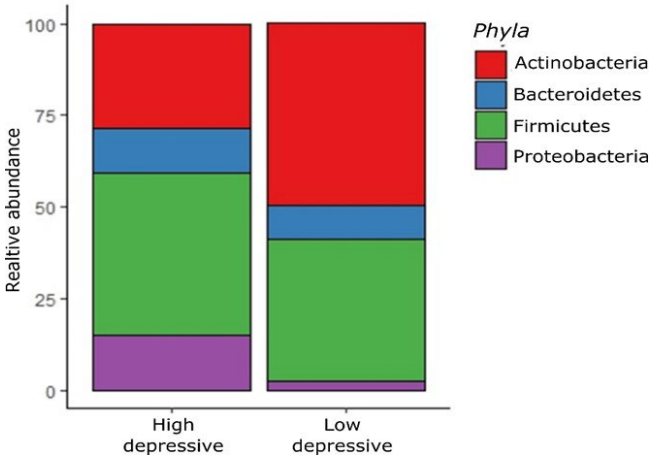

FIGURE 1 Composition in the infant gut microbiome at 1 week old by maternal depressive symptoms.

67 | The herbal medicine STW 5 is efficacious in targeting dyspeptic symptoms in all ages: A meta-analysis of randomized controlled trials

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Introduction: The prokinetics, as eg, metoclopramide and domperidone, are today no longer available for therapy of functional gastrointestinal diseases due to restrictions of their marketing authorizations based on rare but severe side effects. Therefore today other well proven therapeutic options gain increased attention. One of these is STW 5 (Iberogast), for which more than 5 decades of therapeutic experience in more than 73 Mio patients are available. For determining, whether its efficacy in functional dyspepsia shown in meta-analyses is comparable in patients of different age groups, sub-group analyses were conducted.
Aims & Methods: After meta-analyses of randomized placebo-controlled double blind trials have proven compliance to modern standards for a proof of efficacy, now sub-group analyses were conducted. The analyses were based on the original single patient data from the trials, including demografic data and primary endpoints (ANCOVA).
Results: As the primary outcome variable, the validated gastrointestinal symptom score (GIS) [1], as well as the therapeutic dose (3×20 drops/day) were identical in all trials, a uniform evaluation was possible. The full analysis set (FAS) included 557 patients (272 resp. 285 for placebo resp. verum). The mean age (48 resp. 49 years), the mean body size (in both groups 168.7 cm), the mean body weight (72.0 resp. 72.2 kg), the BMI (25.35 resp. 25.54), the gender distribution (67.3 resp. 69.5% females), the duration of the disease at the time of inclusion and the baseline of the GIS (11.6 resp. 11.5 points) were very well comparable between both groups. For the primary variable GIS the difference between placebo and verum after 28 days of treatment showed a highly significant ($P<.0001$) difference between placebo and verum (6.7 resp. 4.7 points). The analyses in different age groups (adults up to 30, 30-40, 40-50, 50-60, above 60) did show a comparable efficacy.
Conclusion: These meta-analyses therefore clearly show the efficacy of STW 5 (Iberogast) and its therapeutic usefulness in patients of all ages. Given also the very good tolerability shown by the study and by pharmacovigilance data, STW 5 is very well suitable also in self-medication. Additional insights can be expected from additional sub group analyses, as eg, the evaluation of subgroups with specific predominant symptoms.
Policy of full disclosure: J. Müller, B.R. Vinson, C. Fink, S. Rabini, H. Abdel-Aziz, and O. Kelber are employees of Phytomedicines Supply and Development Center, Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. M.A. Storr, K. Nieber, and K. Kraft have received honoraries or travel grants from Phytomedicines Supply and Development Center, Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany.

References

[1] Adam et al. Aliment Pharmacol Ther 2005; 22: 357-363.

68 | Neuromuscular changes in asymptomatic diverticulosis and diverticular disease

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Background and aims: Intestinal motility is controlled by the enteric nervous system communicating with smooth muscle cells, the effectors of intestinal movement. As diverticular disease (DD) is associated with an enteric neuropathy and impaired intestinal motility, we analyzed markers of smooth muscle cells and the neurotrophic glial cell line-derived neurotrophic factor (GDNF) system in patients with symptomatic DD, asymptomatic diverticulosis, and controls in order to enlighten the pathologic changes preceding the onset of DD.
Material and methods: Colonic samples obtained from patients with symptomatic DD (n=13), asymptomatic diverticulosis (n=12), and controls (n=19) were subjected to histological analyses. Furthermore, site-specific gene expression analyses were performed for smooth muscle marker genes and genes of the neurotrophic system by real-time-qPCR analysis on mRNA samples extracted from tunica muscularis (TM) and myenteric ganglia harvested by laser microdissection (LMD). Protein expression was assessed and quantified by immunohistochemistry on full-thickness sections of patients and controls.
Results: In contrast to our previous findings on DD, only moderate structural muscular alterations were observed in colonic full thickness sections from patients with asymptomatic diverticulosis compared to controls. mRNA expression of HDAC8 and Tropomyosin 1 was increased in the TM of patients with diverticulosis compared to controls. Although we did not detect signs of hypoganglionosis in samples from diverticulosis patients, mRNA expression of GDNF, GFRα1, and RET was decreased in the TM of patients with both, DD and diverticulosis, compared to controls. Expression of GFRα1 and RET was reduced within myenteric ganglia of patients with diverticulosis and DD compared to controls on both, the transcriptional and protein level.

Conclusion: Our data provide evidence for an impaired neurotrophic system both at gene and protein expression level in DD and also in asymptomatic diverticulosis during early stages of diverticula formation. These findings further add evidence for a primary enteric neuromyopathy contributing to the pathogenesis and disturbed intestinal motility in DD independent from inflammatory events.
Policy of full disclosure: None.

69 | Responsibility of patients towards the management of their Irritable Bowel Syndrome (IBS): A qualitative study

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Introduction: Taking responsibility in the management of the disease is an important feature in the well-being of irritable bowel syndrome patients. However, there is little knowledge with respect to how patients see this responsibility.
Method: We carried out fifteen interviews of irritable bowel patients from Iasi Area, Romania, focused on their responsibility-related experiences. The interviews were analyzed by using the constant comparative method assisted by QSR Nvivo software.
Results: Several important themes emerged about how patients perceive responsibility. Patients told that they took responsibility motivated by the avoidance of consequences like pain, poor access to toilets or extra medical investigations. Respecting the dietary requirements is important, although, poor abstention, travelling or professional life may prevent taking full responsibility in this area. Comorbidities usually enhance responsibility in the management of IBS. The results are discussed in the context of existing normative, and empirical literature and recommendations for clinicians are provided.
Policy of full disclosure: None.

70 | Trivalent chromium suppresses gastrointestinal motility and secretion in experimentally altered gut homeostasis in laboratory rodents

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Diarrhea and fast gut asides from being components of some gut ailments are also responsible for loss of major nutrients. Trivalent chromium was investigated for its impact on gut motility, secretion and smooth muscle contraction in experimentally altered gut homeostasis. Sixty adult male Wistar rats (90.6±4.2 g), and 15 male slc:ddY mice (26.4±2.1 g) were used for this study after exposure to chromium for 12 weeks (n = 5/group/experiment). In in vivo experiments, rats were grouped thus: group 1- negative control (distilled water); group 2- positive control (castor oil); groups 3, 4 and 5 were treated with atropine (5 mg/kg) or loperamide (3 mg/kg), 10 and 100 ppm chromium, respectively depending on the experiment. For in vitro experiments, mice were grouped into 3: control, 10 ppm and 100 ppm respectively. The motility, secretory and contractile functions were evaluated using standard in vivo and in vitro methods. Data were expressed as Mean ± SEM analyzed using one way ANOVA and considered significant at $P<0.05$. Chromium significantly inhibited intestinal transit in 10ppm (23.24%) and 100ppm (21.08%) compared to control. Passage of loose stool and purging index significantly decreased in 100 ppm (2.8±1.2; 3.1) compared with control (6.6±1.0; 15.6), respectively. Enteric fluid pooled significantly reduced in 10 ppm by 47.1% and in 100 ppm by 45.3% compared with control. Colon motility time was significantly prolonged in 10ppm (135.3±6.70 sec) and 100ppm (123.0±6.10) compared with control (95.40±4.26 sec). Intestinal muscle contraction significantly reduced in chromium group compared to control. Chromium exhibits potent anti-motility and anti-secretory effects on destabilized gut homeostasis.
Key words: Trivalent chromium, Motility, Secretion, Diarrhea, Enteropooling.
Policy of full disclosure: None

71 | Innovative technology solutions to explore effects of the microbiome on intestine and brain pathophysiology

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Recent advances in organ-on-chip technology, combined with adult stem cell differentiation protocols, capable of generating a growing list of human organoids, mean that in vitro models have the power to revolutionize drug discovery and disease modelling. To date, animal studies remain one of the sole means of assessing the importance of microbiota on development and well-being, however the use of animals to study human systems is increasingly questioned due to ethics, cost and relevance concerns.
IMBIBE is a newly funded ERC Consolidator grant that seeks to generate an in vitro model of the Gut-Brain-Microbiome axis. Although of necessity reductionist, this model represents one of the first in vitro approaches to study host-microbiome interactions and consequences for pathophysiology, in particular, of the GI tract and brain. IMBIBE is unique in that it will use cutting edge organic electronic technology which will allow real-time monitoring thus enabling iterative improvements in the models employed.
Based on previous work integrating models of the GI tract and the blood brain barrier with in situ electronic monitoring, IMBIBE will use microfluidic technology to generate compartments for hosting the gut and brain modules. Bioelectronic readout parameters will include transepithelial resistance, neuronal firing, metabolites (glucose and lactate), pH, oxygen and optical signatures. In collaboration with the APC Microbiome institute, distinct populations of microbiota will be added and their effects observed.
This platform has the potential to feed in to current research on the microbiome to accelerate discovery in a manner predictive of human physiology.

NEW TECHNOLOGIES IN CLINICAL NEUROGASTROENTEROLOGY

72 | Post-reflux swallow-induced peristaltic wave index and mean nocturnal baseline impedance predict heartburn response to proton pump inhibitors better than acid exposure time in GERD

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Background: Traditionally, acid exposure time (AET) has been regarded as the most useful parameter to predict heartburn improvement with proton pump inhibitor (PPI) therapy. However, recent studies showed high rates of heartburn response to PPIs also in patients with normal AET. Two novel impedance parameters, namely the post-reflux swallow-induced peristaltic wave (PSPW) index and mean nocturnal baseline impedance (MNBI) were found useful in identifying GERD patients. Therefore, they could prove useful in predicting PPI-induced heartburn relief.

Aim: We aimed to investigate demographic, endoscopic and impedance-pH features able to predict PPI response in patients with heartburn.

Methods: Off-therapy impedance-pH tracings from 425 patients, 317 with definitely PPI-responsive heartburn and 108 with PPI-refractory, were blindly and manually re-analyzed. Baseline demographic and endoscopic characteristics, conventional impedance-pH variables including acid exposure time (AET) and number of reflux episodes, PSPW index and MNBI were assessed by means of multivariate logistic regression to identify independent predictors of PPI responsiveness. Models based on independent predictors were then developed and compared for the ability to predict symptom improvement by calculating the area under the curve (AUC) at receiver-operating-characteristic analysis. MNBI values were calculated at 3 cm above the LES, during the overnight rest, for at least 30 minutes after excluding swallows and reflux induced changes. The PSPW index was calculated by dividing the number of refluxes followed within 30 seconds by swallow-induced peristaltic waves with the number of total refluxes.

Results: AET, MNBI and PSPW index were the only factors associated with PPI responsiveness: abnormal values were found in 60%, 76% and 92% of PPI-responsive cases ($P<.017$ for all pairwise comparisons). As shown in the Figure, comparing AUCs, PSPW index (AUC 0.794, $P=.002$) and MNBI (AUC 0.741, $P=.003$), both separately and combined (AUC 0.810, $P<.001$), predicted PPI responsiveness better than AET (AUC 0.687).

Conclusion: AET, PSPW index and MNBI are independent predictors of heartburn responsiveness to PPIs. However, PSPW index and MNBI can link PPI-responsive heartburn to reflux better than AET and should become part of the standard analysis of impedance-pH tracings.

Policy of full disclosure: None.

73 | Dynamic MRI for bowel motility imaging: How fast and how long?

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Objective: Dynamic 'motility' imaging of small intestinal motility is an increasingly common research method to examine bowel physiology in health and disease. However, limited data exists to guide imaging protocols with respect to quantitative imaging analysis. The purpose of this study is to provide guidance information on temporal resolution and scan duration in dynamic MRI for small bowel motility assessment.

Methods: 6 healthy volunteers (3 females, median age 22 [range 21-25]) underwent motility imaging with MR enterography using a breath-hold (BH) protocol. All volunteers ingested 1 L of 2.5% mannitol solution prior to the scan session. A 2D bFFE sequence was

	OR (95% CI)	*P* value
Male gender	0.623 (0.330-1.175)	0.144
Age (years)	1.009 (0.987-1.032)	0.393
BMI	0.983 (0.920-1.049)	0.613
History of reflux esophagitis	1.786 (0.680-4.693)	0.239
Hiatal hernia	0.753 (0.403-1.407)	0.375
Abnormal AET	**0.379 (0.155-0.924)**	**0.033**
Positive SAP	1.310 (0.599-2.868)	0.498
Positive SI	1.854 (0.799-4.300)	0.150
Abnormal number of reflux events	1.576 (0.753-3.295)	0.227
Abnormal PSPW index	**12.499 (5.548-28.157)**	**< 0.001**
Abnormal MNBI	**3.586 (1.885- 6.824)**	**< 0.001**

TABLE

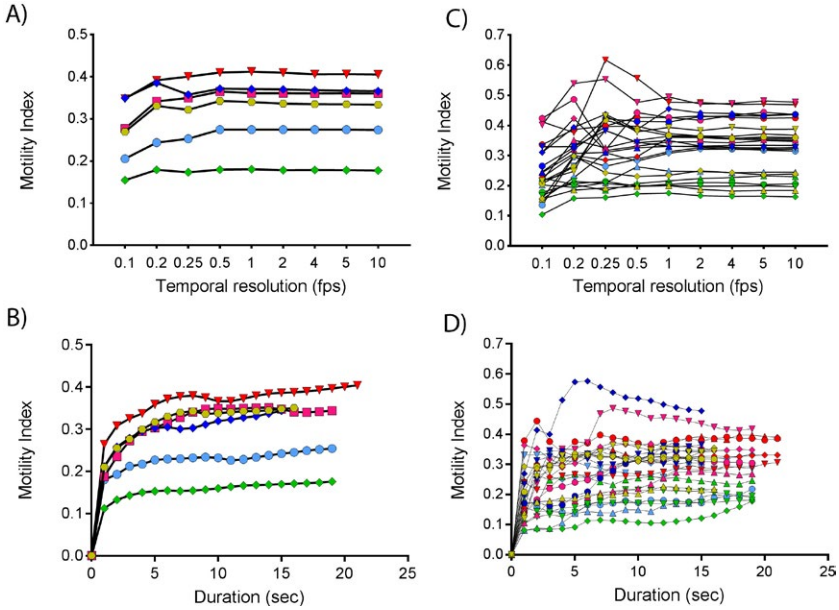

FIGURE 1 Mean motility index from breath hold scans for the global small bowel ROIs at (A) different temporal resolutions and (B) at different scan durations. The subjects are visualized with different colored labels. The same is shown for the four local ROIs with m

used to acquire dynamic data at a high temporal resolution of 10 fps. Motility was quantified using a validated registration algorithm which summarized motility by taking the standard deviation of the deformation fields' Jacobian determinant averaged across a region of interest (ROI) referred to as the motility index. One global ROI and four local ROIs were drawn in every scan. Firstly, to explore temporal resolution, the data was under sampled to different temporal resolutions to examine resulting changes in the motility index. Secondly, to evaluate the impact of scan duration, the dynamic series length was varied to determine the impact on motility score.

Results: The mean motility index stabilizes at a temporal resolution of 1 fps. The mean motility index appears to stabilize for scan durations of 15 seconds.

Conclusion: A temporal resolution of at least 1 fps is necessary for a scan duration of at least 15 seconds for consistent motility observations. This is consistent with the majority of small bowel motility studies to date.

Policy of full disclosure: None.

74 | Ultrasound and wireless motility capsule findings in patients with Familial GUCY2C diarrhea syndrome

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Objectives: Dysmotility and increased intestinal fluid are typical findings in patients with Familial GUCY2C diarrhea syndrome (FGDS) caused by an activating GUCY2C mutation. The aim of this study was to investigate meal-related gut motility using ultrasound and wireless motility capsule (WMC) in FGDS patients and in healthy controls (HC).

Material and Methods: Bristol stool chart and stool frequency (in FGDS patients) was assessed. Occlusive and non-occlusive contractions were recorded using high-frequency (9 MHz) ultrasound before and after a small meal (260 kcal) in 21 FGDS patients and 24 HC. WMC was used to sample gut transit time, pH, contractions and pressure.

Results: The FGDS patients had median 4 (range 1-10) loose stools per day, and those receiving WBC (n=14) had prolonged total gut transit time compared to HC (55.5 hours vs 28.5 hours, *P*=.001), with increased colon transit time (39.5 hours vs 21.5 hours, *P*=.008). Compared to HC, pH in duodenum, small bowel and colon was significantly increased in FGDS patients and the number of contractions and the intraluminal pressure was significantly decreased measured by WMC. Ultrasound showed that the FGDS patients had increased number of non-occlusive contractions compared to HC. "Snowglobes" (non-propulsive, ineffective contractions) were present in the jejunum in 18 of 21 FGDS patients and in 1 of 24 HC, and in ileum in 20 of 21 FGDS patients and in 1 of 24 HC.

Conclusion: Despite having diarrhea, the FGDS patients have delayed transit time through the whole gut, particularly in the colon. WMC revealed more contractions overall in the HC, whereas ultrasound showed that the FGDS patients had more non-occlusive contractions.

Policy of full disclosure: None.

75 | Gastrointestinal peptides during chronic gastric electrical stimulation in patients with intractable vomiting

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Objective: Gastric electrical stimulation (GES) is an alternative therapy to treat patients with intractable vomiting. A preclinical study has demonstrated the modulation of the gastrointestinal (GI) peptide ghrelin by GES but such mechanism has never been investigated in patients. The aim of this work was to assess the effect of GES on GI peptide levels in patients with intractable vomiting.

Methods: Fourteen patients completed the study (10 ON, 4 OFF stimulation). Vomiting episodes, gastric emptying and gastrointestinal quality of life index (GIQLI) were assessed. Gastric and blood samples were collected before and 4 months after the ON period of gastric stimulation. mRNA and/or peptide levels were assessed in gastric biopsies for ghrelin, leptin and NUCB2/nesfatin-1 and in duodenal biopsies for glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) using RT-qPCR and multiplex technology. Ghrelin, leptin, GLP-1, PYY, gastric inhibitory peptide (GIP) and NUCB2/nesfatin-1 levels were also quantified in blood samples.

Results: Among clinical parameters, vomiting episodes were slightly reduced by GES (P=.09). In tissue, mRNA or protein levels were not modified following chronic GES. In blood, a significant reduction of post-prandial PYY levels (P<.05) was observed at M4 and a reduction of NUCB2/nesfatin-1 levels in fasted patients (P<.05). Increased plasma leptin levels after GES were correlated with reduction of vomiting and improvement of GIQLI.

Conclusions: GES reduces NUCB2/nesfatin-1 levels under fasting conditions and post-prandial PYY levels in patients suffering from nausea and/or vomiting refractory to pharmacological therapies. These data bring new insights in the mechanism of action of GES which remains elusive and may explain the beneficial effect of this therapy. Grants, sponsoring: Mathieu Meleine, Chloé Melchior and Philip Prinz were nEUROgastro TANDEM 2015 participants. This idea was partly generated at the nEUROgastro TANDEM 2015 meeting and work was partly supported by a nEUROgastro TANDEM 2015 grant. Authors have no conflict of interest to declare.

Policy of full disclosure: None.

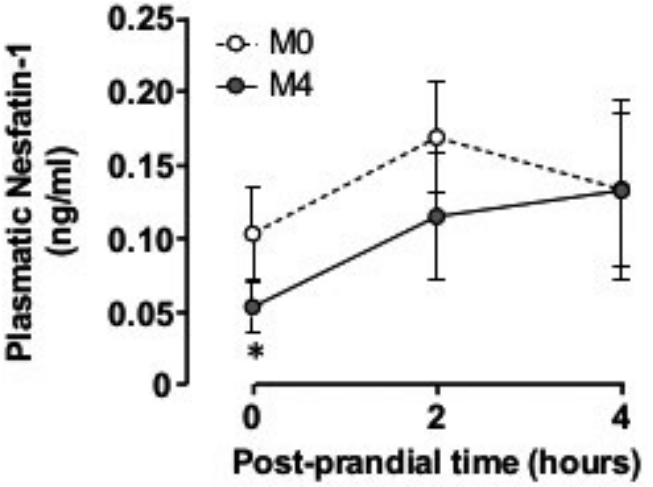

FIGURE 1 Plasma nesfatin-1 concentration before (M0) and after 4 months of gastric stimulation (M4) in fasted patients then 2 and 4 hours after meal

76 | Standardizing parameters of high resolution duodenojejunal manometry in healthy controls

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Objective: Small intestinal manometry historically is performed in low resolution fashion. High resolution methods have dramatically advanced esophageal and anorectal manometry interpretation. We employed high resolution methodologies to characterize duodenojejunal manometry parameters in healthy volunteers including novel propagation and coupling measures not measurable by standard techniques.

Methods: 10 fasting duodenojejunal recordings (MMS Systems) (mean 5.07±0.39 hours) were performed in 8 healthy controls in a bioavailability study. Catheters (Mui Scientific) were fluoroscopically passed to position three manometry clusters in the duodenum and jejunum with 1-2 cm spacing between channels. MMC phase 3 periodicity, duration, amplitude, and complex propagation were quantified. Propagation direction and velocity of individual phase 3 contractions were measured from spatial-temporal contour plots. Coupling was defined by pressure peak continuity across all channels within 3-4 cm clusters.

Results: 46 phase 3 complexes (24 antral, 22 intestinal origin) with 157±75 minute periodicities, 6.99±2.52 minute durations, 10.92±0.81 cpm frequencies, 73.6±20.3 mmHg maximal amplitudes, and 0.078±0.068 cm/s propagation velocities were recorded. Contour plot analyses showed 48.3±23.8% coupling of individual contractions within manometry channel clusters. 67.5±26.5% of contractions were antegrade, 21.5±21.2% were retrograde, and 11.0±14.2% were stationary (Figure). Individual phase 3 contractions propagated faster (1.74±0.57 cm/sec) than complexes themselves. On subgroup analyses, site of origin influenced contractile frequencies (antral

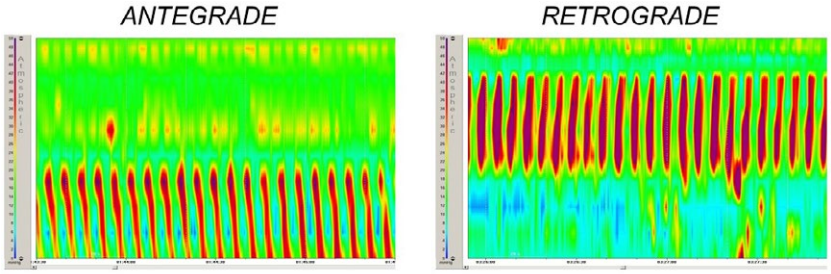

Figure: Propagation of Phase 3 Contractions

origin 11.16±0.80 vs duodenal 10.65±0.79 cpm, *P*=.04). Phase 3 complexes >30 cm distal to the ligament of Treitz were more prolonged (8.79±3.51 vs 6.62±2.12 minutes, *P*=.02), slower propagating (0.036±0.016 vs 0.088±0.072 cm/sec, *P*=.04), and had less coupled individual contractions (33.2±25.0 vs 66.5±27.0%, *P*<.01) than proximal complexes. Coupling was lower with 2 cm channel spacing vs 1 cm (38.6±20.3 vs 67.0±18.7%, *P*<.01).

Conclusions: High resolution small intestinal manometry characterizes novel parameters including relating to both antegrade and retrograde propagation and coupling which degrade in distal segments. Coupling appears greater with more closely spaced recording channels, emphasizing the value of higher resolution. Applying similar methods to dysmotility patients will define the clinical relevance of these findings.

Policy of full disclosure: None.

77 | In vivo assessment of foods that stimulate intestinal secretions using magnetic resonance imaging: Implications for dietary advice in ileostomy care

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Introduction: Plant foods may stimulate intestinal secretion through noxious chemicals designed to deter herbivores such as lactucins in lettuce and rhein in rhubarb. Increased small bowel water increases ileostomy output and may induce diarrhoea in people with intact bowel.

Objective: To investigate the effect of lettuce and rhubarb on intestinal water content. Method: A three treatment crossover trial. Population: Adults ≥18 without bowel disorders. Interventions: 3 meals of 245±3 kcal, with 200 mL water. Meals: 2 slices white bread with 10 g butter; 300 g rhubarb with 60 mL lactose free cream; 300 g lettuce with 30 mL mayonnaise. Meals were eaten 1 week apart. Meal order was randomised using an online tool. **Primary outcome:** water content of the small bowel (SBWC) using magnetic resonance imaging (MRI). Secondary outcomes: ascending colon water content; T1 relaxation time (T1AC); gastric volume; visual analogue scales of bloating and satiety (0-100). MRI analysts were blinded. Scanned fasting and hourly to 180 minutes postprandial.

Results: 15 subjects completed the study. 9 female, 6 male, median age 21(IQR 20,22) and mean body mass index 21.4 kg m^{-2} (SD±2.2). Bread induced a fall in SBWC compared to a rise after lettuce and greater rise after rhubarb, with increased area under the curve (0-3 hours) (both *P*<.01, paired *t*-test). Ascending colon water volumes at 3 hours were significantly higher for both lettuce and rhubarb than bread (*P*<.05 for both, Wilcoxon). Rhubarb induced a rise in T1AC but differences at 3 hours were not significant (*P*=.06). Symptom scores were higher for lettuce>rhubarb>bread.

Conclusion: Lettuce and rhubarb meals increased small bowel water with later effects on the ascending colon on arrival of the meal residue. MRI can be used to demonstrate the mechanism by which food can alter stoma output and stool consistency. Future work is needed to identify the active components of these meals.

Policy of full disclosure: None.

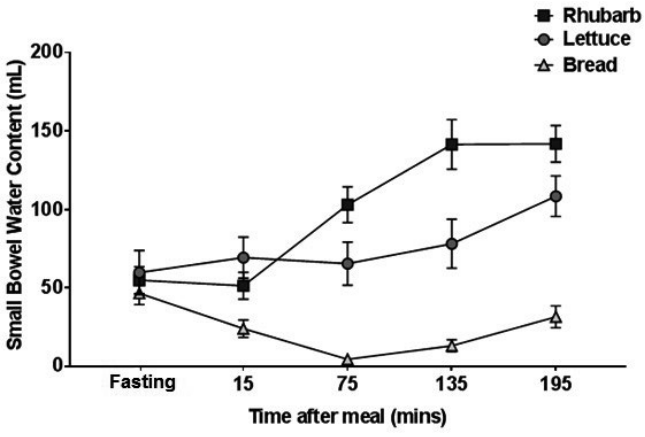

FIGURE 1 Small Bowel Water Content After Test Meals

78 | Volume vs caloric stimulation of small bowel motility in healthy controls

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Objective: Small bowel dysmotility is implicated in a number of high prevalence gastrointestinal diseases and syndromes. Increasingly, MRI has been used in research to objectively and reproducibly evaluate contractility within the small bowel. The objective of this study was to validate a clinically practical (<30 minutes) stimulation test for small bowel motility using a 300 kcal meal in prepared and unprepared healthy subjects.

Methods: Twenty healthy subjects (12 females, median age 23.8, range 19-32 years) underwent dynamic MRI to capture global small bowel motility after a 10 hour overnight fast. The cohort was split into two a (i) prepared and (ii) unprepared group. The 10 subjects in the prepared group ingested 1 L of 2.5% mannitol solution (routine clinical preparation) prior to the scan session. The unprepared subjects received no preparation. Each subject thereafter underwent the same MRI protocol with a (i) baseline motility scan followed by a (ii) food challenge with Nutridrink (iii) a post-challenge scan and a (iv) a second post-challenge scan (after approximately 20 minutes). Motility was quantified using a validated motility assessment technique (GIQuant) to produce a single, numerical motility score. Baseline motility between groups was compared using a Wilcoxon rank-sum test. Motility at baseline and after the food challenge within groups was compared using a Wilcoxon signed-rank test.

Results: Motility in prepared subjects at baseline was statistically different from motility in unprepared subjects at baseline (0.36 [0.18-0.64] vs 0.16 [0.13-0.20, *P*<.001). In the prepared group the food challenge produced a 8% increase in motility (*P*=.4). In the unprepared subjects a significant increase of 46% was observed (*P*=.004), responses to food remained insignificant and significant, respectively at the third scan time point.

Conclusion: Mannitol, a near zero calorie but large volume, bowel preparation produced significantly higher motility in fasted subjects. Additionally the pro-kinetic effect of a caloric challenge could not produce an effect in prepared subjects but did in fasted subjects. The response to calories within a short time frame (<10 minutes) supports the clinical use of this challenge in unprepared bowel to provoke motility changes in a range of diseases with a physiologically 'natural' stimulus where dysmotility might be implicated.

Policy of full disclosure: None.

79 | Fecobionics: A novel integrated bionics test of anorectal function

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Defecation is a complex process that may easily get disturbed. Constipation is common and affects 12%-15% of the population. Defecatory disorders are commonly diagnosed with rectal balloon expulsion where a bag is distended until urge to defecate followed by attempts to expel the bag. Other diagnostic tools are HRAM and defecography. We constructed a new expulsion device named Fecobionics where pressures, orientation and bending could be assessed. We aimed to characterize physiological parameters in healthy volunteers related to pressure signatures during the expulsion. A deformable 10 cm-long 12 mm OD probe was constructed. The properties mimicked those of normal stool. It had pressure sensors at the front and rear ends and inside an 8 cm-long bag mounted on the probe. It contained two gyroscopes for measurement of bending. Wires were threaded inside a thin tube to the external battery and computer. The bag was distended in the rectum of 4 healthy males until urge to defecate. Several expulsions were done in each person. Urge to defecate was felt at 45-60 mL volume. All could easily expel the device after bag filling and reported that it felt like normal defecation. All pressure transducers showed elevated pressures during expulsion but quickly the front pressure plateaued and decreased. The rectal expulsion pressures had values of 90-170 cm H_2O whereas the front pressure reached values between 20-50 cm H_2O above baseline rectal pressure during anal passage. Based on the front and rear pressure and the pressure difference we subdivided the tracings into five phases (Figure 1). The velocity calculated from the front and rear end pressure was 4-14 cm/s. Successful gyroscope experiments clearly

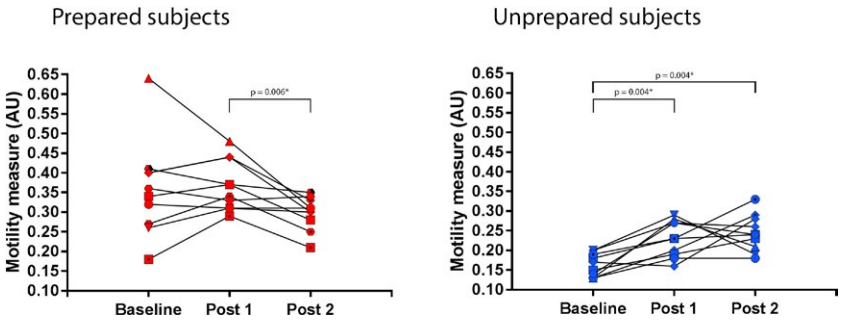

FIGURE 1 Global small bowel motility in prepared subjects (left) and unprepared subjects (right) at baseline, directly after the food challenge (Post 1) and approximately 20 minutes after the food challenge (Post 2)

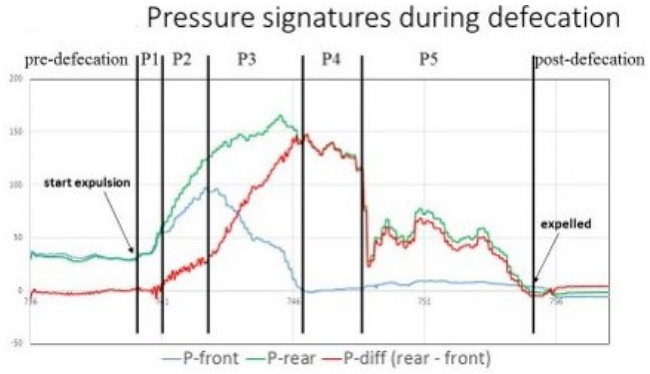

FIGURE 1

showed the shift in orientation when the device was pushed from the rectum into the anal canal, indicating that the anorectal angle could be measured. In conclusion it was possible to obtain promising data at physiological conditions. The pressure signatures defined five defecatory phases and the velocity and bending could be assessed. Hence, it is possible accurately to detect contractions and relaxations of the IAS, EAS and puborectalis during defecation.

Policy of full disclosure: None.

80 | Interobserver and intraobserver agreement in classification of defaecatory disorders on high-resolution anorectal manometry

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Objective: Defaecatory disorders (DD) on anorectal manometry (ARM) are classified into four types based on paradoxical anal contraction, poor anal relaxation, inadequate rectal efforts, or rectal hyposensitivity. There are limited data in intra and interobserver agreement in diagnosis of DD. We assessed variability in intra and inter-observer agreement in classification of defecatory disorder of manometry data.

Methods: ARM tracings of seventy consecutive patients (48 men; mean age 37[15] years) with DD seen over a period of 2 years, were re-analyzed by five observers, blinded to the diagnosis. All observers had minimum 5 years' experience in reporting ARM. One observer re-analyzed the tracings after at least 6 months of initial analysis to assess intra-observer variability. Trace 1.2 software was used for anorectal manometry analysis. K value and Pearson's Correlation Coefficient were used to assess intra- and inter-observer agreement.

Results: The mean duration of symptoms was 3.96 years at presentation, average stool frequency was 2/day with mean basal pressure 70 (21) mmHg, mean squeeze pressure 138 (63) mmHg, and mean defecation index was 0.64 (0.26). At baseline analysis, 42 patients had type 1 DD, 6 had type 2, 18 had type 3, and 4 had type 4. Intra-observer

TABLE 1 shows the kappa values of agreement of various observers

Inter item correlation matrix 'k' value / agreement						
	Expert 1A	**Expert 1B**	**Expert 2**	**Expert 3**	**Expert 4**	**Expert 5**
Expert 1A	1.000	0.862	0.675	0.738	0.650	0.625
Expert 1B	0.862	1.000	0.619	0.712	0.725	0.778
Expert 2	0.675	0.619	1.000	0.718	0.525	0.655
Expert 3	0.738	0.712	0.718	1.000	0.571	0.638
Expert 4	0.650	0.725	0.525	0.571	1.000	0.734
Expert 5	0.625	0.778	0.655	0.638	0.734	1.000

(Expert 1A and 1B) and inter-observer agreement in classification are shown in Table.

Conclusion: HRM has good intraobserver agreement and moderate inter-observer agreement for classification of defecatory disorders.

Policy of full disclosure: None.

81 | Characterization of GERD patients using pressure-flow analysis

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Objective: Pressure-flow analysis (PFA) allows integrated analysis of deglutitive motility and bolus flow. Little is known about PFA metrics in patients with gastro-esophageal reflux disease (GERD), and in particular, in relation to reflux monitoring. The aim of our study was to characterize GERD patients using PFA.

Methods: Combined high-resolution impedance manometry recordings were performed in GERD patients with typical reflux symptoms. Standardized 5 and 10 mL saline boluses were administered (orally). PFA was performed using purpose designed software (AIMplot, T. Omari), calculating the following metrics: impedance at peak pressure (IPP), pressure at nadir impedance (PNI), intra-bolus pressure (IBP), time from nadir impedance to peak pressure (TNIPP), pressure flow index (PFI) and impedance ratio (IR) as a measure of bolus clearance failure. All patients underwent a 24 hours impedance-pH monitoring prior to the manometric investigation, which we analyzed for reflux parameters and baseline impedance. Results were statistically analyzed using Kruskall-Wallis test and correlations were performed using Spearman correlation.

Results: Seventy-four GERD patients (48 years, min 19-max 78, 46 f/30 m) were investigated, 23 of them were taking proton pump inhibitors at the time of the measurement. Using pH-impedance and endoscopy records, we classified patients into erosive esophagitis (EE, n=23), non-erosive reflux disease (NERD, n=24), reflux hypersensitivity (RHS, n=10) and functional heartburn (FH, n=17). When pooling all patients (on and off PPI), using PFA, we found a significant difference in IPP between the 4 groups (P=.0058), with post-hoc tests revealing significantly lower values in EE compared to both NERD (P=.018) and FH (P=.026). We also found a significant difference in IR between the 4 groups (P=.0096), with significantly higher values (post-hoc) in EE compared to NERD (P=.021) and RHS (P=.05). In all GERD patients,

IR and esophageal acid exposure were positively correlated (P=.0045, r=.33). Interestingly, baseline impedance at different levels of the esophagus was highly correlated to PFA metrics (see Table).

Conclusion: This study describes, as a first, PFA metrics in a well-characterized GERD population. Markers of reflux exposure (acid exposure, impedance baseline) are closely correlated with measures of less complete bolus clearance. The data support the role of esophageal clearance function in reflux control.

Policy of full disclosure: None.

TABLE 1

	r (Spearman)	p-value
3 cm above the LES		
PNI	0.28	0.016
IBP	0.27	0.019
IPP	0.30	0.01
IR	-0.52	<0.0001
5 cm above the LES		
PNI	0.23	0.047
IBP	0.27	0.019
IPP	0.40	0.0004
IR	-0.61	<0.0001
15 cm above the LES		
IPP	0.27	0.02
IR	0.34	0.003

PNI = pressure at nadir impedance; IBP = intra-bolus pressure; IPP = impedance at peak pressure; IR = impedance ratio.

82 | Spectral bowel motility assessment using dynamic tagged MRI

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Objective: Abnormal bowel motility is thought to underpin symptoms of several common conditions. Measuring and quantifying motility can help in management and follow-up of these conditions. MRI has been used increasingly to assess small bowel motility. Recently, a tagging MRI sequence was presented for motility measurement in healthy volunteers during free breathing. Assessment during free breathing allows for longer monitoring ie, minutes vs seconds and assessing motility at frequencies as low as 2 contractions per minute (cpm), the slow wave motility pattern range. The objective of this study was to validate a clinically practical test for small bowel motility assessment in the low frequency range using tagged dynamic MRI before and after a 300 kcal meal in healthy subjects.

Methods: After ±11 hours of fasting and no bowel preparation, 6 healthy subjects (3 females, median age 26.5, range 21-30 years) underwent a free breathing, continuously tagged 3D dynamic BFFE sequence with a temporal resolution of 3 images per second. Each subject underwent the same MRI protocol with a (i) baseline motility scan followed by a (ii) food challenge with Nutridrink iii) a post-challenge scan and a (iv) second post-challenge scan (after ±20 minutes). Motility was quantified within a specified region of interest in the small bowel using a validated frequency analysis technique for measuring the spectral power of the strain, referred to as 'motility score'. Motility at baseline and after the food challenge was compared using a Wilcoxon signed-rank test.

Results: The motility score is most dominant in the low frequency regime (slow wave range), and is consistently highest in spectral activity right after caloric intake (see Figure). The difference between baseline motility score and directly after drinking is significant in the slow wave range intervals 2-4 cpm, 6-8 cpm and 8-10 cpm.

Conclusion: Tagged dynamic MRI is capable of assessing bowel motility characteristics during free breathing. The strain related spectral motility measure is capable of assessing changes in motility characteristics as a result of caloric intake in the low frequency regime. This

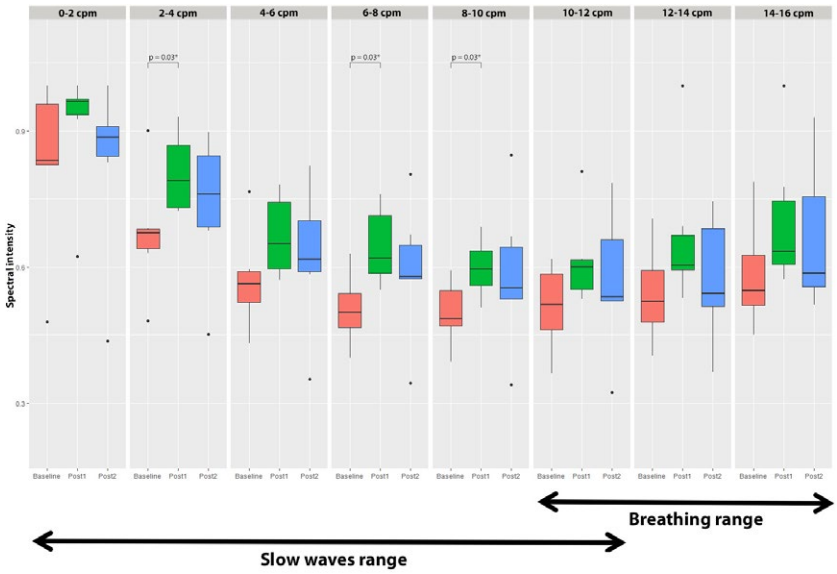

FIGURE 1 Boxplots of the spectral motility scores for all volunteers ranging over 8 spectral intervals (0-2 to 14-16 contractions per minute) visualizing global small bowel motility in unprepared subjects at baseline (red), directly after the food challenge (Post)

presents the opportunity to provoke and assess (patho)physiology within a short time frame (<10 minutes) in a range of diseases causing dysmotility.

Policy of full disclosure: None.

83 | The diagnostic value of esophageal mucosal and baseline impedance measurements in patients with gastroesophageal reflux disease

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Objective: Various biomarkers have been studied to evaluate the integrity of esophageal epithelium in distinguishing phenotypes of gastroesophageal reflux disease (GERD). Baseline impedance (BI) measurement is likely to be one of these and can be measured during the 24-hour ambulatory intra-esophageal impedance-pH study. Mucosal impedance (MI) measurement is a technique introduced in recent years and is a practical method that can be applied during endoscopy, but the validation studies are insufficient. BI and MI measured with the same regular impedance catheter and data from 118 patients with different reflux phenotypes and controls were evaluated.

Methods: Patients were divided into five groups: mild (ERD A-B, n=31), severe erosive esophagitis (ERD C-D, n=11), non-erosive reflux disease (NERD, n=26), functional heartburn-esophageal hypersensitivity (FH-EH, n=17), healthy controls. High resolution manometry, 24-hour MII-pH, upper gastrointestinal endoscopy were performed. BI values were taken at the sleeping period at night where reflux and swallowing did not occur. MI measured during endoscopy, a regular impedance-pH catheter passed through the biopsy channel of the scope. Distal two rings were contacted to the distal and proximal parts of the esophagus approximately 20-30 seconds. MMS Omega ambulatory recorder and Greenfield (6 imp, 1 pH) impedance catheter were used.

Results: MI can differentiate ERD from non-erosive groups but do not have a diagnostic value to discriminate NERD from FH-EH or controls. However, BI can segregate NERD from ERD addition to controls (Table 1).

Conclusions: As a new diagnostic tool, MI needs validation studies and our results failed to show additional diagnostic value in non-erosive patients compared to healthy controls. There was no correlation between BI and MI values. Since regular catheters are failed, new baloon-shaped catheters should be validated. BI might be a better tool to discriminate NERD from controls. This implicates that the esophageal epithelial resistance is impaired in this particular group compared to controls.

Policy of full disclosure: None.

TABLE 1

Groups	Baseline impedance	Distal mucosal impedance	Mucosal impedance (proximal esophagus)
Control (n=15/n=18)	2267±393#	2673±547#	3190 ±515
FH and EH (n=17)	1906±716#	2654±721#	3350±880
NERD (n=26)	1305±759*#	2423±852#	3407±1074
ERD A-B (n=31)	868±481*	1538±646*	3096±928
ERD C-D (n=11)	441 ±301*	1355±672*	3236 ±1653

* p<0.05 compared to controls. # compared to ERD C-D. Mean/std dev.

84 | Morpho-functional evaluation of the gut in cystic fibrosis

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Background: Cystic fibrosis (CF) is a multisystem disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The loss of CFTR in the gut results in a dehydrated and acidified intestinal milieu, which consequently impairs small bowel motility. However, intestinal motor dysfunction in CF has only been evidenced by indirect transit tests. In our laboratory we have developed automatic diagnostic methods for morpho-functional evaluation of the gut using images obtained by the endoscopic capsule and by abdominal magnetic resonance (MR).

Objective: To evaluate intestinal motor dysfunction in patients with CF using automatic and non-invasive techniques and to explore the relation between the structural and functional findings detected with these techniques.

Methods: Ten patients (4 men, 6 women; 23-59 years) with CF were studied with the endoscopic motility capsule procedure (PillCam SB2, Given Imaging) and with an abdominal MR. Studies were performed after a 48 hours low-residue diet and without bowel preparation. Morpho-functional analysis of the images was performed with specific, previously validated, computer programs based on computer vision and machine learning techniques.

Results: Intestinal hypomotility was detected in seven patients (70%) by capsule endoluminal image analysis (EIA). As compared to a historical cohort of gender and age matched healthy subjects, patients with CF exhibited less intestinal contractions (2.4±0.4 vs 5.2±0.2%; P<.001) and greater retention of turbid secretions (55±8 vs 25±3%; P<.001). Morpho-volumetric analysis of MRI images detected significantly larger ileal and colonic volumes in patients with CF, specifically due to increased solid content in the ileum, ascendant and transverse colon. We found significant correlations between the parameters detected by both techniques: contractile activity detected by EIA correlated inversely with the volume of the transverse colon (R=−.73; P=.008) and with ileal perimeter (R=−.83; P=.003) measured by MRI. The amount of turbid secretions in the proximal bowel detected by

EIA correlated with the volume of solid content in the colon measured by MRI (R=.71; *P*=.023).

Conclusion: Automatic and non-invasive analysis of external and internal images of the gut objectively quantifies small bowel dysmotility in cystic fibrosis, with high correlation between the outcomes of both techniques.

Policy of full disclosure: None.

85 | A case for developing a preventative swallow health maintenance program in the elderly

D. Agrawal[1]; M. Kern[1]; F. Edeani[1]; P. Sanvanson[1]; R. Shaker[1]

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Objective: Oropharyngeal dysphagia (OPD) associated with frailty is a worldwide devastating clinical problem. With increasing life expectancy and high prevalence of aging-associated OPD, challenges for the future include developing strategies to prevent OPD. Despite positive health outcomes with strength training exercises for locomotive health maintenance in the elderly, there has been no systematic approach for an exercise-based strategy to maintain a healthy swallow function to date. Since oropharyngeal swallowing musculature is comprised of striated muscle and amenable to voluntary manipulation using a novel swallowing exercise technique of Swallowing Against Laryngeal Restriction (SALR); the aim of this study was to determine the effect of a SALR enabled six-week swallow exercise program on deglutitive biomechanics in healthy elderly.

Methods: 28 elderly (75±7 years, 10 Male) underwent videofluoroscopic assessment of swallowing (30 frame/second; 1,3,5 mL mL 40% W/V Barium X 3 each) before and after 6 weeks of real exercise. 16 of these volunteers also underwent pre-and post-exercise high resolution pharyngeal manometry (Dry, 5,10 mL water X 5 each). An additional group of 10 elderly (81±6 years, 1 Male) were studied by videofluoroscopy before and after 6 weeks of sham exercise. Real Exercise consisted of 30 swallows at 15 seconds' intervals while wearing a handmade device hindering deglutitive laryngeal excursion. Exercises were done TID for 6 weeks. Sham exercise consisted of simple tongue protrusion X 5 TID. Statistical analysis was performed using paired *t*-test with *P*<.05 considered as statistically significant.

Results: Real exercise but not the sham exercise resulted in significant increase in maximum upper esophageal sphincter (UES) opening diameter, anterosuperior laryngeal excursion, posterior pharyngeal wall thickness for all tested volumes as well as anterior hyoid excursion for 3 mL and 5 mL swallows. Real exercise resulted in a significant increase in pharyngeal contractile integral for all tested volumes.

Conclusions: Strength training of oropharyngeal swallowing muscles facilitated by SALR technique improves UES opening, strengthens suprahyoid muscle, and reverses pharyngeal constrictor sarcopenia. These findings provide the basis for developing an exercise-based preventive swallow health maintenance program for the elderly.

Policy of full disclosure: None.

86 | Rehabilitation of a heterogeneous group of dysphagic patients by a novel exercise technique of swallowing against laryngeal restriction

D. Agrawal[1]; M. Kern[1]; F. Edeani[1]; P. Sanvanson[1]; R. Shaker[1]

[1]*Medical College of Wisconsin, Milwaukee, USA*

Objective: Oropharyngeal dysphagia (OPD) commonly develops following stroke, radiotherapy to head and neck area as well as in frail elderly. Only a minority of exercises prescribed for OPD have sufficient evidence of long-term improvement in swallowing. The oropharyngeal swallow musculature is comprised of striated muscle and can be overloaded, fatigued and strengthened using a novel technique of Swallowing Against Laryngeal Restriction (SALR). Since muscle weakness is a prevalent cause of dysphagia, the aim of this exploratory study was to test the effect of SALR-enabled exercise program on the oropharyngeal function of a non-selected group of OPD patients.

Methods: We studied 29 dysphagic patients age 65±10 years (14Females) with variable severity, duration (6 month-15 years) and diverse etiology (8 post radiation therapy, 3 post CVA, 5 cervical-spine surgery, 5 aging, 4 proximal esophageal hypo-motility, 1 post-esophagectomy, 1 pharyngeal stab injury, 2 other causes). These patients had remained symptomatic despite being in long-term deglutitive rehabilitation. Exercises were done TID for 6 weeks and consisted of 30 swallows at 15 seconds' intervals while wearing a device around their neck hindering deglutitive laryngeal excursion. All patients underwent videofluoroscopic evaluation of swallowing (1,3,5 mL 40% W/V Barium swallows X 3 as tolerated) before and after completion of 6 weeks of exercise. Exercise period was extended to 12 and 18 weeks if no effect was seen. Statistical analysis was performed using paired *t*-test and McNemar's exact test.

Results: Maximum upper esophageal sphincter (UES) opening and anterior hyolaryngeal excursion increased significantly (*P*<.05) after exercise period for all tested volumes. 17 patients showed aspiration at baseline and 8 of these demonstrated no aspiration on follow-up (*P*<.01). Symptom-specific outcome of swallowing (EAT-10) improved significantly (*P*<.01).

Conclusions: SALR-enabled exercise program reduces aspiration, improves oropharyngeal swallowing function, deglutitive UES opening, and biomechanics in patients with diverse etiology, variable severity, and duration of OPD. This improvement may take up to 18 weeks to achieve. These findings provide the foundation for future targeted studies.

Policy of full disclosure: None.

87 | The role of biofeedback therapy in functional disorders

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[1]*State Center of coloproctology, Moscow, Russia*

Objective: Purpose is to evaluate the results of conservative treatment of functional disorders of defecation (FDD) in patients with rectocele.

Materials and methods: In 2015-2016, 75 women with obstructive defecation syndrome due to a combination of rectocele and FDD were examined and treated. The rectoceles were detected in 48/75 (64%), the combination with rectal intussusceptions in 27 (36%). The average age is 47.8±13.2 years. FDD (Rome III criteria) were diagnosed using HRAM, defecography and evacuation test. The function of continence was evaluated by the results of anorectal manometry. Biofeedback therapy was performed.

Results: Based on the results of HRAM, a type I pattern was detected in 58 (77.3%) patients, type II (inadequate propulsion, intrarectal pressure <45 mmHg) in 5 (6.7%) and type III—in 12 (16%); the percentage of relaxation (PR) was (−25.6)±18.1, 10.2±32.1 and 8.8±4.2%, respectively. After biofeedback therapy, average PR was 11.9±9.4 in patients with type I FDD, while relaxation of the pelvic floor muscles during straining over 20% was demonstrated by 17/58 (29.3%) patients; the transition of type I to type III was registered in 25/58 (43.1%); without effect—16/58 (27.6%). In patients with type II: 4 (80%) a normal with adequate propulsion, PR 38.3±13.6%, without dynamics—1 (20%). An average PR was 24.8±10.5% in patients with type III; relaxation more than 20% detected in 8 (66.7%) patients, lack of dynamics—4 (33.3%). Symptoms of anal incontinence were recorded in 30/75 (40%) patients. Anal rest pressure were reduced to 34±3.6 mmHg (norma-52.0±5.6). After treatment, was increase of pressure to an average of 44.1±7 mmHg, with normalization in 9 (30%) patients.

Conclusions: Biofeedback therapy has a positive effect in 72% of cases in patients with rectocele and FDD. In 38.7% of cases there is a normalization with elimination of the FDD.

Policy of full disclosure: None.

88 | Digestive functional scintigraphy for digestive autonomic neuropathy diagnosis: About a case

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Objective: A 33 years old female, suffering from celiac disease diagnosed in 2010 and partially improved by a properly followed gluten-free diet, presented in 2014 a food-intake triggered painful abdominal acute syndrome with nausea, vomiting, intense asthenia, malaise, joint pains and tendency to constipation.

Methods: During a breakfast test (300 kcal with a scrambled egg radiolabelled with Tc99m SAH-nanocolloïde), imaging was performed in upright position to access esophageal transit (thoracic dynamic mode) and then calculation of gastric retention (abdominal static mode; geometric mean percentage of stomach on total abdominal counts).

Results: First scintigraphy carried out in February 2016 was suggestive of autonomic digestive neuropathy: esophageal transit showed failure rate 59% by stopping or splitting at the TZ and gastric phase showed no gastroparesis but accelerated emptying with prandial emptying 11%, no lag phase, evacuation phase as linear pattern at the rate of 32% meal per 30 minutes and emptying completed at 2 h 30. The patient exhibited a dumping syndrome at the end of the test-meal, documented using automatic blood pressure recording. Autonomic cardiovascular testing (Ewing's battery) identified a postural tachycardia syndrome (PoTS) associated with possible cardiovascular autonomic neuropathy. In October 2016, a second scintigraphy, performed while patient receiving immunosuppressive treatment, showed esophageal transit failure rate 55% and 37% improved gastric phase with appearance of a lag-phase, prandial emptying 2.6%, slower evacuation phase, 20% meal per 30 minutes and increased gastric retention at 2 hours (41%meal vs 17%meal initially). Clinically, patient improved with less nausea and vomiting, abdominal pain replaced by discomfort and no more dumping syndrome. The complete ROME III questionnaire highlighted only 4 items out of the 8 initially identified, with disappearance of Functional Chest Pain of Presumed Esophageal Origin, Unspecified Excessive Belching, Chronic Idiopathic Nausea and Cyclic Vomiting Syndrome.

Conclusion: This case report illustrates the place of digestive functional scintigraphy for the diagnosis of autonomic digestive neuropathy and the association of celiac disease and autonomic dysfunction.

Policy of full disclosure: None.

89 | Gastric emptying patterns in diabetic patients with functional digestive symptoms: First results in 45 patients

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Objective/Background: Motility disorder induced by gastric neuropathy can lead to gastrointestinal symptoms and impact on glycaemia regulation.

Purpose: To investigate the gastric emptying pattern of diabetic patients and explore the relationship between symptoms, glycaemia and gastric motility.

Methods: Forty-five diabetic patients were explored by digestive transit scintigraphy. During a breakfast test (300 kcal with a scrambled egg radiolabelled with Tc99m SAH-nanocolloïde), imaging was performed in upright position to calculate gastric retention by geometric mean percentage of stomach on total abdominal counts. All patients fulfilled the complete Rome III survey at the time of scintigraphy.

Results: Gastric emptying curves for the solid meal expressed 5 scintigraphic patterns: type 1 (n=12, 27%), exponential pattern with maximum caloric intestinal clearance during the early postprandial phase ; type 2 (n=14, 31%), triphasic pattern with a fast initial emptying phase followed by an absent or slow emptying phase for over 2 hours, then by a low-flow emptying phase ; type 3 (n=11, 24%), delayed emptying pattern with 2 to 3 hours of retention phase followed by a fast emptying phase with late residual food residues ; type 4 (n=4, 9%), lag-phase pattern with an absent emptying for 3 to 4 hours and a late low-flow emptying phase ; type 5 (n=1, 2%), linear

low-flow emptying pattern starting at the end of the meal. Three (7%) patients expressed a mixed pattern: 2 (5%) exponential pattern with late food residues and 1 (2%) exponential pattern with draft of triphasic mode. Regarding glycaemia, the type 1 pattern may be associated with early hyperglycaemia and late hypoglycaemia, the type 2 pattern with interdigestive hypoglycemia, the type 3 and 4 patterns with early postprandial hypoglycemia and the type 5 pattern with recurrent hypoglycemia.

Conclusion: Diabetic patients expressed 5 different gastric emptying patterns, which impact on glycaemia and are associated with different severity of symptoms.

Policy of full disclosure: None.

90 | Per oral endoscopic myotomy for the management of pediatric achalasia

T. Zangen[1]

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Objective: Tsili Zangen*, Tiberiu Hershcovici**, Harold Jacobs**, Ronit Brodie MPAS**, Yoav Mintz** * Wolfson Medical Center, Holon, Isreal ** Hadassah Medical Center, Jerusalem, Israel Objective Per oral endoscopic myotomy (POEM) is a novel technique for the management of achalasia with encouraging results in adults. Data and outcomes in children are limited. The aim of this study was to evaluate the safety and efficacy of POEM and the incidence of post treatment reflux symptoms in children with achalasia.

Methods: Prospectively maintained data of children 18 years of age and under who underwent POEM in a single center was retrospectively analyzed. Diagnosis of achalasia was based on symptoms, radiology, and high resolution esophageal manometry (HRM). Preoperative and postoperative symptoms scores (Eckardt score) and manometry outcomes were recorded and analyzed.

Results: POEM was successfully performed in five patients, three female, with a mean age of 15.4 (10-18) years. All were diagnosed with type II achalasia based on HRM results. The mean operative time was 62 (43-73) minutes, the mean myotomy length was 11.2 (10-14) centimeters. No serious complications related to the procedure were encountered. All the children had complete resolution of their symptoms during a mean follow up period of 6 months. One patient experienced recurrence of mild dysphagia 3 months after the POEM. Only one patient (20%) had reflux related symptoms after POEM and is currently on oral PPI treatment. One patient did not perform a post treatment HRM. The median preoperative and postoperative Eckardt score was 8.8 and 0.2 respectively (P<.005). The mean basal LES pressure decreased from 36+14.3 mmHg to 14.4+2.2 mmHg (P<.04), and the mean Integrated Relaxation Pressure (IRP) of LES decreased from 18.02+2.25 mmHg to 7+0.5 mmHg after the procedure (P<.009).

Conclusions: Our study suggests that POEM is a safe and effective method for treatment of achalasia in children and adolescents. Further trials with larger sample size and a long term follow up are warranted to confirm the role of POEM in pediatric achalasia.

Policy of full disclosure: None.

91 | Higher baseline cardiac vagal tone implicates a subcortical functional brain network during acute oesophageal pain

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[1]*Queen Mary University of London, London, UK;* [2]*University College London, London, UK;* [3]*King's College London, London, UK;* [4]*University Hospitals Midlands NHS Trust, Staffordshire, UK*

Objective: INTRODUCTION & AIM: Differences in parasympathetic cardiac vagal tone (CVT) has been suggested to have a physiological role in the regulation and modulation of painful sensory signalling, to the extent of vagal nerve stimulation (to raise subject CVT) being tested as a possible anti-nociceptive. To date, no studies have explored the brain functional connectivity or network properties of CVT in relation to a painful stimulus, and thus this was our aim.

Methods: 21 healthy participants underwent functional MRI scanning during painful oesophageal balloon distention, along with resting CVT acquisition. The effect of resting CVT on brain networks during acute oesophageal pain were determined by means of network based statistics. Brain nodes were selected a priori of previous autonomic/visceral pain literature (∑Nodes=11). Participants were dichotomised by means of median split based upon resting CVT value and t-tested (primary threshold [t=1.65; P<.05], permutation tested 50 000 times).

Results: We identified a unique subcortical brain connectivity network in the high resting CVT individuals when exposed to acute oesophageal pain. This network comprised all 11 nodes with a total of 18 edges (significant functional connections) (Figure 1). These interconnections included the following: thalamus-amygdala, thalamus-hypothalamus, hypothalamus-NAc, amygdala-putamen amygdala-NAc and insula-putamen. No significant network was identified for the low CVT group.

Conclusions: High resting CVT yields a strengthened pain-specific functional subcortical network, comprising numerous regions associated with either visceral pain or modulation of baseline autonomics. Previous research has suggested that a high resting CVT may be protective of nociceptive signalling, and furthermore studies investigating vagal nerve stimulation have included and report that of anti-nociception. Given the well-established role of these subcortical regions in pain processing, we suggest that this network identified may be of significance as to the neurophysiological process of parasympathetic modulation of pain. To date, no studies have undertaken real-time assessment of the ANS (including CVT) during functional brain imaging and acute visceral pain, which future studies should investigate this.

Policy of full disclosure: None.

[Correction added on 4 September 2017, after first online publication: The first author name and affiliations have been updated in this version.]

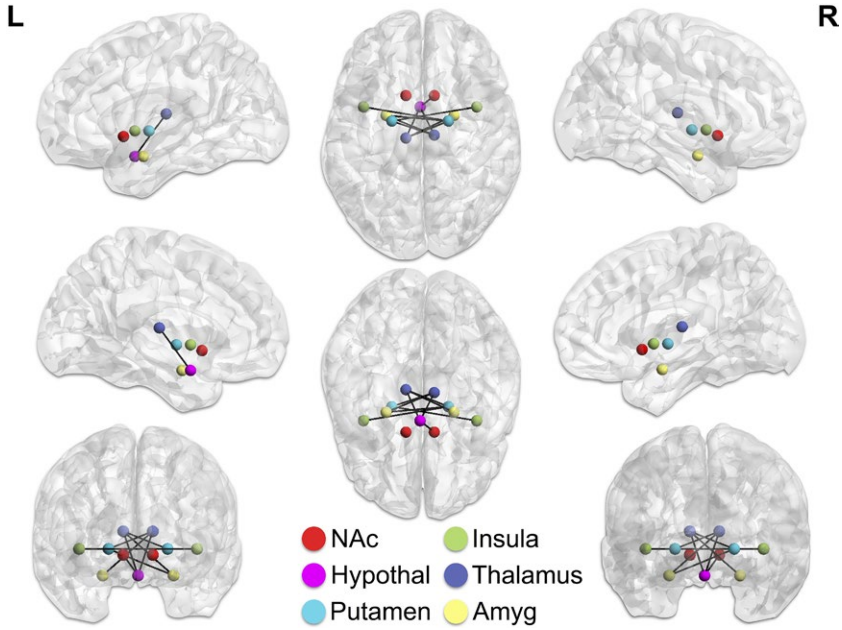

FIGURE 1 High baseline CVT confers a strengthened subcortical network

BIOMARKERS IN IRRITABLE BOWEL SYNDROME/TREATMENT OF VISCERAL PAIN

92 | Longitudinal analysis of IBS patients reveals that acquired immune responses are inhibited in symptom flare vs symptom free

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[1]University of Adelaide, Australia; [2]Royal Adelaide Hospital, Australia; [3]Flinders Medical Centre, Adelaide, Australia

Objective: Immune profiles correlate with symptoms in Irritable Bowel Syndrome (IBS), however the type of immune response involved remains controversial (Hughes et al. Am. J. Gastro. 2013). This appears to be due to 2 main methodological issues: grouping all IBS patients together rather than stratifying according to bowel habit, and an over-reliance on cross-sectional rather than longitudinal data. We aimed to compare the circulating immune profile of IBS patients longitudinally, comparing symptom-flare to symptom-free periods.

Methods: 11 IBS patients (ROME II) (5 IBS-D, 4 IBS-A, 2 IBS-C) completed IBS-S symptom questionnaire and provided a venous blood sample when in self-reported symptom-flare and when symptom-free. Peripheral Blood Mononuclear Cell (PBMC) were isolated from blood and frozen. Thawed PBMC were stimulated with CD3/CD28 beads for 96 hours and supernatant collected for cytokine analysis (multiplex), or loaded with CellTracker violet, stimulated with CD3/CD28 for 96 hours and then stained with CD4-PECy7 before proliferation analysis by flow cytometry.

Results: IBS-S scores were significantly higher in IBS patients with symptom flare compared to symptom free. In IBS-D, IFN-γ concentrations were significantly decreased in symptom-flare vs symptom-free. Concentrations of other cytokines did not differ between symptom-flare and symptom-free periods in any IBS patient group. Again, in patients with IBS-D only, THELPER proliferation was significantly inhibited during symptom-flare compared to symptom-free.

Conclusions: THELPER cell responsiveness to stimulation is inhibited in IBS-D only when they are in symptom-flare relative to symptom-free. These results tend to confirm our hypothesis that the controversy surrounding immune activation in IBS reflects examining all IBS patient together cross-sectionally. Future studies should ensure that symptom profiles at the time of tissue sampling are reported. Supported by NHMRC Australia.

Policy of full disclosure: None.

93 | Heart rate variability characteristics of patients with Irritable Bowel Syndrome (IBS) and association with symptoms

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[1]Göteborg University, Dept. of Medicine, Sweden; [2]Göteborg University, Sweden; [3]Danone Nutricia Research, Palaiseau, France

Objective: Annikka Polster1, Peter Friberg2, Boris Le Nevé3, Hans Törnblom1, Magnus Simren1 1 Department of Internal Medicine and Clinical Nutrition, Sahlgrenska University Hospital, Göteborg, Sweden 2 Department of Molecular and Clinical Medicine, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden 3 Danone Nutricia Research, Palaiseau, France.

Background: Disturbed brain-gut interactions, mediated by the Autonomic Nervous System (ANS), are assumed to be of importance for symptom generation in patients with IBS, but studies show diverging results.

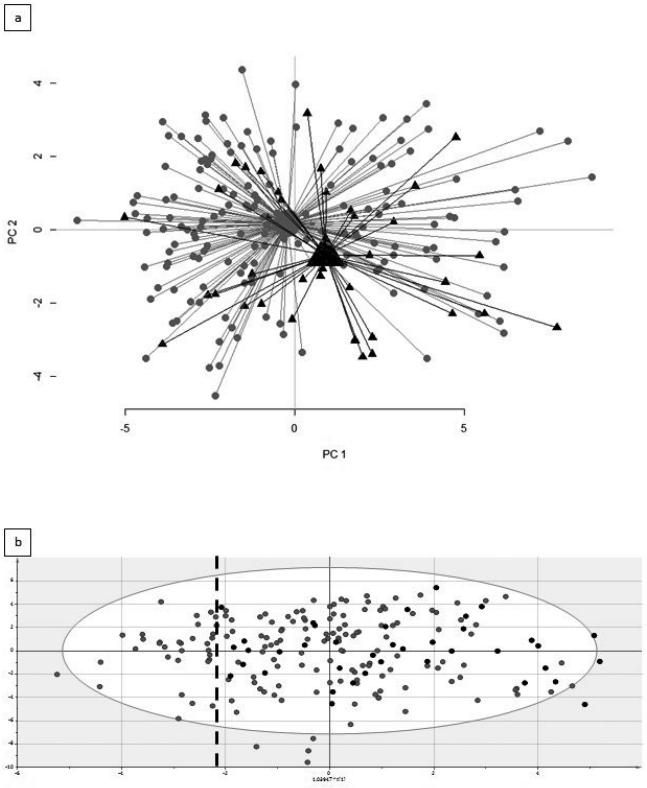

Figure 1: Principal Component analysis (a) and OPLS-DA (b) of IBS (grey) and HC (black). a) the large symbols reflect the groups centroids (weighted means of the multivariate dataset). b) the scatterplot is arranged such that the maximum variance between patients and HC is shown. While the majority of patients overlap with the HC, a subset of patients (left of the dotted line) differs significantly from the other patients and HC regarding their HRV status

Aim: To compare ANS characteristics of patients with IBS and healthy controls (HC) and investigate associations of ANS-status with symptoms.

Materials and Methods: Heart rate variability (HRV) was measured in patients and HC with Holter monitoring during controlled respiration in both supine and standing positions as well as during an ambulatory 24-hour period. Frequency (5 minutes intervals, supine and standing) and time domains (24 hours, day (8 AM-9 PM), night (11 PM-6 AM)) were analyzed. Validated questionnaires (GSRS-IBS, HAD) were used to measure gastrointestinal and psychological symptoms in patients. Groups were compared on a univariate and multivariate level (Principal Component Analysis (PCA), Orthogonal Partial Least Squares Discriminatory Analysis (OPLS-DA)).

Results: We analyzed 158 IBS patients (Rome III, 71.5% females, mean age 35 (range 19-64)) and 30 HC (56.5% females, mean age 29 (range 19-49)). Patients differed significantly from HC regarding several HRV parameters measured on a 24 hours interval and during daytime as well as in standing position. In the PCA a majority of patients overlapped with HC, but the weighted means (taking into account all measurements of every individual) differed clearly (Figure 1A). A subset of patients (n=30, Figure 1B) was identified through PCA and OPLS-DA which differed significantly from HC and the other patients on all HRV-variables. This subgroup had higher scores for Diarrhoea ($P<.05$), Loose stools ($P=.03$) and Urgency ($P=.01$), but had no significant difference in Anxiety or Depression.

Conclusion: Patients with IBS and HC differ on many single HRV variables and partly on a multivariate level, suggesting lower cardiac vagal activity in patients. An ANS profile differing significantly from HC is associated with diarrhoea-related symptoms.

Policy of full disclosure: None.

94 | IBS brain signature: Cerebral microstructure in Irritable Bowel Syndrome (IBS)

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Objective/Introduction: Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder associated with abdominal pain and alternated bowel habits. This is linked to dysfunctional brain-gut communication. Therefore, attention has been directed towards the brain-gut axis role, with multiple neuroimaging studies focusing on cerebral differences between healthy controls (HC) and IBS patients.

Objective: We hypothesized that IBS patients have an altered cerebral microstructure.

Methods: 15 IBS patients and 15 HCs went through our magnetic resonance imaging protocol. With diffusion tensor imaging (DTI) it is possible to evaluate white matter microstructure in the brain through estimation of directional water transfusion (fractional anisotropy (FA)). Using FreeSurfer we segmented the brain, while calculation of FA and whole-brain tractography was performed with Diffusion Toolkit and TrackVis. Integrated analysis at voxel level was performed in MATLAB.

Results: Pooled FA values across all 73 white matter subregions show median value of 0.375 for HCs and 0.370 for IBS, which is highly significant ($P<2.2\times10^{-16}$). IBS patients had significantly lower insular FA values both in the left ($P<2.2\times10^{-16}$) and right ($P<2.2\times10^{-16}$) hemisphere. Permutation testing (1000) confirm our highly significant results. Bilaterally in the middle temporal lobe and isthmus part of cingulate gyrus IBS patients had a reduction in FA values. In subregions of the cingulate gyrus FA values were typically bimodal distributed with less consistent group results than in previously mentioned regions.

Conclusions: Our highly significant results indicate that IBS patients have altered microstructure in insula. As we know that insula is a key region for interoception, this finding may either reflect an underlying difference contributing to symptom development, or is a result of abdominal symptoms over time. The FA changes observed in white matter around the insular regions pose the important question of this measures role in IBS pathophysiology and etiology.

Policy of full disclosure: None.

95 | IBS brain signature: Cortical thickness in the salience network

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Objective: Introduction: A core network in the irritable bowel syndrome (IBS) brain is the salience network, which responds to subjective salience of stimulus of the expectation of stimulus. Over time chronic pain might inflict morphological changes, such as altered cortical thickness.

Objective: We aimed to distinguish between IBS patients and healthy controls (HC) using a standardized classification machine-learning framework based on cortical thickness data from the salience network.

Methods: 15 IBS patients and 15 HCs underwent two successive 3D T1-weighted cerebral magnetic resonance imaging acquisitions. Using Freesurfer segmentation we obtained mean values for cortical thickness, which we analyzed in R with Neural Network and Random Forest. These repeated cross-validation classification methods enable prediction of IBS vs HCs.

Results: Neural Network classification gave mean classification accuracy of 0.69 (SD 0.13), mean sensitivity 0.70 (SD 0.16), and mean specificity 0.69 (SD 0.16). Random Forest classification gave a mean classification accuracy 0.67 (SD 0.11), mean sensitivity 0.62 (SD 0.17), and mean specificity 0.71 (SD 0.15). There were no regions in either hemisphere who could be singled out as most important for discrimination. However, with logistic regression the left rostral middle frontal region (P=.017), right insula (P=.029) and left insula (P=.005) obtained significant scores.

Conclusions: There seem to be a structural difference within the salience network between IBS patients and HCs in terms of cortical thickness patterns. This could be several minor non-significant alterations in cortical thickness which structurally reflects altered interoceptive function in IBS patients. The left rostral middle frontal region and insula bilaterally seem to be important regions in the IBS brain. Thus, we show that chronic syndromes might influence whole-brain structure in such ways that researchers are required to observe the brain at both regional and network level. The cause of such alterations might be prolonged abdominal discomfort, or it might be an innate component in the IBS brain which predispose for this gastrointestinal disorder.

Policy of full disclosure: None.

96 | A functional SNP of the serotonin transporter gene promoter is associated with IBS

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The serotonin transporter gene SLC6A4 has been implicated in irritable bowel syndrome (IBS) pathophysiology. We performed sequencing analysis of an alternative promoter claimed to primarily drive expression in the gut in a discovery sample from the UK. Several single nucleotide polymorphisms (SNPs) were strongly linked to each other and associated with IBS. All SNPs constituted two main haplotypes and the tagging SNP rs2020938 associated with female IBS-C (C-constipation). For validation, we genotyped rs2020938 in more than 2000 IBS patients and 8000 controls in eleven cohorts from eight countries. Meta-analysis of the data revealed replication of the initial finding. Gene reporter assays showed increased expression levels for the major haplotype. Comparative expression analysis in different intestinal regions confirmed the alternative promoter to drive the expression within the small intestine. Further follow up studies in tissue samples of IBS patients and controls revealed that the major allele of rs2020938 correlates with changed expression levels in the jejunum and corroborates a functional impact of the SNP on SERT expression. In conclusion, we confirmed a novel functionally relevant promoter SNP to be associated with female IBS-C underlining the relevance of SERT in IBS pathogenesis.

Policy of full disclosure: None.

97 | Comparative expression profiling in rectal biopsies of giardia-induced post-infectious IBS: A pilot study

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To date, evidence accumulated pointing to differential microRNA (miRNA) mediated gene expression regulation in irritable bowel syndrome (IBS). Complementing analysis of miRNA target genes revealed differential expression of genes relevant for immune and mast cell signalling as well as intestinal barrier function. In order to further enlighten the role of differential miRNA and target gene expression in IBS, we performed comparative expression profiling of miRNAs in microdissected gut biopsies from Giardia lamblia post-infectious IBS patients (PI-IBS) vs healthy controls (HC) in an extraordinary entity of patients from Bergen in Norway. miRNA profiling on rectal biopsy samples revealed several miRNA expression changes in the epithelial layer of PI-IBS samples. Biological functions related to differentially expressed miRNAs included gastrointestinal disease, inflammatory response and immunological disease. Validation was performed by nCounter miRGE code set where the expression of the top 5 candidate miRNAs and 100 potential mRNA targets was assessed in both, the epithelium layer and lamina propria. De-regulation of the selected miRNAs could not be verified in a larger sample set; however, target genes involved in immunology (Tryptase, TGFB1, ZEB2, NR3C1), barrier function (HNF4A, CLDN3, OCLN, MUC1-2) and epigenetic modulation (HDAC1, DNMT1-3A) were differentially expressed in both the epithelial layer and lamina propria of PI-IBS samples. So far, upregulation of tryptase and downregulation of MUC2 in PI-IBS samples could be validated at the protein level. In conclusion, genes relevant to immune and barrier function as well as stress response and epigenetic modulation are differentially expressed in PI-IBS and presumably contribute to the manifestation of the disease.
Policy of full disclosure: None.

98 | Serum proteomics in African American females with IBS: A pilot investigation

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Objective: Sex and subtype differences within patients with irritable bowel syndrome (IBS) complicate the understanding of disorder pathogenesis, and hinder the design of efficacious, therapeutic interventions. Furthermore, the influence of race on IBS symptomatology has not been fully explored nor explained. The purpose of this pilot investigation was to harness the power of shotgun proteomic analysis, to identify circulating proteins that differentiate African American female IBS patients from healthy controls (HC), and gain biological insight on symptomatology.

Methods: Serum proteome analysis was performed upon a cohort of African American overweight, IBS-Constipation predominant-subtyped females (n=5) and HC (n=5), matched on age, sex, years of education, BMI and 11 physiological markers. Tandem Mass Tags (TMT) for multiplexed proteomic analysis was performed, incorporating reverse-phase liquid chromatography (RPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: Participants with IBS did not differ from HC in demographic or clinical characteristics, and initial proteomic analysis of 10 samples did not detect significant differences in protein expression between groups. Nested case control analysis of six samples (IBS: n=3, HC: n=3), hierarchically clustered into two main groups, with 12 out of 1317 proteins found to significantly differ in levels of expression: TGFB1, PF4V1, PF4, APP, MMP9, PPBP, CTGF, SRGN, THBS1, WRN, LTBP1 (isoform 3) and IGLV5-48 (false discovery rate of 0.05, fold cutoff of 2, $P<.05$). Identified proteins were uploaded into DAVID and STRING resources, with top associations (upregulated in HC vs IBS) involving platelet alpha granule lumen, platelet activation/degranulation, extracellular region and secretion by cell.

Conclusions: The predominance of differentially expressed proteins between African American IBS participants and HC involving platelet related associations prompts inquiry as to differences in serotonergic signaling, inflammatory or immunomodulatory mechanisms underlying IBS symptomatology. Although these findings are preliminary and require validation in larger cohorts, they bear relevance to understanding pathogenic processes of IBS, contribute to the dialogue on racial differences in IBS symptomatology, and warrant further exploration.
Policy of full disclosure: None.

99 | miRNA-16 and miR-103 impact 5-HT4 receptor signalling and correlate with symptom profile in Irritable Bowel Syndrome (IBS)

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Irritable bowel syndrome (IBS) is a gut-brain disorder characterized by alterations in intestinal sensitivity and motility. 5-HT4 receptors regulate motor function and represent beneficial therapeutic targets in the treatments of IBS with constipation. We hypothesized that disturbed 5-HT4 receptor regulation or functional defects in the HTR4 gene may be involved in the motor dysfunction seen in IBS patients. We have identified the single nucleotide polymorphism (SNP) c.*61T>C within HTR4 to be significantly enriched in IBS-D patients (D-diarrhoea). The SNP is located in the 3′ untranslated region (3′UTR) and affects a miRNA binding site (miR-16 family, miR-103/miR-107) within the isoforms HTR4b/i. This leads to a compromised fine-tuning of miRNAs and their target HTR4 resulting in a downregulation of HTR4b/i. Furthermore, we could identify a novel isoform of HTR4b with an alternatively spliced 3′UTR, which escapes miRNA regulation when the SNP is present, suggesting a significant increase of 5-HT4 receptor expression in SNP carriers. Complementary to this, miR-16 and miR-103 were found to be significantly downregulated in jejunal biopsies of IBS-D patients compared to controls and to correlate with bowel movements and stool consistency. In conclusion, we have shown that HTR4 expression is regulated by miRNAs either by the presence of the SNP or by diminished levels of miR-16 and miR-103, thereby making people susceptible to develop IBS-D.

Policy of full disclosure: None.

100 | IBS subgroups based on combination of GI and non-GI symptoms in a general population study

A. Polster[1]; H. Törnblom[2]; O. Palsson[3]; W. Whitehead[4]; M. Simren[2]

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Objective: Annikka Polster1, Olafur S Palsson2, Hans Tornblöm1, William E Whitehead2, Magnus Simrén1,2 1. Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. 2. Center for Functional Gastrointestinal and Motility Disorders, University of North Carolina, Chapel Hill, North Carolina, United States. Background/Aim IBS is a heterogeneous disorder. In a previous study(1) our group identified subgroups in IBS outpatients, characterized by predominant GI symptoms and the presence or absence of additional extraintestinal somatic and psychological symptoms. We aimed to replicate these subgroups and symptom associations in participants fulfilling IBS diagnostic criteria in a general population survey.

Methods: An internet-based health survey was completed by general population adults from the US, Canada, and UK. The respondents fulfilling IBS diagnosis (Rome IV and III) were analyzed for latent subgroups using Mixture Model analysis. Symptom measures were derived from validated questionnaires: IBS related GI symptoms (Rome IV), extraintestinal somatic symptoms (PHQ-12) and psychological symptoms (SF-8).

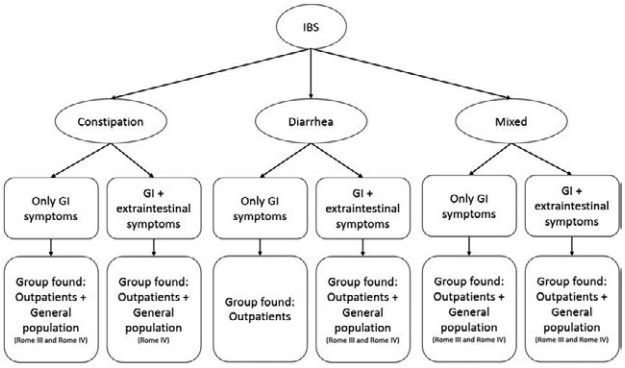

Figure 1: Schematic representation of identified groups and group characteristics in the previous (IBS outpatients) and present study (Rome III and Rome IV IBS from the general population)

Results: 341 respondents fulfilled Rome IV diagnostic criteria for IBS (average age 44, range 18-77, 64% female) and 637 Rome III diagnostic criteria (average age 46 years, range 18-87, 66% females). The Rome IV set showed five latent subgroups characterized by: (I) constipation with mild/no comorbid non-GI symptoms, (II) constipation with high severity of comorbid non-GI symptoms, (III) diarrhea and abdominal pain with moderate comorbid non-GI symptoms, (IV) mix of GI symptoms with moderate comorbid non-GI symptoms, (V) a mix of GI and non-GI symptoms of overall mild severity. The Rome III set showed four latent subgroups: (I) constipation with mild/no non-GI comorbidities, (II) diarrhea and pain with moderate non-GI comorbidities, (III) mixed GI symptoms with high non-GI comorbidities, (IV) a mix of GI and non-GI symptoms with overall mild severity.

Conclusion: We were able to replicate all but one of the subgroups previously demonstrated in clinical IBS patients in subjects with Rome IV IBS from the general population (Figure 1). The Rome III patients also showed similar but less distinct grouping and associations, likely reflecting less stringent diagnostic criteria. The distinct associations of symptoms present in these subgroups may be of importance for individual treatment decisions and future pathophysiological studies. (1) Polster et al. 2017.

Policy of full disclosure: None.

101 | Imaging mast cells by confocal microscopy without fluorescent staining for the diagnosis of Irritable Bowel Syndrome (IBS)

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Objective: Background and aims: Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal (GI) disorders and is diagnosed based on subjective symptoms because no specific disease markers have yet been found. Recent research has shown that IBS is related to intestinal mast cells located in the lamina propria and

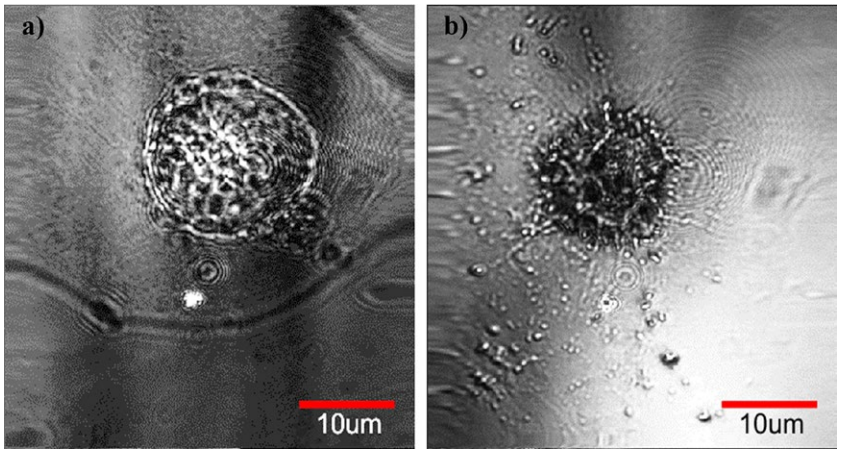

FIGURE 1 Mast cells imaging with with both scattering and reflecting signal (a), and with interferometric scattering signal (b)

submucosal layers of the GI tract. These mast cells might be visualized by confocal laser endoscopy (CLE) where there is a need of injecting a fluorescent dye with potential adverse effects and also limitation of observation time. We postulated mast cell imaging could be achieved by confocal microscopy without fluorescent dye. The aim of this study was to visualize mast cells using two different modes of confocal microscopy without fluorescent staining.

Methods: Mast cell line of MC/9 (ATCC® CRL¬8306™) was purchased and utilized for the experiments. One group of mast cells were not stained with any dye but the other group stained with DAPI for confirmation. In these groups, we investigated two different modes of confocal microscopy, auto-fluorescence and reflection modes, for imaging mast cells without staining.

Results: Reflection confocal mode allowed us high spatial resolution and image contrast for observing mast cells. In reflection mode where confocal reflection microscopy (CRM) with the laser wavelength of 532 nm (pinhole diameter: 25 μm) was used, we could detect both scattering and reflecting signal from the mast cells, and interferometric scattering signal provided high image contrast (Figure 1). In auto-fluorescence mode, no signal was detected with excitation sources in visible-wavelengths.

Conclusions: These results indicate that mast cell can be visualized by confocal microscopy without staining. After incorporating these results with CLE, we might improve an objective diagnosis of IBS.

Policy of full disclosure: None.

102 | Global metabolite profiling of multiple sample types to identify markers for Irritable Bowel Syndrome (IBS)

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Objective: Irritable Bowel Syndrome (IBS) is a functional gastrointestinal (GI) disorder characterised by chronic or recurrent abdominal discomfort mostly associated with changes in GI habit in the absence of a detectable organic cause. The aetiology and pathogenesis of IBS is poorly understood. To date, plasma samples have been used to identify metabolites that can differentiate IBS phenotypes from healthy controls using a small cohort size only. Recent advancement in the analysis of exhaled breath offers a promising tool to identify potential biomarkers of host and microbial interactions occurring during IBS. Larger cohorts are needed to increase statistical power to detect biomarkers in various biological samples. Furthermore a combination of analysis types is required to maximise coverage of the metabolome.

Objective: In a case-control study, we aimed to identify microbial and host factors that provide mechanistic insights into functional IBS and increase the predictability of phenotypes for use in nutrition intervention studies.

Methods: Individuals undergoing a colonoscopy were recruited. Diet diaries, questionnaires and biological samples were collected. Exhaled breath from each individual was trapped on graphitised carbon tubes and analysed using thermal desorption-gas chromatography mass spectrometry. Metabolomics analyses of plasma samples from the same cohort were carried out using biphasic extraction and liquid chromatography mass spectrometry to monitor both polar and non-polar plasma metabolites.

Results: Among the individuals recruited, participants were classified as IBS, control, or with a mixed phenotype. The breath analysis detected alcohols, ketones, aldehydes and alkanes. Multivariate analysis of the breathograms using partial least squares discriminant analysis revealed clustering by the classification, with the mixed phenotype individuals displaying an intermediate grouping that overlapped with control and IBS subjects. These results will be correlated with those obtained from plasma samples.

Conclusions: Profiling of the metabolomes of various body samples has the potential to identify biomarkers relevant to host and microbe interactions underlying IBS.

Policy of full disclosure: None.

103 | Single nucleotide polymorphisms in the control region of mitochondrial genome are associated with Irritable Bowel Syndrome: A preliminary study

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Objective: Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal diseases (FGIDs), however its pathogenesis remains unclear. It has been noted that FGIDs may be related with single nucleotide polymorphisms (SNPs) of mitochondrial genome [1,2]. The D-loop, as the control region, is found to be the most polymorphic region of the human mitochondrial genome. We aimed to explore whether SNPs of the D-loop are associated with IBS.

Methods: 40 participants were recruited and classified into 3 groups: 20 cases fulfilled with Rome III criteria for IBS with diarrhea (IBS-D), 10 cases with IBS with constipation (IBS-C) and 10 healthy volunteers acting as controls. DNA was extracted from biopsy specimens of the colon during routine colonoscopy. The mitochondrial D-loop were sequenced and variants were identified in comparison with the reference sequence from Genbank (NC-012920). We searched on Genbank and MitoMap (www.mitomap.org) to determine if a variant can be considered as an SNP.

Results: No significant differences were found regarding sex, age and BMI between IBS and controls. The average number of SNPs is 12.2±2.7, 9.8±1.8 and 9.9±2.1 in IBS-D group, IBS-C group and controls, respectively. The frequency of SNPs in IBS-D group is significantly higher than both IBS-C group and the control group (Kruskal-Wallis test, P=.01). However, no significant difference was found between the latter two groups. Each SNP was compared among three groups and the frequency of 199C was found significantly higher in the control group compared with IBS-D group (chi-square test, P=.03), but no significant difference was found between IBS-C group and controls.

Conclusions: Patients with IBS-D have a higher incidence of polymorphisms in the D-loop than healthy controls, which suggests that SNPs of the control region may play a role in the pathogenesis of IBS-D. Meanwhile, genotype 199C may be associated with lower risk of IBS-D, not IBS-C. Further studies involving larger samples are needed.

Policy of full disclosure: None.

References:

1. Camilleri M, et al. 2009, PMID:19147801

2. van Tilburg MA, et al. 2014,PMID:24500451.

104 | Identification of an analgesic lipopeptide produced by the probiotic Escherichia coli strain Nissle 1917: Role in visceral hypersensitivity

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Among the probiotic bacteria tested in the treatment of Irritable Bowel Syndrome (IBS), *Escherichia coli* Nissle 1917 (EcN) decreases the visceral pain associated with IBS. The mutation of clbA gene encoding an enzyme PPTase (EcN ΔclbA) inhibits the probiotic effect of EcN in animal colitis. The aim of our study was to characterize the lipid molecules produced by wild-type and mutated probiotic bacteria EcN in order to assess their effect on visceral hypersensitivity. Lipids from EcN and EcN ΔclbA bacteria have been extracted and analyzed by liquid chromatography coupled to mass spectrometry. Their production by chemical synthesis allowed us to determine their ability to cross $CaCo_2$ cells monolayer in vitro and the colonic wall in vivo. Their roles on calcium mobilization in primary culture of sensory neurons from mouse dorsal root ganglia (DRG) have been tested in basal condition and stimulated by TRPV1 agonist: capsaicin or by an agonist mix of G-protein coupled receptors (bradykinin, histamine, and serotonin. Ex vivo, the effect of bacterial lipid compounds on intestinal contractility has been evaluated using mice duodenal fragment. Finally, the role of bacterial lipids on capsaicin-induced visceral hypersensitivity has been assessed by colorectal distension in mice. The comparison of lipid compounds of EcN and EcN ΔclbA by mass spectrometry has allowed the characterization of 31 lipopeptides produced by probiotic bacteria under the dependence of clbA and in particular the C12-Asn-GABA. This lipopeptide was able to cross the intestinal epithelial barrier in vitro and in vivo. Moreover, this lipopeptide did not induce intracellular calcium flux in primary culture of sensory neurons. However, the C12-Asn-GABA (0, 1-10 µmol/L) decreased in dose dependent manner the calcium flux induced by the two types of nociceptor. This effect was inhibited by saclofen (100 µmol/L) an antagonist of GABA B receptor. Furthermore, the C12-Asn-GABA (10 µmol/L) inhibited capsaicin-induced visceral hypersensitivity without altering intestinal contraction. In conclusion, this study shows that probiotic bacteria produce lipopeptides able to cross the intestinal barrier and to inhibit visceral hypersensitivity induced by nociceptor activation. The C12-Asn-GABA could represent a new therapeutic tool against visceral pain.

Policy of full disclosure: None.

CHALLENGES IN SEVERE DIGESTIVE DISORDERS

105 | Prostacyclin reverses colitis through the down regulation of intestinal epithelial permeability

C. Pochard[1]; P. Aubert[1]; C. Gesret[1]; J. Bregeon[1]; N. Cenac[2]; A. Bourreille[1]; G. Meurette[1]; M. Neunlist[1]; M. Rolli Derkinderen[1]

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Objective: In inflammatory bowel disease (IBD) both intestinal epithelial barrier (IEB) permeability and PTGIS expression are altered. Nevertheless the role of the lipid mediator PGI2 produced by PTGIS in IEB regulation is unknown. The present study concerns the control of IEB permeability by PGI2 and its involvement in the development of colitis.

Method: PGI2 production from control or IBD biopsies was established using high sensitivity liquid chromatography tandem mass

spectrometry. Consequences of Flolan PGI2 analog supplementation were evaluated in a DSS-induced mice model of colitis, measuring disease activity index (DAI), inflammation (pro-inflammatory cytokine mRNA) and IEB permeability (sulfonic acid flux). Molecular mechanisms involved were assessed by quantification of junctional and pro-proliferative vs pro-apoptotic protein expression (western blot and immunostaining). Eventually PGI2 impact on reversing IEB breakdown was assessed ex vivo measuring permeability of mice or human mucosal explants treated with staurosporine apopstosis inducer, or permeability of IBD biopsies both treated or not with Flolan.

Results: Biopsies from IBD patients had lower PGI2 production compared to control patients, and addition of Flolan reduced their permeability. In vivo PGI2 supplementation significantly reduced DAI, and inflammation (IL-1β and IL-6_mRNA) as well as reduced IEB permeability. DSS-induced cleavage of Caspase 3 is normalized by Flolan. Ex vivo, staurosporine-induced permeability of mice or human mucosal explants is entirely inhibited by Flolan.

Conclusions: This study not only presents a role of PGI2 in controlling IEB permeability through the regulation of apoptosis mechanisms, but also reveals that increased permeability in IBD patients can be fixed by PGI2 supplementation.

Policy of full disclosure: None.

106 | Dynamics of liver dysfunction in children with hereditary tyrosineemia type 1 on the background of pathogenetic therapy

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Objective/Background: Hereditary tyrosinemia type I (HTI) is a genetically determined disease in which the disintegration of tyrosine is carried out along an alternative pathological pathway with the formation of highly toxic and carcinogenic products, which leads to damage to the liver, kidneys, peripheral nerves. Purpose based on a multifactorial statistical analysis of clinical and diagnostic indicators and their changes assess the dynamics of changes in the severity of the structure and function of the liver on the background of specific therapy.

Patients and methods: 17 children (8 boys and 9 girls) with type I tyrosinemia: 5 patients (29.4%) with type IA tyrosinemia and 12 patients (70.6%) with type I tyrosinemia. To assess the degree of liver function abnormality, a biochemical blood test was performed: the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), de Rytis coefficient, bilirubin, glucose, albumin, cholesterol, lactate, ammonia, urea, transferrin, ceruloplasmin, Prothrombin by Kviku, fibrinogen, gamma-glutamyltranspeptidase (GGTP), alkaline phosphatase (AFP).

Results: The liver function was reduced by 34.1±11.3% with a range of fluctuations from 12.0% to 48.0%, and in 12 children the degree of its reduction was moderate, in 2 patients it was insignificant. After 6 months of therapy, the liver function improved significantly (P=.026) and was reduced by (26.1±6.9)% with a range of fluctuations from 11.0% to 39.0%; In 10 patients the degree of its reduction was moderate, in 4 patients it was insignificant.

Conclusion: On the basis of multifactorial statistical analysis, the effectiveness of pathogenetic therapy of genetic disease—hereditary type I tyrosinemia—has been proved.

Policy of full disclosure: None.

107 | Influence of nitizinon on the performance of mineralexchange in children with type 1 tyrosinemia

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Objective: The aim of the study was to evaluate the effect of nitizinon on the level of cal- Phosphorus and alkaline phosphatase in children with tyrosinemia 1 Type.

Scope and methods of research: Under supervision were 13 Children (6 boys and 7 girls) with type 1 tyrosinemia: 5 patients (38.5%) with type 1a and 8 patients (61.5%) with 1b type. All children Received pathogenetic therapy with nitizinone. In dynamics On the background of a six-month treatment, the level Calcium, phosphorus and alkaline phosphatase in biochemical analogues. Lys of blood.

Results: Against the background of the use of nitizinon for 6 months in Biochemical blood tests there was a decrease in the level of alkaline phosphatase (Before treatment (778.8±408.6) units/L, after 6 months of therapy (427.8±277.3) units/L, P=.016), serum calcium levels due Changed insignificantly (before treatment (2.4±0.2) mmol/L, after 6 months, (2.5±0.2) mmol/L, P=.520), but there was an increase The level of phosphorus in the blood serum (before treatment (1.2±0.6) mmol/L, After 6 months of therapy (1.8±0.2) mmol/L; P=.021).

Conclusions: The use of nitinosin in the treatment of tyrosinemia 1 Type in children leads to improved mineral metabolism, increased The level of phosphorus in the blood serum and decreasing the level of Lactic phosphatase, which can positively influence the development of of rickets in this disease.

Policy of full disclosure: None.

108 | Peripheral and central nervous system defects are associated with altered neuronal projections and early neonatal lethality in a mouse model of Goldberg-Shprintzen megacolon syndrome

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Objective: Background and Objectives: Goldberg-Shprintzen megacolon syndrome (GOSHS) is a rare congenital disorder that is characterised by severe intellectual disability, craniofacial dysmorphisms, microcephaly, and Hirschsprung disease. GOSHS is caused by mutations in the gene encoding Kinesin Binding Protein (KBP; KIA1279). Studies of kbp mutant zebrafish have shown a role for kbp in axonal outgrowth and maintenance. The objectives of this study were to develop a mouse model of GOSHS, and to examine the role of KBP in the development of the extrinsic and intrinsic innervation of the gut, and the brain.

Methods: Kbp knockout mice were generated by CRISPR/Cas9 genome editing, using guide-RNA probes to target exon 1 of murine Kiaa1279. The phenotype of mutant mice was examined using immunohistochemistry.

Results: Although GOSHS patients have Hirschsprung disease, neurons were present along the entire large intestine of P0 Kbp−/− mice. However, we found a significant delay in enteric neural crest cell migration in E12.5 Kbp−/− embryos. The projections of vagal fibres into the stomach were significantly reduced in Kbp−/− mice, as was the vagal innervation to the pancreas and lungs. The development of the sympathetic innervation of the intestine was also delayed in Kbp−/− mice. Newborn Kbp−/− mice were cyanotic, had breathing difficulties and died within several hours of birth. There were no obvious defects in the development of the phrenic nerve projections to the diaphragm or in the respiratory centres in the brainstem of P0 Kbp−/− mice, however some of the white matter tracts in the brain were reduced in size. The olfactory bulbs were also significantly smaller in Kbp−/− mice.

Conclusions: Defects in the vagal innervation to the stomach, pancreas and lungs, as well as defects in white matter tracts in the brain indicates an important role for KBP in axonal projection in the peripheral and central nervous system and etiology of GOSHS.

Policy of full disclosure: None.

109 | Inflammatory and oxidative impairment of antral motility in obese patients

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Background and aim: Obesity is related to oxidative stress and chronic low-grade systemic inflammation. Oxidative stress activates inflammasomes NLRP3 component with parallel secretion of interleukin-1β (IL-1β). In addition a reduction of endothelial nitric oxide synthase (eNOS) has been reported in obese, human tissues. eNOS is a key molecule in the transduction signaling of human antral gastric relaxation in response to Vasoactive intestinal peptide (VIP). The aim of this study was to investigate the inflammatory-oxidative induced damage on human obese gastric antrum.

Methods: Muscle strips and smooth muscle cells (SMC) were isolated from gastric antrum from 31 obese (OB) (40.9<BMI<52.0 Kg/m^2) and 11 normal weight (NW) (19.0<BMI<25.0 Kg/m^2) subjects. Antioxidant capacity and IL-1β were measured by ELISA and the expression of inflammasome and mRNA transcript for NLRP3, caspase-1, pycard, IL-1β and eNOS by qPCR, and data normalized to β-actin mRNA of Relative Quantification (RQ) vs NW. VIP effects were tested on muscle preparations. The effect of NADPH inhibitor apocynin (APO) (60 mM) in reverting oxidative stress was evaluated. Data are expressed as mean±SE.

Results: In respect to NW, OB SMC presented a statistically significant inhibition of antioxidant capacity by 4000%±0.46 and a stronger increase of mRNA encoding for NLRP3 inflammasome (4.66±1.18) and co-molecules caspase-1 (6.77±2.66), pycard (3.33±1.11) and IL-1β transcript (1.72±0.44), associated to IL-1β secretion (pg/mL: 9.09±2.17) in OB SMC. This oxidative-inflammatory status causes in OB SMC a stronger reduction of transcript for eNOS (0.10±0.05) and an impairment in VIP-induced relaxation, that resulted inhibit vs NW by 57.15% in strips and 81.2% in SMC. APO treatment of OB gastric SMC restored by 84.71%±0.2 antioxidant capacity and reduced IL-1β secretion (pg/mL: 3.78±0.5). Associated with inhibition of the oxidative-inflammatory status, APO completely restored the transcript for eNOS (4.82±0.06) and VIP-induced relaxations in obese SMC by 100%±0.24.

Conclusion: These results demonstrate that in obese the presence of myogenic pro-oxidative status via the activation of NLRP3 and loss of eNOS expression causes a reduced relaxation of human gastric antrum.

Policy of full disclosure: None.

110 | Correlation between symptoms, quality of life and gastric empting among dyspectic patients

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Objective/Introduction: Correlation between symptoms, quality of life and gastric emptying (GE) is discrepant throughout the literature. The aim of the study was to investigate whether GE is associated or not with symptoms and quality of life in a cohort of dyspeptic patients.

Patients and Methods: Two hundred patients with dyspepsia were studied prospectively. Gastrointestinal symptoms were assessed using a Likert scale of 5 points, and by a symptomatic composite score (TSS) and the quality of life measured by the GIQLI score. GE half time (T1/2) was measured by 13C octanoate breath test, for more than 6 hours. Association between symptoms, quality of life and T1/2 was analyzed according to established standards in healthy volunteers from the literature (Dig Dis Sci 47; 1657-), eg, T1/2 higher to 166 minutes or not. Association between symptoms, quality of life and T1/2 in patients was also assessed in patients with severe delay of GE, define as T1/2 above 200 minutes (75th percentile).

Results: In our cohort, 91 patients (45%) had T1/2>166 minutes. There was no difference in symptoms, TSS and GIQLI between patients with T1/2>166 minutes and T1/2<166 minutes. Patients with severe delay of GE (eg, T1/2>200 minutes) had increased gastric fullness (*P*=.02), epigastric pain (*P*=.05), bloating (*P*=.01), nausea (*P*=.02), early satiety (*P*=.01) and TSS (*P*=.005) compared to patients with T1/2<200 minutes. GIQLI score was decreased in patients with severe delay of GE compared to patients with T1/2<200 minutes. T1/2 correlated with symptoms and quality of life only in patients with T1/2>200 minutes, while such correlation was not observed for patients with T1/2≤200 minutes. Finally, association between symptoms, quality of life and T1/2 was no longer observed in patients with T1/2>200 minutes if the calculation of T1/2 was limited on the first 4 hours samples.

Conclusion: Our study demonstrates that there is no association between symptoms, quality of life and GE measured with breath test in an overall dyspeptic patients population. In patients with severe delay GE however, GE remains associated with symptoms, quality of life, but this requires an 8 hours measurement.

Policy of full disclosure: None.

111 | The effect of intraperitoneal placenta-derived mesenchymal stem cell injection in stomachs of diabetic mice

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Objective: Diabetic gastropathy is caused by loss of Interstitial cells of Cajal (ICCs) and destruction of neural plexus. Effective management for diabetic gastropathy is still unavailable. This study was designed to confirm the pathogenetic changes in diabetic gastropathy and to examine the effect of treatment with placental derived mesenchymal stem cells (PDMSCs) in animal models.

Methods: Fourteen NOD/LtJ mice of 8 weeks were bled until week 30. Diabetes mellitus developed in 10 out of 14 mice which all survived with insulin. The mice were grouped into three groups: non-diabetic group (n=4), diabetic sham group (n=5), diabetic PDMSC group (n=5) all of which were treated with intraperitoneal PDMSCs injection at week 30. All mice were sacrificed at week 34 and the stomachs were examined by immunohistochemical stain with c-kit and neuronal nitric oxide synthase (nNOS) antibodies.

Results: The number of c-kit positive cells in stomach decreased significantly in the diabetic sham group compared with that in the non-diabetic group (21.2±6.7 vs 88.0±29.3, *P*=.006) but increased with PDMSC treatment (21.2±6.7 vs 64.0±15.1, *P*=.02). The positive rate of nNOS in neural plexus was also significantly lower in the diabetic sham group than in the non-diabetic group (22.3±18.5% vs 48.0±22.7%, *P*=.003) but increased with PDMSC treatment (22.3±18.5% vs 43.3±20.5%, *P*=.03).

Conclusions: ICC and neural plexus decreased in stomachs of mice with diabetes mellitus but were significantly repaired with intraperitoneal injection of PDMSC.

Policy of full disclosure: None.

STRESS AND FUNCTIONAL GASTROINTESTINAL DISORDERS

112 | Brain-gut miscommunication: Biopsychosocial predictors of quality of life in IBS

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Objective: The biopsychosocial model of IBS describes a number of predisposing, precipitating, and perpetuating factors, such as personality traits, affective status, altered autonomic, and/or immune response, which contribute to the onset and maintenance of symptoms and consequently to quality of life impairment. The aim of this study was to examine the impact of various psychological (personality traits and affect, daily mood, stress intensity, perceived symptom severity) and biological (calprotectin, cortisol and HRV) factors on the physical and mental components of quality of life in IBS patients.

Methods: A total of 46 IBS patients completed a set of questionnaires (Big Five Personality Inventory, State-Trait Anxiety Inventory, Beck Depression Inventory-II, Medical Outcome Study Short-Form 36) and kept a diary of their mood, daily stress, and symptoms over a period of 2 weeks. Patients' heart rate variability, serum cortisol, and fecal calprotectin levels were also measured.

Results: The results of regression analyses showed that depression (β=−.41*) and negative mood (β=−.32*) significantly predicted physical quality of life, while depression (β=−.43**) and positive mood (β=.33**) predicted mental quality of life. The model, which included calprotectin, cortisol, depression, positive and negative mood, and stress intensity, explained a total of 48% of variance of physical and 57% of variance of mental quality of life. Additional analyses showed that, even though they were not significant individual predictors in the total model, calprotectin and cortisol explained 9% of physical quality of life, over and above the psychological measures.

Conclusion: Our results confirm the role of negative affect, depression and negative mood in IBS quality of life impairment. In addition, they indicate that biological factors, calprotectin, and cortisol seem to play a role in the impairment of physical quality of life in IBS patients. Additionally, the role of positive mood as a protective factor for mental quality of life might be of significant importance for psychological interventions with IBS patients.

Policy of full disclosure: None.

113 | Gastroesophageal reflux disease after radiofrequency catheter ablation of atrial fibrillation

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Objective: Patients undergoing atrial fibrillation (AF) ablation (by pulmonary vein isolation) seem to have an increasing risk to develop gastroesophageal reflux disease (GERD) due to the proximal vicinity of esophagus with posterior left atrium and inflammation induced by radiofrequency lesions during the procedure. We prospectively studied the presence of GERD in patients who underwent paroxysmal AF ablation.

Methods: All patients were investigated both by a cardiologist and a gastroenterologist on the basis of patients' complaints. GERD diagnosis was clinically assessed by gastroenterologist before and at 3 months after ablation. In addition all patients underwent upper gastrointestinal endoscopy and transthoracic echocardiography. Therapy with proton pump inhibitors was interrupted within 8 weeks before ablation.

Results: Seventy five patients were included in 2 groups: 46 patients with AF ablation (study group) and 29 patients without AF ablation (control group). Patients with AF ablation were younger (57.76±7.66 years vs 67.81±8.52 years; *P*=.001), predominantly men (62.2% vs 33.3%; *P*=.030) and with higher body mass index (28.96±3.12 kg/m^2 vs 26.81±5.19 kg/m^2; *P*=.046). After ablation, symptomatic GERD was not more frequent in the study group (42.2% vs 61.9%; *P*=.220). On the contrary, esophagitis was more frequently (55.0% vs 43.8%; *P*=.050). In study and control group were 88.9% respectively 57.1% patients with sinus rhythm (*P*=.009), at 3 months after ablation, at the time of upper gastrointestinal endoscopy. There was no difference in left atrium area as marker of structural remodeling in patients with or without AF ablation, irrespective of GERD presence (26.51±5.31 cm^2 vs 26.47±5.33 cm^2, *P*=.766) and GERD with AF ablation (n=20) vs GERD without AF (n=16) ablation (25.34±5.06 vs 26.31±5.33; *P*=.585). On the contrary, pro-BNP was significantly different (344±180 vs 760±253 pg/mL, *P*=.001).

Conclusion: Gastroesophageal reflux disease seems to be not more frequently after atrial fibrillation ablation, if it is clinically assessed based on patient symptoms. However, esophagitis seems to be more frequent in these patients.

Policy of full disclosure: None.

114 | The prevalence and impact of overlapping Rome IV functional gastrointestinal disorders on somatisation, quality of life, and healthcare utilisation: Results from a three-country general population study

I. Aziz[1]; O. Palsson[2]; H. Tornblom[3]; A. Sperber[3]; W. Whitehead[2]; M. Simren[3]

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Objective: The population prevalence of Rome IV functional gastrointestinal disorders (FGIDs) and their cumulative effect on health impairment is unknown.

Design: An Internet-based health survey was completed by 5931 of 6300 general population adults from three English-speaking countries (2100 each from US, Canada, and UK). The survey included questions on demographics, medication, surgical history, somatisation, quality of life, doctor-diagnosed organic GI disease, and criteria for the Rome IV FGIDs. Comparisons were made between those with Rome IV FGIDs against non-GI and organic GI disease controls.

Results: The number of subjects having symptoms compatible with a FGID was 2083 (35%) compared to 3421 (57.7%) non-GI and 427 (7.2%) organic GI disease controls. The most frequently met diagnostic criteria for FGIDs was bowel disorders (n=1665, 28.1%), followed by gastroduodenal (n=627, 10.6%), anorectal (n=440, 7.4%), oesophageal (n=414, 7%), and gallbladder disorders (n=10, 0.2%). On average, the 2083 individuals who met FGID criteria qualified for 1.5 FGID diagnoses, and 742 of them (36%) qualified for FGID diagnoses in more than one anatomic region. The presence of FGIDs in multiple regions was associated with increasing somatisation, worse mental and physical quality of life, greater use of medical therapies, and a higher prevalence of abdominal surgeries (all *P*<.001, Table 1). Notably, individuals with FGIDs in multiple regions had worse somatisation and quality of life scores than organic GI disease controls.

Conclusion: Roughly a third of the general adult population fulfils diagnostic criteria for a Rome IV FGID. In a third of this subset multiple GI regions are involved and this overlap is associated with increased health impairment.

Policy of full disclosure: None.

Table 1: Impact of increasing numbers of organ regions with functional gastrointestinal disorders (FGIDs) on somatic symptoms, quality of life (QOL), GI related medication and abdominal surgery

	No FGID (healthy)	One FGID region	Two FGID regions	Three FGID regions	Four FGID regions	Organic GI-disease
Number of subjects	3421	1341	493	166	83	427
Mean number of somatic symptoms	2.8	4.6	5.6	6.3	7.3	4.7
Mean PHQ-12 score	3.3	6	7.5	9.1	10.9	6.3
Mean SF-8 MCS QOL	52.1	45.7	42.8	38.6	37.2	47.9
Mean SF-8 PCS QOL	51.9	47.4	44.1	40.2	38.6	43.8
GI-related medication (%)	35%	59%	74%	84%	93%	71%
Abdominal surgery (%)	19%	26%	31%	37%	54%	53%

The PHQ-12 instrument tool was used to calculate a) the number of sites reporting somatic symptoms and b) the overall severity of somatic symptoms (PHQ-12 score).

The SF8-QOL provides norm-based mental component score (MCS) and physical component score (PCS)

115 | The prevalence of Rome IV functional dyspepsia and its impact on health impairment: Results from a three-country general population study

I. Aziz[1]; O. Palsson[2]; H. Tornblom[3]; A. Sperber[4]; W. Whitehead[2]; M. Simren[3]

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Objective/Background: The population prevalence of Rome IV functional dyspepsia and its effect on health impairment is unknown. We used data from a population-based study to address this, and compared somatisation, quality of life, healthcare utilisation, and presence of overlapping irritable syndrome and functional heartburn in individuals fulfilling criteria for Rome IV functional dyspepsia against non-dyspeptic controls.

Methods: An internet-based health survey was completed by 6300 general population adults from three English-speaking countries (2100 each from United States, Canada, and United Kingdom). Quota-based sampling was used to ensure equal sex, age, and education distribution across the countries. The survey included questions on demographics, healthcare visits, medication use, criteria for the Rome IV functional dyspepsia as well as for irritable bowel syndrome and functional heartburn, the patient health questionnaire-12 somatisation measure, and the Short Form-8 quality of life questionnaire.

Results: Data was available for analysis from 5931 subjects (49.2% female; mean age 47.4 years, range 18-92). Overall, 551 (9.3%) of the population fulfilled criteria for Rome IV functional dyspepsia. This comprised 339 (61.5%) with postprandial distress syndrome-PDS, 97 (17.6%) with epigastric pain syndrome-EPS, and 115 (20.9%) with both. Subjects with functional dyspepsia had significantly greater health impairment than non-dyspeptic controls, with on average 73% seeking healthcare more than once yearly and 77% taking GI-relevant medication (ie, GI-specific, psychotropics, analgesia, or complementary medicine). Notably, those with overlapping Rome IV EPS and PDS had higher frequency of co-existing irritable bowel syndrome and functional heartburn, worse somatisation, and poorer mental and physical quality of life scores, compared to EPS- or PDS-alone (Table 1). Somatisation and quality of life scores were similar between EPS- and PDS- alone.

Conclusion: Almost 10% of the population fulfil criteria for Rome IV functional dyspepsia. Subjects with Rome IV functional dyspepsia, in particular those with overlapping PDS and EPS, have considerable health impairment. This is despite frequent healthcare visits and use of GI-relevant medication.

Policy of full disclosure: None.

Table 1: Comparison between functional dyspepsia subtypes

	Postprandial distress syndrome (PDS), n=339	Epigastric pain syndrome (EPS), n=92	Overlapping PDS & EPS, n=115	P-value
Demographics				
Mean-age (SD)	45 (16.8)	42.5 (15.6)	41.2 (14.4)	0.07
Female	211 (62.2%)	52 (53.6%)	68 (59.1%)	0.3
White Ethnicity	242 (71.4%)	70 (72.2%)	77 (67%)	0.6
Symptom scores				
PHQ-12 somatisation score (SD)	7.97 (4.0)	8.5 (4.3)	11.3 (4.7)	<0.001[a,b]
Number of somatic symptoms (SD)	5.8 (2.5)	6.1 (2.6)	7.5 (2.5)	<0.001[a,b]
Short form (SF)-8 quality of life, SD				
Physical component score (PCS)	43.8 (11.2)	40.9 (11.2)	37 (10.1)	<0.001[a,b]
Mental component score (MCS)	41.5 (12.8)	40.0 (13.3)	37.6 (11.3)	0.02[a]
Overlapping FGIDS				
Irritable bowel syndrome	50 (14.7%)	41 (42.3%)	83 (72.2%)	<0.001[a,b,c]
Functional Heartburn	21 (6.2%)	11 (11.3%)	35 (30.4%)	<0.001[a,b]

[a] indicates the overlap group is significantly different compared to PDS group
[b] indicates the overlap group is significantly different compared to EPS group
[c] indicates EPS group is significantly different compared to PDS group

116 | Cav3.2 calcium channels: Targets to relieve colonic hypersensitivity encountered in Irritable Bowel Syndrome (IBD)

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Objective: Chronic abdominal pain associated with colonic hypersensitivity (CHS) encountered in the irritable bowel syndrome (IBS) has a negative impact on the quality of life of patients. Current drug treatments are generally ineffective or are associated with strong adverse effects. Therefore, a therapeutic innovation is expected. T-type calcium channels, mainly the 3.2 isoform (Cav3.2), constitute good targets in the treatment of chronic pain. Thus, the aim of this study was to evaluate the consequence of Cav3.2 channels inhibition in a murine model of IBS.

Methods: Mice received water or 0.5% dextran sulfate sodium (DSS) during 12 days to induce IBS like symptoms. CHS was evaluated by colorectal distension, low grade inflammation by quantification of fecal lipocalin-2 with ELISA assay and spinal glial (microglia and astrocyte) activation by immunohistochemistry. The involvement of Cav3.2 channels was analyzed using genetic and pharmacological blockage strategies.

Results: The use of a total Cav3.2 knock-out (KO) in mice and also a pharmacological Cav3.2 blocker: TTA-A2, showed a high reduction of CHS, indicating Cav3.2 implication in CHS DSS-induced. To understand the Cav3.2 involvement on CHS, two strategies were used: conditional KO mice (specific on dorsal root ganglion, DRG) and a peripherally acting Cav3.2 blocker, ABT-639. Results indicated a reduction of HSC only in conditional KO mice, with no effect of ABT-639, suggesting a requirement of Cav3.2 channels localized on DRG to induce HSC in our IBS model. Chronic administration of ethosuximide (ETX), a T-type blocker commonly used in childhood absence epilepsy treatment, was also studied. ETX reduced both CHS, spinal glial activation and low grade inflammation induced by DSS.

Conclusion: These results demonstrated an analgesic effect of Cav3.2 channels inhibition with an implication of Cav3.2 located at DRG level. Therefore, the inhibition of these channels constitutes an attractive therapeutic strategy for the treatment of CHS encountered in IBS.

Policy of full disclosure: None.

117 | Microbiota-related changes in bile acid and serotonin metabolism are associated with gastrointestinal dysfunction in a mouse model of autism

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Autism spectrum disorder (ASD) is one of the most serious neurodevelopmental conditions, characterized by impaired social communication, compulsive behaviour, attention deficit and intellectual decline. There is growing awareness that ASD is comorbid with gastrointestinal dysfunction and altered intestinal microbiome, and that host-microbiome interactions may contribute to the disease symptoms. The BTBR T+ Itpr3tf/J mouse, a widely used animal model of ASD, exhibits a robust deficit in sociability. However, there is limited information on the gut-brain axis signalling in this model of ASD. To this end, we investigated gut physiology and intestinal microbiota composition in "unsocial" BTBR as compared to "social" C57BL/6 male mice. Here we show that BTBR mice display a substantial impairment of epithelial barrier function in the intestine ("leaky" gut) in conjunction with delayed intestinal transit. These symptoms were associated with a significant reduction in serotonin production, as well as a decrease in secondary bile acid pool in BTBR intestine. Synthesis of serotonin from dietary tryptophan and transformation of host bile are both controlled by intestinal microbiota. In agreement with metabolic data, BTBR microbiota was characterised by a dramatic decrease in the relative abundance of a few bacterial genera in the Clostridiales order, the latter known to activate serotonin synthesis in the intestine. Furthermore, we observed a reduction in the abundance of bile-metabolising bacterial taxa in BTBR gut. Together, these data provide novel plausible targets for microbiota-based interventions aiming to improve gastrointestinal and behavioural symptomatology in ASD.

Policy of full disclosure: None.

118 | Outcome of breath tests in adult patients with suspected small intestinal bacterial overgrowth

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Objective: Background Breath testing is used to detect small intestinal bacterial overgrowth (SIBO) by measuring hydrogen and methane produced by intestinal bacteria. Aims The aim was to investigate breath test outcomes in patients with suspected SIBO and indicative symptoms of SIBO, diagnosed by breath testing.

Methods: A retrospective cross sectional study carried out at Celiac Disease Center at Columbia University in New York of 311 patients undergoing breath testing.

Results: 50% had a positive lactulose breath result and 37% had a positive glucose breath result (*P*=.036). The most common symptom of the patients was bloating. In total 46% had a positive breath test: 18% positive for methane, 24% positive for hydrogen and 4% positive for both gases (*P*=.014). The only statistically significant predictor of positive glucose breath test was increased gas (*P*=.028).

Conclusion: Lactulose breath test was more often positive than glucose breath test. Positivity for hydrogen was more common than methane. Bloating was the most frequently perceived symptom. The only statistically significant symptom for a positive glucose breath test was increased gas.

Policy of full disclosure: None.

119 | Stress differentially alters the plasma and brain metabolomes and caecal microbiome in Wistar Kyoto and Sprague Dawley rats

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Objective: Stress negatively impacts the brain and gastrointestinal tract. Wistar Kyoto (WKY) rats demonstrate hyper-responsiveness to stress and are commonly used to study gastrointestinal disorders, while Sprague Dawley (SD) rats are routinely used to model 'normal' physiology. To date, there is limited knowledge of which metabolic and microbial signatures underpin physiological responses to stress in either WKY or SD rats.

Objective: To examine the effect of stress on plasma and brain metabolomes, and caecal microbiome composition, in WKY and SD rats.

Methods: Age-matched WKY and SD rats subjected to stress (Open Field, Novel Object Recognition and Forced Swim tests) were compared to WKY and SD non-stressed controls (n=12). Plasma and brain metabolome profiles were analysed by LC-MS. Caecal microbiome composition was analysed using 16S amplicon sequencing.

Results: Plasma and brain metabolomes were differentiated between stressed vs control, and WKY and SD rats. The concentration of plasma metabolites involved in neurotransmission pathways were decreased in response to stress for both WKY and SD rats, while plasma triacylgyceride levels increased; particularly in SD rats where most were upregulated by >30%. Free fatty acids levels (vaccenic, linoleic and palmitic) were also increased in stressed SD rats. Brain sphingomyelin levels were lower in stressed WKY rats (vs stressed SD rats), whereas glutamine, tyrosine, phosphocholine and carnitine levels were elevated. Stressed groups also had increased relative abundances of caecal Ruminococcus and Desulfovibrio, and decreased Akkermansia. Caecal Lactobacillus and Allobaculum proportions were also altered by stress.

Conclusions: Analysis of brain and plasma metabolomes, and caecal microbiomes, has provided new information regarding putative novel biomarkers of physiological responses to stress, some of which are host specific. This provides further evidence that physiological, behavioural, and microbiome responses are intimately linked.

Policy of full disclosure: None.

120 | The effect of glycoursodeoxycholic acid on epithelial integrity and bacterial uptake in duodenal biopsies of patients with functional dyspepsia and healthy volunteers

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Objective: Functional dyspepsia (FD) is an extremely common disorder of gastrointestinal function. Impaired duodenal mucosal integrity was reported as a potential pathophysiological mechanism in FD. Recently, our group showed that duodenal primary bile salt (BS) concentrations were decreased in FD patients compared with healthy volunteers (HV), that the secondary BS glycoursodeoxycholic acid (GUDCA) correlated positively with duodenal permeability in HV and that the ratio of primary/secondary bile salts correlated negatively with duodenal mucosal permeability in FD (unpublished study). In this study, we hypothesized that the GUDCA-induced impairment of duodenal integrity allows duodenal bacterial uptake in FD patients and HV.
Methods: Duodenal biopsies were obtained from five FD patients (1 man, 35±18 years) and five HV (1 man, 36±16 years). Biopsies were mounted in Ussing chambers for 120 minutes to measure transepithelial resistance (TEER) and bacterial uptake using fluorescein labelled chemically killed *E. coli* (K12-strain) and were exposed to GUDCA (1, 2 or 5 mM; pH 7.8±0.3) or to a standard Krebs-Ringer bicarbonate buffer.
Results: In standard conditions, TEER values from biopsies of FD patients did not differ with those of HV with the current sample size (21.4±5.5 Ω cm^2 and 27.4±8.1 Ω cm^2 respectively; P=.1054). In HV, there was a significant difference in TEER after GUDCA exposure (21.8±6.5 Ω cm^2) compared to control (27.4±8.1 Ω cm^2; P=.0042). However, TEER values of biopsies of FD patients with (21.8±3.7 Ω cm^2) and without (21.4±5.5 Ω cm^2) GUDCA exposure didn't differ (P=.8678). In FD patients (n=3), bacterial uptake occurred on average in 9% of biopsies without GUDCA exposure and in 55% of biopsies with GUDCA exposure (1mM; P=.0153). The proportion of biopsies showing bacterial uptake in HV (n=3) did not differ between with (25%) and without (45%) GUDCA exposure (1mM; P=.2077).
Conclusion: GUDCA exposure decreased duodenal permeability in HV and may increase duodenal bacterial uptake in FD patients.
Policy of full disclosure: None.

121 | NUCB2/nesfatin-1 is associated with the severity of eating disorder symptoms in female obese patients

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Objective: Nesfatin-1, an 82-amino acid neuropeptide, has been described as a potent anorexigenic peptide. Over the last years, comprehensive evidence points towards the involvement of nesfatin-1 in the modulation of emotional pathways. We previously reported a negative correlation of NUCB2/nesfatin-1 levels with anxiety in obese males and positive correlations with anxiety, depression and perceived stress in obese females as well as with anxiety in women with anorexia nervosa suggesting a sex-specific regulation of NUCB2/nesfatin-1. Although the implication of nesfatin-1's role in the regulation of food intake is well-established in animals, data in humans are lacking so far. Therefore, the aim of the study was to investigate a possible association of circulating NUCB2/nesfatin-1 with disordered eating habits in patients displaying a wide range of body weight.
Methods: For our study we enrolled 243 inpatients hospitalized due to medical treatment of anorexia nervosa (n=66) and obesity (n=143). Normal-weight inpatients (n=33) underwent medical treatment for somatoform, depressive and anxiety disorders. Blood samples were taken and analyzed by commercial ELISA. Eating disorder symptoms were psychometrically assessed using the Eating Disorder Inventory-2 (EDI-2, range 0-100).
Results: The study population displayed distinct eating disorder symptoms with a mean EDI-2 total score of 42.7±14.6 (ranging from 5 to 80), with women displaying significantly higher EDI-2 scores (44.7±14.8) than men (37.7±12.8, P<.001). We observed a significant positive correlation between NUCB2/nesfatin-1 plasma levels and EDI-2 total scores in obese female patients (r=.293, P=.013), whereas no associations were found in other BMI subgroups or in male populations.
Conclusions: NUCB2/nesfatin-1 plasma levels were positively associated with EDI-2 total scores in obese women. Interestingly, no association was visible in men. Whether NUCB2/nesfatin-1 is selectively involved in eating behavior in women will have to be further investigated. Studies with a longitudinal design will be helpful in order to examine alterations of NUCB2/nesfatin-1 plasma levels in relation to changes in EDI-2 as well as after treatment of eating disorders.
Funding: Charité University Funding UFF 89/441-176, DFG STE 1765/3-1 (AS) Presenter: E.W.
Policy of full disclosure: None.

122 | Irritable Bowel Syndrome (IBS) and associated health problems: Insights from a primary care registry

E. Clevers[1]; B. Vaes[2]; S. Henrard[2]; G. Goderis[2]; J. Tack[2]; H. Törnblom[3]; M. Simrén[4]; L. van Oudenhove[5]

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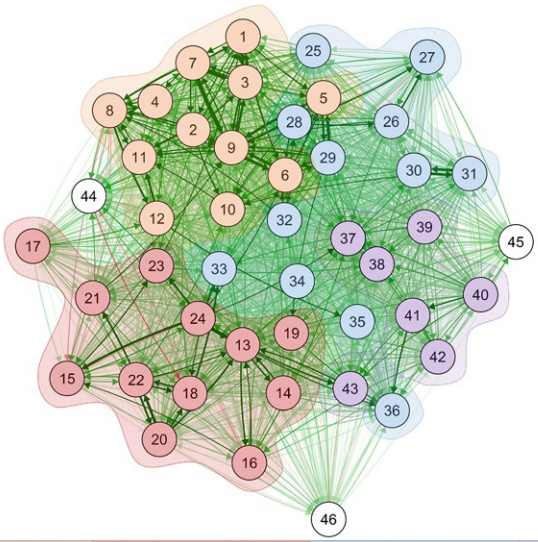
Objective: Although associations between irritable bowel syndrome (IBS) and other health problems are well-described, studies showing how these health problems cluster together are missing. We aimed to investigate what health problems share a lifetime association with IBS, and to show their relations within the "diseasome" of IBS.



Psychosocial health problems		Urogenital symptoms / infections		Miscellaneous symptoms		Musculoskeletal symptoms	
1	Feeling anxious/nervous	13	Urination symptom	25	General weakness	37	Chest pain NOS
2	Acute stress reaction	14	Urinary incontinence	26	Headache	38	Chest symptom
3	Feeling depressed	15	Urinary infection	27	Migraine	39	Muscle pain/symptom
4	Sleep disturbance	16	Urinary calculus	28	Tension headache	40	Hip/leg/foot symptom
5	Anxiety disorder	17	Menstruation symptom	29	Hyperventilation syndrome	41	Shoulder symptom
6	Somatisation disorder	18	Genital symptom female	30	Vertigo / dizziness	42	Back symptom
7	Depressive disorder	19	Breast symptom female	31	Vertiginous syndrome	43	Arm/hand symptom
8	Neurasthenia/surmenage	20	Genital candidiasis female	32	Heart symptom		
9	Psychological disorder	21	Genital infection female	33	Sensation disturbance		Other diagnoses
10	Fear of disease	22	Vaginitis/vulvitis NOS	34	Throat symptom	44	Concussion
11	Relationship problem	23	Genital symptom male	35	Respiratory symptom	45	Peripheral neuritis
12	Health issue other person	24	Genital infection male	36	Pruritus	46	Haemorrhoids

FIGURE 1 Weighted network of 46 health problems prevalent in IBS

Methods: We used the Flemish morbidity registry Intego. Briefly, GPs in Flanders recorded 4 000 000 health problems for 300 000 patients between 1999 and 2015. We compared the lifetime prevalence of health problems between IBS patients (n=13 701) and matched controls free of gastrointestinal (GI) disorders (matched by age, sex, GP), and summarised the results using functional enrichment analysis. Matched pairs were also used for all health problems within IBS (ie, IBS+X+ vs IBS+X–). Risk ratios were entered in a network.

Results: Aside from GI disorders, the following categories of health problems were enriched in IBS compared to controls: psychosocial health problems ($P<.001$), urogenital symptoms and infections ($P<.05$), musculoskeletal symptoms ($P<.001$), and other, miscellaneous symptoms ($P<.05$). These four categories clustered together in the "disease-some" of IBS (Figure 1).

Conclusions: IBS shares lifetime associations with many health problems in primary care, specifically psychosocial health problems, urogenital symptoms and infections, musculoskeletal symptoms, and a number of miscellaneous symptoms. Our findings highlight the multidimensional nature of IBS, and the importance of a holistic view when managing patients.

Policy of full disclosure: Magnus Simrén has received unrestricted research grants from Danone and Ferring Pharmaceuticals, and served as a Consultant/Advisory Board member for AstraZeneca, Danone, Nestlé, Menarini, Almirall, Allergan, Albireo, Glycom and Shire, and as a speaker for Tillotts, Takeda, Menarini, Allergan Shire and Almirall. Jan Tack has given Scientific advice to Abide Therapeutics, AlfaWassermann, Allergan, Christian Hansen, Danone, Genfit, Ironwood, Janssen, Kiowa Kirin, Menarini, Mylan, Novartis, Nutricia, Ono Pharma, Rhythm, Shionogi, Shire, SK Life Sciences, Takeda, Theravance, Tsumura, Yuhan, Zealand and Zeria pharmaceuticals, has received Research grant or support from Abide Therapeutics, Shire, Zeria and has served on the Speaker bureau for Abbott, Allergan, AstraZeneca, Janssen, Kiowa Kirin, Menarini, Mylan, Novartis, Shire, Takeda and Zeria. Lukas Van Oudenhove has received grant support from Abide Therapeutics and Nestlé and has given scientific advice to Grünenthal. Hans Törnblom has served as Consultant/ Advisory Board member for Almirall and Allergan as a speaker for Tillotts, Takeda, Shire and Almirall.

123 | Irritable Bowel Syndrome (IBS) and its comorbidities: What comes first?

E. Clevers[1]; B. Vaes[2]; S. Henrard[2]; G. Goderis[2]; J. Tack[2]; H. Törnblom[3]; M. Simrén[4]; L. van Oudenhove[5]

[1]Sahlgrenska Sjukhuset, Mag- och Tarmlaboratorium, Göteborg, Sweden; [2]KU Leuven, Belgium; [3]University of Gothenburg, Göteborg, Sweden; [4]University of Gothenburg, Dept. of Internal Medicine, Göteborg, Sweden; [5]Katholieke Universiteit Leuven, TARGID, Belgium

Objective: Irritable bowel syndrome (IBS) aetiology may be studied by the order of onset of IBS and its comorbidities. We investigated the incidence of health problems in the years before and after the IBS diagnosis.

Methods: We used the Flemish morbidity registry "Intego". Briefly, GPs in Flanders recorded 4 000 000 health problems for 300 000

patients between 1999 and 2015. Patients with an IBS diagnosis (n=6730) were matched with controls without IBS, by age, sex, and GP. Incidence rates were computed for four categories of health problems that we previously demonstrated to be enriched in IBS: psychosocial health problems, urogenital symptoms and infections, musculoskeletal symptoms, and other miscellaneous symptoms. We subsequently adjusted for the total number of health problems, and finally calculated the ratio between IBS patients and controls (ie, adjusted incidence rate ratios, AIRR).

Results: Psychosocial health problems tended to be diagnosed in the same year as the IBS diagnosis (AIRR ~1.6). Musculoskeletal symptoms and urogenital symptoms and infections had a high AIRR throughout the period, and did not favour incidence before vs after IBS. The AIRR of other, miscellaneous symptoms tended to decrease somewhat after the IBS diagnosis. However, no comorbidity clearly preceded or followed IBS. Results are shown in Figure 1.

Conclusions: We illustrate that there is no consistent answer to the question "what comes first" for IBS and its comorbidities in primary care. Our work highlights the heterogeneity of IBS, and the complexity of its comorbidity spectrum.

Policy of full disclosure: Magnus Simrén has received unrestricted research grants from Danone and Ferring Pharmaceuticals, and served as a Consultant/ Advisory Board member for AstraZeneca, Danone, Nestlé, Menarini, Almirall, Allergan, Albireo, Glycom and Shire, and as a speaker for Tillotts, Takeda, Menarini, Allergan Shire and Almirall. Jan Tack has given Scientific advice to Abide Therapeutics, AlfaWassermann, Allergan, Christian Hansen, Danone, Genfit, Ironwood, Janssen, Kiowa Kirin, Menarini, Mylan, Novartis, Nutricia, Ono Pharma, Rhythm, Shionogi, Shire, SK Life Sciences, Takeda, Theravance, Tsumura, Yuhan, Zealand and Zeria pharmaceuticals, has received Research grant or support from Abide Therapeutics, Shire, Zeria and has served on the Speaker bureau for Abbott, Allergan, AstraZeneca, Janssen, Kiowa Kirin, Menarini, Mylan, Novartis, Shire, Takeda and Zeria. Lukas Van Oudenhove has received grant support from Abide Therapeutics and Nestlé and has given scientific advice to Grünenthal. Hans Törnblom has served as Consultant/Advisory Board member for Almirall and Allergan as a speaker for Tillotts, Takeda, Shire and Almirall.

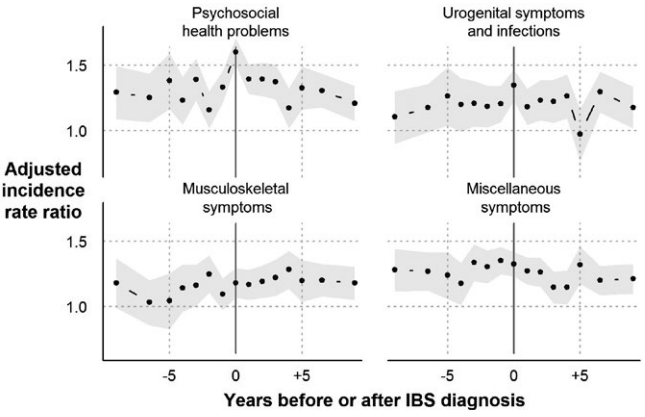


FIGURE 1 Adjusted incidence rate ratios of health problems commonly seen in IBS

124 | Within-person correlations between gastrointestinal and psychological features of the Irritable Bowel Syndrome (IBS)

E. Clevers[1]; J. Tack[2]; H. Törnblom[3]; G. Ringström[3]; L. van Oudenhove[4]; M. Simrén[5]

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Objective: Although correlations between features of irritable bowel syndrome (IBS) have been reported, these were based on between-person rather than within-person variation. We investigated the longitudinal within-person correlations between features of IBS.

Methods: We used a longitudinal cohort of 276 IBS patients, who filled out questionnaires once annually over 5 years on the following features: gastrointestinal (GI) symptom severity (GSRS), quality of life (QOL), GI specific anxiety (VSI), general anxiety and depression (HADS), coping resources (CRI), and sense of coherence (KASAM). For each participant, scores were centered on their own mean, and within-person correlations were computed for all pairs of features.

Results: Aggregate within-person correlations are shown in Figure 1. Within-person correlations were strong for the triad GI symptom severity, GI specific anxiety, and QOL (|*r*|: .47 to .64). Another set of features was comprised of general anxiety, depression, coping resources, and sense of coherence (|*r*|: .39 to .57). Within-person correlations between the two sets were weak (|*r*|: .00 to .37). However, within-person correlations tended towards bimodal distributions across the population, especially for GI symptom severity and depression (*r* ~.6 for half of participants, and *r* ~−.4 for the other half).

Conclusions: Here we show that, within individual IBS patients, GI symptom severity is strongly correlated with GI specific anxiety and QOL, but not with four other psychological features. The presence of negative within-person correlations in some individuals may imply a lack of relation, but could also signal long-term causative processes.

Policy of full disclosure: Magnus Simrén has received unrestricted research grants from Danone and Ferring Pharmaceuticals, and served as a Consultant/ Advisory Board member for AstraZeneca, Danone, Nestlé, Menarini, Almirall, Allergan, Albireo, Glycom and Shire, and as a speaker for Tillotts, Takeda, Menarini, Allergan Shire and Almirall. Jan Tack has given Scientific advice to Abide Therapeutics, AlfaWassermann, Allergan, Christian Hansen, Danone, Genfit, Ironwood, Janssen, Kiowa Kirin, Menarini, Mylan, Novartis, Nutricia, Ono Pharma, Rhythm, Shionogi, Shire, SK Life Sciences, Takeda, Theravance, Tsumura, Yuhan, Zealand and Zeria pharmaceuticals, has received Research grant or support from Abide Therapeutics, Shire, Zeria and has served on the Speaker bureau for Abbott, Allergan, AstraZeneca, Janssen, Kiowa Kirin, Menarini, Mylan, Novartis, Shire, Takeda and Zeria. Lukas Van Oudenhove has received grant support from Abide Therapeutics and Nestlé and has given scientific advice to Grünenthal. Hans Törnblom has served as Consultant/ Advisory Board member for Almirall and Allergan as a speaker for Tillotts, Takeda, Shire and Almirall.

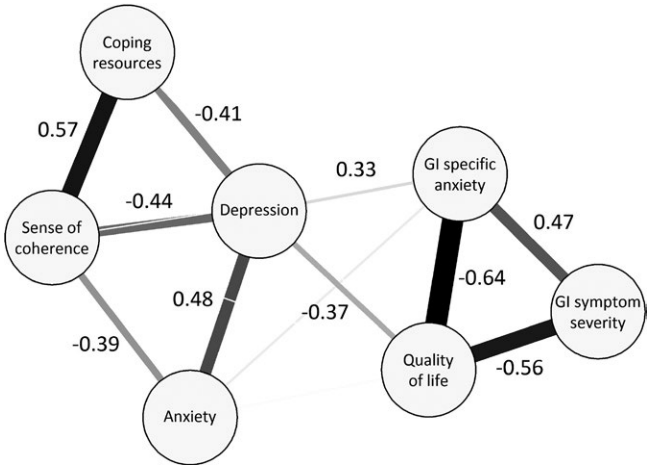

FIGURE 1 Population aggregate of within-person correlations (*r*) between several IBS features

125 | The effect of intragastric fructose infusion on homeostatic and hedonic brain regions interacts with the effect of emotional state

J. Iven[1]; J. Biesiekierski[2]; D. Zhao[2]; J. Tack[2]; L. van Oudenhove[1]

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Objective: Not only exteroceptive properties of food, but also purely interoceptive properties can influence emotional state and its neural basis. This effect has been shown for fatty acids, but whether it also applies to other nutrients including carbohydrates, remains unknown. We aimed to study the effects of intragastric administration of fructose, compared to placebo, on brain activity in homeostatic and hedonic regions, and its interaction with the effect of sad emotion induction.

Methods: Fifteen healthy subjects were studied after an overnight fast. Brain activity before and up to 40 minutes after infusion of fructose (25 g) or saline (placebo), was recorded using functional magnetic resonance imaging. Sad or neutral emotional states were induced using a combination of validated classical music pieces and facial expressions. Emotional state was assessed every 10 minutes using the Self-Assessment Manikin. Brain responses over time to fructose vs placebo, sad vs neutral emotion, and their interaction were analysed in a priori defined regions of interest (ROI) at a voxel-level threshold of pFWEcorrected<0.05. Similar effects on emotional ratings were tested using mixed models.

Results: We did not observe significant main effects of nutrient and emotion, and no significant nutrient-by-emotion interaction effect, on emotional ratings. However, significant main effects of fructose, emotional state, and a significant nutrient-by-emotion interaction effect on brain responses in pre-hypothesized ROIs, including the medulla, midbrain, hypothalamus, basal ganglia, anterior insula, orbitofrontal cortex and amygdala (see Table 1) were found.

Conclusion: These findings indicate that 25 g of fructose is not able to influence emotional state at the behavioural level, but had a significant effect on brain responses in most hedonic and homeostatic regions.

Policy of full disclosure: None.

126 | STW 5 prevents changes in intestinal permeability induced by psychological stress in mice

M. Neunlist[1]; P. Aubert[2]; J. Chevalier[3]; T. Durand[3]; A. Bessard[3]; O. Kelber[4]; H. Abdel-Aziz[5]

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Objective/Introduction: The herbal preparation STW5 has been reported to increase intestinal chloride secretion. However, the ability of STW5 to modulate paracellular and transcellular permeability remains currently unknown. Therefore, we aimed to study the ability of STW5 to modulate intestinal permeability under basal and repeated acute stress conditions.

Material and methods: C57 bl6 mices were gavaged for 14 days with STW5 (3 mL/kg). After 10 days of treatment, mice were subjected to water avoidance stress (WAS) during 4 consecutive days. In vivo permeability to FITC—Sulfonic Acid (FSA, 400 Da) and Horse Radish Peroxydase (HRP, 44 KDa), total transit time and colonic transit (fecal pellet output—FPO) were assessed at Day 0 (D0), D10 and D14 of STW5 treatment. Ex vivo permeability to FSA and HRP was assessed on jejunum, ileum, proximal colon and distal colon at D14 using Ussing chambers. Corticosterone blood level was measured at D11 and D14.

Results: In vivo permeability to FSA and HRP as well as total transit time were not modified by STW5 in basal and WAS conditions. However, STW5 prevented the increase in permeability to FSA induced by WAS in the distal colon. Conversely, STW5 prevented the increase in permeability to HRP induced by WAS in the jejunum and proximal colon. Furthermore, while STW5 tended to increase colonic transit as compared to control in basal condition it the increase in colonic transit induced by WAS. Finally, STW5 did not modify the changes in corticosterone induced by WAS.

Conclusion: Our study suggests that SWT5 can prevent WAS induced changes in paracellular and transcellular permeability in specific regions of the gastrointestinal tract. Such effects could contribute to STW5 therapeutical effects in irritable bowel syndrome and support

novel therapeutical indications for pathologies in which intestinal barrier functions are altered.

Policy of full disclosure: This work was funded by a research grant from Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH.

127 | Light therapy (LED 940 nm) recovers colonic motility in experimental colitis of mice

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[1]Queen Mary University London, Blizard Institute, United Kingdom; [2]Queen Mary University of London, Blizard Institute, United Kingdom; [3]Londrina State University, Londrina-PR, Brazil; [4]Blizard Institute, London, United Kingdom

Objectives: Inflammatory bowel disease (IBD) is an important clinical problem which still needs the development of new treatments. Previously we have demonstrated that a specific wavelength of LED light (940 nm) has anti-inflammatory potential due to reduction of pro-inflammatory cytokines (1). Our aim was to evaluate if the impaired colonic motility in acetic acid (7%)-induced experimental colitis could be recovered by LED therapy.

Methods: Male Swiss mice were allocated into 5 groups: control, control with LED light, colitis, colitis plus prednisolone treatment and finally colitis with LED light therapy. The colitis induction and LED light therapy was applied in sedated mice as previously described (i). In-vitro colonic manometry was performed using a multi-lumen perfused assembly, assessing propagating colonic activity from the proximal to the distal colon at four locations as previously shown in rats (ii). Spontaneous pressure waves were recorded and analysed using Spike2 software to obtain: number of peaks, amplitude, area under the curve (AUC) and the duration of colonic migrating motor complex (CMMC).

Results: The amplitude of contractions was increased in the colitis group only. LED treatment normalized the amplitude similar to control (Table 1. *P*<.05). The AUC was reduced in the colitis group, and treatment with either prednisolone or LED therapy reversed it. A trend towards a shorter CMMC was observed only in the colitis group (21.26 5.02 seconds) which then normalised post LED therapy (47.36 6.29) (*P*<.05). Across all 5 groups there was no change in the average number of peaks/min.

Conclusion: These data suggest that LED therapy (940 nm) may be a useful tool in rescuing impaired motility observed in inflammation, but its mechanism of action remains to be determined. (1). Belém, M. O. et al. Light-emitting diodes at 940nm attenuate colitis-induced inflammatory process in mice. J. Photochem. Photobiol. B. 162, 367-73 (2016). (2). Fraser, R. et al. Small intestinal dysmotility following abdominal irradiation in the rat small intestine. Neurogastroenterol. Motil. 10, 413-9 (1998).

Policy of full disclosure: None.

TABLE 1. Spontaneous contraction amplitude in mmHg of each segment of the colon.

Segment / Groups	Proximal	Mid Proximal	Mid Distal	Distal
Control	58.5 ± 18.4	74.1 ± 8.1	53.8 ± 9	52.2 ± 1.9
Control with LED	50.2 ± 12.3	55.5 ± 9.1	61.1 ± 7	54.3 ± 3.3
Colitis	374.8 ± 109 a	245.4 ± 90.3	202 ± 50 a	161 ± 40.2 a
Colitis prednisolone treatment	65.9 ± 17.4	21.4 ± 8.6	16.2 ± 5 ab	6.8 ± 3 ab
Colitis with LED	32.1 ± 10 a	43.3 ± 8.5	11.8 ± 4.1 b	11.5 ± 5 a

Equal letters in the same column represent significant difference ($p<0.05$)

128 | Antibiotic treatment prevents stress-induced plasticity in the PVN

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A threat to survival triggers a defense mechanism call the stress response. This response is initiated by corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) of the hypothalamus. Recent studies demonstrate that manipulation of the gut microbiome affects the behavioural response to stress. However, the mechanisms by which gut flora affects the stress response is not clear. Here we investigated whether a brief, antibiotic-induced disturbance of gut flora affects synaptic transmission and homecage behavior following stress. An antibiotic cocktail (neomycin, vancomycin, ampicillin) was administered in the drinking water for 7 days. On day 7, coronal brain slices were prepared for electrophysiological experiments. To examine the effects of antibiotics on the neural response to stress, one littermate was removed from the cage and exposed to a stressor for 5 minutes (10 footshocks, 0.5 mA). Antibiotic treatment did not change basal synaptic properties of CRH neurons. Consistent with previous findings, exposure to a single acute stress allowed glutamate synapses to undergo short-term potentiation following a burst of high frequency afferent activity. Antibiotic-treated mice showed no short-term plasticity. In addition, antibiotic-treated mice showed differences in homecage behavior after stress. Immediately after stress, water-treated mice exhibited ano-genital sniffing of their cagemate. This behavior was reduced in antibiotic-treated mice. These results suggest that antibiotic treatment disrupts the normal behavioural and neural response to stress.

Policy of full disclosure: None.

129 | Handling of complex data in neurogastroenterology: Multi-step clustering of preclinical data for a combination phytomedicine, STW 5

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A multitude of concomitant causes and likewise also targets for therapeutic interventions have been identified in functional gastrointestinal diseases (FGDs) with their heterogeneous and overlapping pathomechanisms (Allescher 2006). Therefore, a multi-target approach is a promising therapeutic strategy, as is exemplified by the herbal combination medicinal product STW 5 (Iberogast), which has been proven to be effective in a large number of randomized controlled clinical studies, while likewise exhibiting a highly favourable safety profile (Storr et al. 2016). The fact, that STW 5 consists of nine plant extracts, makes the combination an ideal candidate for the use of the newly described method of step-wise cluster analysis, a standardized procedure to transfer heterogeneous pharmacological data, from different models, into effect size categories. This method is based on a step-wise cluster formation starting from the level of single tests up to the level of different pathomechanisms involved in the development of a certain disease, in this case functional dyspepsia (FD) and irritable bowel syndrome (IBS). The result is based on an overview on the pharmacological data on STW 5 and its single components, analysed using step-wise cluster formation. The different modes of action address multiple etiologic factors involved in functional dyspepsia and irritable bowel syndrome, like hyper- and hypo-motility, acidity, inflammation and hypersensitivity, and also inflammatory gastrointestinal diseases. The results on STW 5 and the contribution of the single constituents to the overall multi-target action of this herbal combination preparation are evaluated and visualized by 2D histograms. Thus, by making the allocation of specific actions to the different components of STW 5 manageable, the results also give support to the clinical use of the combination in patients with different symptom clusters.

References: Storr et al. 2016, Pharmakon 4: 356; Allescher et al. 2006, Phytomedicine 13 SV:2.

Policy of full disclosure: H. Abdel-Aziz and O. Kelber are employees of Innovation and Development, Phytomedicines Supply and Development Center, Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany; G. Lorkowski, K. Nieber and M. Storr have received honoraries or travel grants from Innovation and Development, Phytomedicines Supply and Development Center, Bayer Consumer Health, Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany.

130 | Evaluating the efficacy of mixture of *Boswellia carterii*, *Zingiber officinale* and *Achillea millefolium* on severity of symptoms, anxiety and depression in Irritable Bowel Syndrome (IBS) patients

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Objective: Irritable bowel syndrome (IBS) is one of the most prevalent functional gastrointestinal disorders (FGID) that affects in different aspect of life and IBS patients experienced depression and anxiety more than others. There are several herbal medicines with positive effects in these patients. The aim of this study is to evaluate the effects of mixture of *Boswellia Carterii*, *Zingiber officinale* and *Achillea millefolium* on severity of symptoms, anxiety and depression in IBS patients.

Methods: This study was done in 60 IBS patients divided in to two case and control groups. In cases and controls Memogut capsules and placebo, respectively administered to patients. They were assessed at the beginning, 1 month and 3 months after by Irritable Bowel Syndrome-Severity Scoring System (IBS-SSS) and Hospital Anxiety and Depression Scale (HADS).

Results: About 60 IBS patients with mean age of 38.75±11.74 participated that about 55.4% of cases and 72.8% of controls were men. The most prevalent type of IBS was mixed type of IBS. The mean score of abdominal pain severity and frequency, bloating score, depression and anxiety score were decreased in patients administered Memogut significantly but changes in these variables in controls were not statistically significant.

Conclusion: A mixture of *Boswellia carterii*, *Zingiber officinale* and *Achillea millefolium* is effective in eliminating IBS symptoms and its related depression and anxiety and using herbal medicine in IBS treatment is suggested.

Policy of full disclosure: None.

131 | Anhedonia is related to more severe abdominal pain in Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD)

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Objective: Object Anhedonia is the lowered ability to experience pleasure from rewarding or enjoyable activities such as hobbies, friends, work—even food and sex. It is considered a symptom of depression. Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD) are frequently accompanied by psychiatric disorders, such as depression. However, in our knowledge no studies investigated the presence of anhedonia in these patients.

Methods: Sixty-nine patients with IBD (35 individuals (16 women, 38.49±15.26 years) suffered from Crohn disease (CD), 34 (18 women, 41.74±16.02 years) had ulcerative colitis (RCU)) and 54 with IBS (37 women, 41.37±12.41 years) were enrolled in this study. All patients fulfilled the Snaith-Hamilton Pleasure Scale (SHAPS), a self-rating scale consisting of 14 items that cover the domains of social interaction, food and drink, sensory experiences, achievement and pastimes. The Beck Depression Inventory-II (BDI-II) was used to screen for depression. Moreover, we calculated abdominal pain on a (0-100) Visual Analog Scale (VAS) in all patients.

Results: All patients had significantly higher Pleasure Scale score and BDI-II score than healthy controls (HC) (n=81) (1.30±0.2 vs 0.80±0.1; *P*=.017 and 10.5±7.5 vs 5.9±4.9; *P*=.0001, respectively). And there is a significant correlation between them (*r*=.26, *P*=.0001). The multiple linear regression analysis showed that the Pleasure Scale score is significantly related to the severity of abdominal pain (0-100 VAS) (Table 1_Multiple Linear Regression).

Conclusions: Anhedonia is related to the severity of abdominal pain. This study confirms that Anhedonia should be considered a nuclear symptom in IBS and IBD patients.

Policy of full disclosure: None.

TABLE 1 Multiple Linear Regression

Coefficient[a]

Model		Unstandardized coefficients		Standardized coefficients		
		B	Standard deviation Error	Beta	t	Sig.
1	(Constant)	1,338	,557		2,402	,017
	SEX	,216	,216	,072	1,001	,318
	AGE	-,008	,008	-,068	-,936	,350
	VAS_Abdominal Pain	,079	,039	,152	2,021	,045
	CD, RCU, IBS, HC	-,132	,103	-,096	-1,280	,202

a.Dependent variable: PLEASURE_SCALE

132 | Homozygous variants in MYLK and LMOD1 cause Megacystis Microcolon Intestinal Hypoperistalsis Syndrome by disruption of smooth muscle contractility

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Objective/Introduction: Megacystis Microcolon Intestinal Hypoperistalsis Syndrome (MMIHS) is a congenital disorder characterized by severe dilation of the bladder and intestinal obstruction. To date, two genes are known to be involved in MMIHS pathogenesis, ACTG2 and MYH11, but in some patients the genetic etiology of this disease is still unknown.

Material and Methods: Homozygosity mapping and whole exome sequencing were performed in four MMIHS patients derived from three consanguineous families for which no variant in ACTG2 and MYH11 was identified. Pathogenicity of the variants identified was determined by a series of expression studies, in vitro and in vivo assays.

Results: Homozygous variants in two different genes where identified in the four patients analyzed. In one family a nonsense variant in the Leimodin-1 gene (LMOD1) was found, while the other two families carried two different homozygous variants in the Myosin light chain kinase gene (MYLK). Expression studies showed that the variants in LMOD1 and MYLK affected protein expression, and in vitro data confirmed pathogenicity of these variants due to impairment of smooth muscle contractility. Moreover, Lmod1 knock out mice generated using CRISPR-Cas9 genome editing, and homozygous mutant mice for Mylk previously described1, showed pathology consistent with MMIHS.

Conclusions: Our results present LMOD1 and MYLK as two new disease causing genes for the recessive form of MMIHS, confirming that MMIHS is a myopathy with multiple patterns of inheritance caused by disruption of the smooth muscle contractile apparatus.

Policy of full disclosure: None.

133 | Stress gone viral: Chronic social stress induces marked changes in the gut virome in mice

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In every gut, there are billions of organisms that live with the host and contribute to digestion, metabolism, immune function, and stress response. Current research now indicates that the microbiome-gut-brain axis plays a critical role in mood and behavior. Changes in gut bacteria are seen during chronic stress, hypothalamic-pituitary-adrenal (HPA) axis dysfunction, and psychiatric disorders such as anxiety and depression. Research into the role of gastrointestinal microbiota in health focuses almost exclusively on bacteria yet the number of commensal viruses, most notably bacteriophage, vastly outnumber bacteria in the gut. This study sought to investigate changes in viral gut composition following chronic social stress. Stress animals were exposed to 3 consecutive weeks of chronic unpredictable social stress. Faecal and caecal samples from control and stress animals were sequenced for bacteria (16S) and viruses (metagenomic). Following chronic social stress, stressed animals had significant increases in immobility time during the forced swim test, basal corticosterone, and cytokine IL-6 compared to non-stressed controls. Bioinformatic analysis revealed marked differences in bacteriome and virome between control and stressed animals. Moreover, viral species richness increased in the stress group alongside reductions in bacterial richness. This is the first study to investigate changes in virome in relation

to the microbiota-gut-brain axis. Findings from this research further elucidate the impact of psychological stress on the microbiome and suggest that viruses may play a role in HPA axis function.

Funding sources: 12/RC/2273.

Policy of full disclosure: None.

LUMINAL SIGNALLING/FOOD ALLERGIES AND INTOLERANCES

134 | Abnormal stem cells and differentiation progeny into enteroendocrine cells in the colon of patients with IBS

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Objective: The densities of enteroendocrine cells have been reported to be reduced in IBS patients. It is believed that this reduction plays an important role in the pathophysiology of IBS. The cause of this reduction is not known. The present study investigated the possibility that the reduction in the enteroendocrine cells in IBS is caused by abnormalities in the stem cells and their differentiation activities.

Methods: Sixty-one patients with IBS according to the Rome III criteria (48 females and 13 males with a mean age of 51 years, range, 27-67 years). These patients included 22 with IBS-D, 18 with IBS-M, and 21 with IBS-C. Twenty-four healthy subjects were included in the study as controls. Patients and controls underwent standard colonoscopy and 4 biopsies were taken from sigmoid colon. Sections from colonic biopsies were immunostained for Musashi 1 (transcription factor expressed by intestinal stem cells and their early progeny), neurogenin 3 (expressed in endocrine progenitor cells), serotonin, PYY, oxyntomodulin (enteroglucagon), PP, and somatostatin. Immunoreactive cells were quantified by computer image analysis.

Results: The densities of Musashi 1, neurogenin 3, serotonin, PYY, and oxyntomodulin cells (Figure) were significantly lower than that of controls (P<.0001, <.0001, =.0002, <.0001, =.0009, respectively). There was no difference in the densities of PP and somatostatin between IBS patients and controls (P=.7, and .6, respectively).

Conclusion: The reduction in the density of stem cells as indicated by the reduction in the density of Musashi expressing cells and their differentiation progeny towards enteroendocrine cells as indicated by the reduction in neurogenin 3 expressing cells can explain the reduction in the several enteroendocrine cell types observed here.

Policy of full disclosure: None.

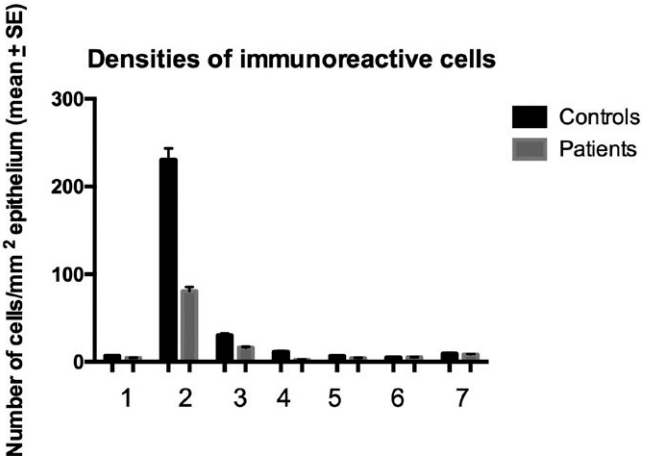

FIGURE 1 The densities of Musashi 1, neurogenin 3 and different types of enteroendocrine cell in controls and patients with IBS. 1; Musashi 1, 2; neurogenin 3, 3; serotonin, 4; PYY, 5; oxyntomodulin, 6; PP, and 7; somatostatin

135 | Gluten causes symptom relapse in a small group of patients with suspected non-coeliac gluten sensitivity: A randomized, double-blind placebo controlled challenge

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Objective: Non-coeliac gluten sensitivity (NCGS) is a new entity with unknown prevalence and mechanisms, and there is a need for a standardized procedure to confirm the diagnosis. The objective of this study was to identify NCGS in a patient group without coeliac disease or wheat allergy, who reported improvement of symptoms while on a gluten-free diet.

Methods: Twenty patients with suspected NCGS (14 F/6 M, age range: 21-62 year) were included. All patients went through four periods of double-blinded provocation with gluten/placebo. They randomly received two packages of muffin-mix with gluten, and two packages without gluten (placebo). They were instructed to eat two muffins a day (11 g gluten) for 4 days, followed by a 3 days' wash-out. Gastrointestinal symptoms were recorded with the symptom questionnaires IBS-SSS and IBS-SQ after each provocation, while fatigue and quality of life were registered at baseline and end of the trial.

Results: Four out of twenty patients (20%) correctly identified the two periods when they received muffins containing gluten as relapse of abdominal symptoms, hence were diagnosed with NCGS. The diagnosed group showed higher symptom scores than the not-diagnosed group both at baseline, after gluten exposure and after placebo, and there was a significant difference in gastrointestinal symptoms between the two groups after provocation with gluten (P=.047). The not-diagnosed group showed the most severe symptoms after placebo. Symptom severity increased significantly from baseline to after gluten for the diagnosed group (P=.045) and from baseline to after placebo for the

not-diagnosed group (*P*=.032). Symptom severity at baseline was significantly correlated with fatigue and reduced quality of life.

Conclusions: This randomized, double-blind placebo-controlled challenge identified symptom relapse in accordance with gluten intake in a small group of patients with suspected NCGS. Baseline symptom severity may identify reliably the subgroup of patients confirmed to be true non-coeliac gluten intolerant.

Policy of full disclosure: None.

136 | Different proton pump inhibitors are equally effective in inducing endoscopic and histologic remission in patients with proton pump inhibitor-response esophageal eosinophilia

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Objective: Introduction: Proton Pump Inhibitor-response esophageal eosinophilia (PPI-REE) is an emerging condition characterized by a constellation of clinical, endoscopic, and histopathologic features in the setting of eosinophilic inflammation responding to a course of proton pump inhibitor (PPI). A recent meta-analysis explored the role of different PPIs in inducing clinical and histological remission in patients with esophageal eosinophilia without observing significant differences among them due to several limitations. Aim: We aimed to prospectively compare the effect of different PPIs in inducing endoscopic and histologic remission in patients with PPI-REE.

Methods: Consecutive patients with symptoms suggestive of EoE underwent upper endoscopy to assess the presence of at least 15 eos/hpf on oesophageal biopsies at mid/proximal esophagus and, then, were treated with twice-daily PPI for at least 8 weeks. Patients were assigned to receive esomeprazole 20 mg bid, rabeprazole 20 mg bid and pantoprazole 40 mg bid in a 1:1:1 ratio. Thereafter, patients repeated upper endoscopy and PPI-REE was identified in case of less than 15 eos/hpf and a 50% decrease from baseline. Endoscopic according to Endoscopic Reference Score (EREFS) and histologic features were blindly reviewed for each patient and treatment.

Results: Twenty-eight patients [23 M/5 F; mean age 35] reporting dysphagia (93%), bolus impaction (68%) and chestpain (25%) were diagnosed with PPI-REE. According to treatment allocation, 8 (29%) patients received esomeprazole, 9 (32%) rabeprazole and 11 (39%) pantoprazole. At baseline, demographic and clinical data, including age, sex, BMI, H. pylori infection, concomitant allergy conditions and latency from diagnosis were similar (*P*=ns). Moreover, no differences were found in terms of frequency of symptoms reported (*P*=ns). Endoscopic and histologic features at baseline and after PPI therapy are shown in the Table. Esomeprazole, rabeprazole and pantoprazole reached the same degree of efficacy in inducing endoscopic and histologic changes in PPI-REE patients (*P*=ns).

Conclusions: Various PPIs administered at double daily dose were equally effective in determining endoscopic and histologic remission in patients with PPI-REE. These data, although obtained in a small sample of patients, seem to suggest that the pharmacokinetic and pharmacodynamic differences among these drugs do not affect the final diagnosis of PPI-REE.

Policy of full disclosure: None.

TABLE. Endoscopic and histologic features at baseline and after PPI therapy in PPI-REE

	Esomeprazole Group (n=8)		Rabeprazole Group (n=9)		Pantoprazole Group (n=11)	
	Baseline	After-Therapy	Baseline	After-Therapy	Baseline	After-Therapy
Endoscopic Features, %						
Rings	75%	26%	67%	22%	63%	18%
Linear furrows	62%	13%	55%	11%	54%	9%
Whitish exudates	50%	13%	44%	11%	54%	18%
Edema	26%	13%	33%	11%	27%	9%
Crepe paper	13%	0%	0%	0%	9%	0%
Strictures	13%	13%	22%	11%	18%	9%
Mean EREFS Score	7.3	1.2	8.1	1.6	8.4	1.7
Histologic Features, n or %						
Median maximum eos count*	75	6	87	5	95	6
Median eos count (of 5 HPFs)*	55	4	68	3	62	5
Degranulation present	88%	13%	100%	11%	90%	18%
Microabscess present	63%	13%	55%	11%	48%	9%
Basal layer present	100%	26%	100%	22%	100%	18%
Spongiosis present	88%	26%	88%	11%	90%	18%
Subepithelial tissue present	75%	13%	55%	11%	81%	9%
Lamina propria fibrosis present	0%	0%	0%	0%	9%	0%

Eos=esosinophils
*Calculated for an HPF area = 0.24 mm^2

137 | Proximal esophageal baseline impedance levels are able to discriminate between scleroderma patients with and without esophageal involvement

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Objective/Background: Patients with non-erosive reflux disease (NERD) showed lower distal esophageal baseline impedance BI levels (ie, a measure of mucosal integrity) compared to healthy controls (HCs) due to the presence of abnormal acid exposure time (AET). In contrast, no differences were found in proximal esophagus. Systemic sclerosis (SSc) is a systemic disease characterized by the deposition of collagen and matrix proteins in the connective tissue of the gastrointestinal tract. This event could potentially affect the conductivity of the oesophageal wall and consequently reduce BI levels.

Aim: We aimed to prospectively compare BI levels between a group of NERD patients and two groups of SSc patients, one with a clear manometric picture of scleroderma esophagus and one without esophageal involvement.

Methods: Consecutive patients with heartburn and those with a definite diagnosis of SSc underwent upper endoscopy in order to assess the presence of esophageal mucosal lesions. Further, a group of healthy subjects was used as controls (HCs). All endoscopy-negative and SSc patients underwent esophageal manometry and impedance-pH testing off-therapy. Ee measured distal AET and BI values at 3, 5, 7, 9, 15 and 17 cm above the lower esophageal sphincter, during the overnight rest, for at least 30 minutes after excluding swallows and reflux induced changes.

Results: Fifty patients [38 F; mean age 51 years] with NERD, 50 SSc patients [44 F; mean age 48 years] with esophageal involvement, 30 SSc patients [28 F; mean age 49 years] without esophageal involvement and 50 HCs [37 F; mean age 49years] were enrolled. All 50/50 (100%) NERD patients and 44/50 (88%) SSc patients with esophageal involvement showed an abnormal AET compared to 8/30 (26%) SSc patients without esophageal involvement and 0/50 (0%) HCs (P<.01). Proximal median BI levels (ie, at 9, 15 and 17) were similar between NERD, SSc patients without esophageal involvement and HCs (P=ns), whereas they were much lower in SSc patients with esophageal involvement (P<.05).

Conclusions: Proximal BI levels are able to segregate between scleroderma patients with and without esophageal involvement. The advent of novel and poorly invasive methods for mucosal impedance assessment will allow us to perform this measurement without the need of prolonged probe insertion.

Median BI Values	NERD	SSc patients with esophageal involvement	SSc patients without esophageal involvement	HCs
at 3 cm, Ω	1180	961	1890	2965
at 5 cm, Ω	1250	1114	2310	2688
at 7 cm, Ω	1684	1230	2670	3110
at 9 cm, Ω	2480	1640	2540	2890
at 15 cm, Ω	2770	1520	2960	3320
at 17 cm, Ω	2840	1980	3100	3250

Policy of full disclosure: None.

138 | Low FODMAP diet improves symptoms and quality of life in patients with radiation-induced small bowel disease: A pilot study

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Rationale: Patients suffering from chronic radiation induced small bowel diseases (RISBD) after cancer treatment have similar symptoms as IBS (irritable bowel syndrome) patients despite dissimilar pathological origin. The low FODMAP (fermentable oligo-, di-, monosaccharides and polyols) diet (LFD) is a widespread management strategy for IBS. The aim of the conducted study was to investigate the effects of LFD on symptoms and health related quality of life (HRQOL) for RISBD patients.

Methods: Eleven patients (mean age 46.6 years) with RISBD related IBS symptoms, were instructed to follow LFD throughout a 4-week intervention period. IBS Severity Scoring System (IBS-SSS) and IBS Symptom Questionnaire (IBS-SQ) were used to evaluate symptoms. An ad hoc questionnaire measured grade of damage and typical RISBD complaints. Short Form Nepean Dyspepsia Index (SF-NDI) and 12-item Short Form Health Survey (SF-12) were used to evaluate HRQOL. Baseline FODMAP intake, additional dietary changes and adherence to the diet were estimated from a 3-day food record. All forms were filled at baseline and at 4 weeks.

Results: FODMAP intake was successfully reduced, and main additional dietary changes were reduced intake of energy, carbohydrates and fiber. The adherence to the diet was high (mean 94.8%). IBS symptoms improved significantly based on mean total score of IBS-SSS and IBS-SQ, which changed from 310.2±60.7 to 171.4±107.2 (P=.001) and 27.4±4.1 to 15.7±10.1 (P=.002), respectively. HRQOL improved according to SF-NDI (P=.001), and based on the mental component score (P=.047) and the physical component score (P=.134) of SF-12.

Conclusions: The low FODMAP diet seems effective in alleviating IBS symptoms, and improving HRQOL in RISPBD patients. High compliance to LFD is possible with adequate diet counselling and continuous guidance. Further research should be conducted to enable implementation of LFD as a future management strategy for chronic RISBD.

Policy of full disclosure: None.

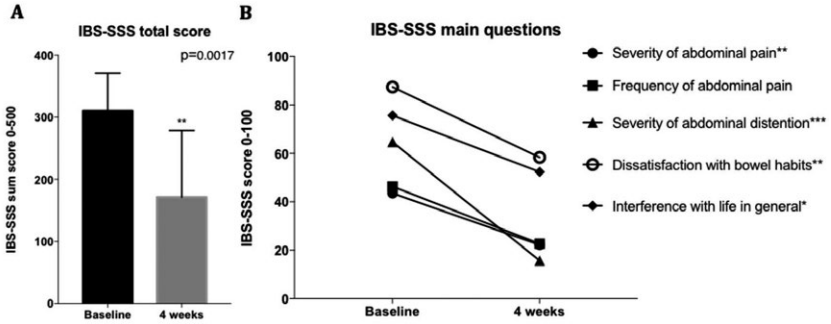

Figure 1: IBS-SSS total score (A) and individual score of main questions (B) at baseline and at 4 weeks. IBS-SSS; Irritable bowel syndrome severity scoring system

139 | Effect of faecal microbiota transplantation on the enteroendocrine cells of the colon in patients with Irritable Bowel Syndrome (IBS): Double blinded-placebo controlled study

M. El-Salhy[1]; P. Holger Johnsen[2]; T. Mazzawi[3]; M. El-Salhy[4]; T. Hausken[5]; R. Goll[6]; P. C. Valle[7]

[1]University of Bergen, Stord Hospital, Norway; [2]University Hospital North Norway, Harstad, Norway; [3]University of Bergen, Dept. of Clinical Medicine K1, Norway; [4]Helse Fonna Stord Hospital, Norway; [5]K1/University of Bergen, Bergen/Norway, Norway; [6]Arctic university of Norway, Tromsø, Norway; [7]University Hospital North Norw, Harstad, Norway

Objective: Gut microbiota and enteroendocrine cells alterations are believed to play an important role in the pathophysiology of irritable bowel syndrome (IBS). The aim of the study was to investigate the effect of faecal microbiota transplantation (FMT) on the enteroendocrine cell densities of the colon in IBS patients.

Methods: This study is connected to the REFIT study, where the treatment of IBS with FMT is investigated in a double blind randomized controlled trial (ClinicalTrials.gov Identifier NCT02154867). Eighty-three subjects were allocated to receive donor FMT or placebo FMT (own feces) by colonoscope to cecum. We obtained biopsies from sigmoideum as part of the FMT procedure. Ten amongst our best responders, defined by improvement in IBS-symptom severity score (IBS-SSS), and ten non-responders without any changes in IBS-SSS, consented to a new biopsy one-year post FMT. Out of these participants (n=20), 16 received donor FMT and 4 received placebo FMT. Biopsies were immunostained by Avidin biotin complex method for chromogranin A, serotonin, somatostatin, peptide YY, enteroglucagon and pancreatic polypeptide, and quantified using computerized image analysis. Allocation sequence for FMT was revealed after obtaining re-biopsies and quantification of enteroendocrine cells.

Results: Eight of ten responders and non-responders received donor FMT with a change in IBS-SSS scores (mean±SEM) by comparing baseline to 12 months, (280.6±10.3 and 180.5±25.8, *P*=.0015) and (291.2±16 and 270±17, *P*=.15), respectively. Using Paired *t*-test to compare cells densities 1 year post FMT to baseline showed only significant change in somatostatin cells in responders group (*P*=.023) and no significant change the non-responders' group. A subanalysis of recipients of donor FMT showed significant changes in Peptide YY (*P*=.04) and Enteroglucagon (*P*=.035) cells. No significant changes were noted in the placebo FMT subgroup.

Conclusion: This study shows that the densities of enteroendocrine cells change following FMT in the responders group and in those who received donor FMT. This observation emphasizes the interaction between gut microbiota and enterendocrine cells. The mechanisms behind these interactions remain to be investigated.

Policy of full disclosure: None.

140 | Severe system reactions at children with intolerance to fish

A. Khavkin[1]; N. Esakova[2]; E. Varlamov[2]; A. Pampura[2]; O. Komarova[2]

[1]Pirogov Russin National Research, Moscow, Russia; [2]Pirogov Russin National Resear, Moscow, Russia

Objective/Background: The aim of this study is to evaluate clinical features of severe systemic reactions at children with intolerance to fish.

Methods: 160 children with intolerance to fish were included in study. Age of children varied from 8 months to 18 years with median at 6 years ([Q1 3 years; Q3 10 years]). All the children were interviewed by the authors developed questionnaire. Clinical data associated with the allergy history and the reports were evaluated according to European Academy of Allergy and Clinical Immunology diagnostic criteria of anaphylaxis. An allergy survey was performed in all children. Specific IgE concentration to fish in serum was tested with a fluoro-immunoassay (ImmunoCAP 100, Phadia AB, Sweden).

Results: Anaphylaxis to fish was retrospectively diagnosed in 16.8% (n=27) children. Specific IgE levels≥0.35 kUA/l to fish was identified in all children with anaphylaxis to fish (Me=34.5 kU/L [Q1-15,4; Q3-100]). Single episode of anaphylaxis to fish was diagnosed in 77.8% of children, more than one episode in 22.2% of patients. 3 children had anaphylaxis after the first in a life of eating fish. During the first 15 minutes anaphylaxis was developed in 21 (77, 8%) children, 15-30 minutes—in 5 cases (18.5%), in 1 patient the time of development anaphylactic reaction was more than 1 hour. Anaphylaxis was developed after eating fish in all patients. In addition, symptoms of anaphylaxis was occurred

after inhalation and skin contact in 38.5% (n=10) and 4 (14.8%) patients, respectively. Skin/mucosa and respiratory system involvement were the most frequent (97.5% and 92% respectively). Cardiovascular systems were involved in 44.4% of cases. Severe anaphylaxis was recorded in 22% (n=6) patients. Moderate and mild anaphylaxis founded in 66% (n=18) and 11% (n=3) patients, respectively.

Conclusion: Anaphylaxis is diagnosed in 16.8% of patients with intolerance to fish. Anaphylaxis to fish may occur during the first in the life eating fish. The management of children with anaphylaxis to fish should include not only the complete elimination to fish, but also preventing any possibility of exposure (inhalation, skin contact) of the fish allergen.

Policy of full disclosure: None.

141 | Peculiarities of the upper gastrointestinal tract motility in children with atopic dermatitis

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Objective/Background: The aim is studying the motility state of the upper gastrointestinal tract in children with atopic dermatitis.

Materials and methods: There were investigated 30 children aged 10-17 years (mean age 13.4±2.9) suffering from chronic gastritis (CG) and atopic dermatitis (AD). The comparison group included 33 children with CG. The main group included patients with AD in mild and moderate stages. Clinical examination was carried out using SCORAD. All the patients underwent standard gastroenterological examination which included gastrofibroscopy (GFS). Statistical analysis was performed with Statistica package for Windows (Version 6.0).

Results: In 83.3% of children with AD there was detected sensibilisation to cow milk protein, to goat milk protein—in 66.6%, to eggs—in 79%. The reaction to fish was detected in 66.6% of children, to chicken—in 50%, to carrot—in 56.6%, to wheat—in 36.6%, to oat—in 23.3%. The sensibilisation to domestic and epidermal allergens was found in 6.67% of children with AD and CG; to arborescent pollen—in 15.6% and to grass pollen—in 6.6% of patients. There were no reliable differences in estimating physical condition in both groups. The patients from both groups equally complained of epigastric burning (40.00% and 30.30% $P>.05$). According to FGS data, erosive esophagitis was more often detected in patients with AD in gastroesophageal reflux (26.67% and 6.06%, $P<.05$) and incompetence of cardia (20.0% and 3.03%, $P<0.05$). Nonetheless, no reliable differences in the presence of duodenogastric reflux were detected (26.67% and 27.09%, $P>0.05$).

Conclusions: In children with AD, the presence of erosive reflux esophagitis was detected more often. A fundamental study is required to study the relationship between atopic dermatitis and motility disorders of the upper gastrointestinal tract in children.

Policy of full disclosure: None.

142 | Innervation and chemical taxonomy of gastric enteroendocrine cells

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The stomach is a major center for the integration of responses to a meal. It detects volume, nutrient content and luminal chemistry. Luminal receptors expressed by gastric enteroendocrine cells (EEC) are in contact with gastric content and release hormones that signal to vagal afferent neurons. In turn, efferent nerves control secretion from at least some EEC. Despite their obvious importance, there is no inventory of gastric EEC or the neurons that connect with them. To address this, we are characterising the EEC taxonomy of rat and human stomach and measuring the proximity of populations of nerve fibers. We have utilised triple label immunohistochemistry of cryosections of antral and oxyntic mucosa with antibodies to ghrelin and gastrin (for EEC) and VIP, CGRP or VAChT, for axons. In rat, gastrin cells were evenly distributed in the base of the antral mucosa with an open type morphology, ghrelin cells were distributed throughout the length of the antral and oxyntic mucosa with a closed morphology. In human antral tissue gastrin was less evenly distributed with some glands containing many gastrin and somaostatin cells and other glands containing none. Ghrelin and gastrin cells had an inverse relationship in human antrum. Co-storage of EEC hormones in either tissue was rare. Nerve fiber proximity to each of the EEC populations was measured in confocal images using Imaris 3-D reconstruction. In rat, ~25% of ghrelin cells had VIP fibres appear to contact the cell and 48% were within 2 µm of a VIP fiber whereas only 5% of the same cells were within 2 µm of a CGRP fiber (158 ghrelin cells). Characterisation of the chemical taxonomies of target cells of vagal innervation in the stomach will assist with the identification of optimal sites for disease-controlling vagal neuromodulation.

Policy of full disclosure: None.

143 | The influence of gluten free diet on the enteric nervous system and intestinal microbiota of mice

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Gluten is a complex mixture of hundreds of related, but distinct proteins, which serve as main storage proteins of wheat grains. It is responsible for distinct diseases and symptom complexes in the gastrointestinal tract (GIT), such as celiac disease, non-celiac gluten sensitivity or wheat allergy. Especially celiac disease can lead to intestinal inflammation and a gluten free diet is often considered the only treatment. So far it is not clear, whether and how the intrinsic innervation of the GIT is involved in the disease complex. We therefore investigated the impact of gluten upon gut motility and the enteric nervous system (ENS). Mice were fed with either a chow that contained 25% of gluten, or with a gluten-free diet. Gastrointestinal motility analyses of individual gut segments were performed. Additionally, differentiated myenteric plexus cultures were exposed to gluten in vitro. Finally, the intestinal microbiota of different GIT sections was investigated by massive 16S rRNA gene sequencing. Intestinal motility was changed in mice receiving the gluten free diet compared to mice fed with gluten. There was an increased gut diameter in mice with gluten free diet compared to gluten diet. A reduced neural outgrowth and clustering of the enteric nervous system cells was found in gluten stimulated compared to untreated cultures. The intestinal microbiota composition also showed significant shifts under the influence of gluten: The bacterial microbiota of animals fed with gluten showed a significantly increased share of Proteobacteria. In addition, the Firmicutes to Bacteroidetes- ratio decreased with the gluten-content of the diet, and an overall decrease in alpha diversity was observed. In conclusion, gluten directly impacted the enteric nervous system cells as well as gut physiology and microbiota composition.

Policy of full disclosure: None.

144 | Co-storage of enteroendocrine hormones evaluated at the cell and subcellular levels

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Recent studies reveal complex patterns of hormone co-expression within enteroendocrine cells (EEC), contrary to the traditional view that gut hormones are almost always expressed individually in EEC. Moreover, some co-expressed hormones have been found in separate subcellular vesicles. However, detailed analysis of relative expression and storage of multiple hormones has not been made, nor has the functional significance of hormone co-expression been explored. Co-expression of 5-HT, CgA, secretin, CCK, ghrelin, and GLP-1 in mouse duodenum was quantified at a cellular and subcellular level by semi-automated cell counting and quantitative vesicle measurements, and the co-localisation of these hormones at a vesicle level was analysed. Separate subcellular stores of 5-HT, CgA, secretin, CCK, ghrelin, and GLP-1 were identified. The indolamine, 5-HT, and the neuroendocrine protein, chromogranin A (CgA) were frequently expressed in EEC that contained peptide hormones. Almost all combinations that were investigated occurred, for example co-localisation of ghrelin, 5-HT and GLP-1 revealed cells with only ghrelin, only 5-HT, ghrelin plus 5-HT, ghrelin plus GLP-1 and with all three hormones. In some cases, high resolution analysis revealed small numbers of immunoreactive vesicles in cells dominated by a different hormone. Thus the observed incidence of cells with co-localised hormones is greater when analysed at a subcellular, compared with a cell level, where a small number of vesicles may not reach the threshold for detection. We are investigating whether endogenous hormones are co-released, and whether they have synergistic effects on ion transport. We conclude that EEC exhibit substantial heterogeneity, including the co-localisation of hormones that were formerly thought to be in cells of different lineages. Co-localised hormones are presumed act together to provide an integrated response to stimulation. Separate packaging of hormones that are co-localised is a general feature of EEC, suggesting that differential release of hormones from individual cells may occur.

Policy of full disclosure: None.

MIXED TOPICS I

145 | Effect of faecal microbiota transplantation on the symptoms and gut microbiota in patients with Irritable Bowel Syndrome (IBS)

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Objective: Alterations in the gut microbiota play an important role in irritable bowel syndrome (IBS). The aim was to investigate the effect of faecal microbiota transplantation (FMT) on the gut microbiota and symptoms of patients with IBS.

Methods: The study included 13 patients (4 females and 9 males, age range 20-44 years) with IBS according to Rome III criteria and 13 healthy donors. The patients received freshly donated feces from a relative and was administered in to the descending part of the duodenum via a gastroscope. Faeces were collected from the donors and the patients before FMT and from the patients after 1, 3, 12 and 20/28 weeks and were stored in special freezers (80°C) until analysis. Microbiota analysis was performed using the GA-map Dysbiosis test (Genetic Analysis AS, Oslo, Norway). The patients completed the following questionnaires before FMT and again at 1, 3, 12, 20/28 weeks after FMT: IBS symptom questionnaire (IBS-SQ), IBS-symptom severity scoring system (IBS-SSS), short form of Nepean Dyspepsia Index (SF-NDI) and Bristol stool scale.

TABLE 1 Mean normalized signal for probes sorted by significant difference between donors and patients with irritable bowel syndrome before faecal microbiota transplantation (FMT) and 1, 3, 12 and 20/28 weeks after FMT

Bacteria strain		Donors, Beginning of study, *n*=13	Recipients					Donors End of study, *n*=10	p* Before FMT	p** After 1 week	p*** After 3 weeks	p**** After 12 weeks	p***** After 20/28 weeks	p****** Donors
Phyla	Genus		FMT day, *n*=9	After 1 week, *n*=12	After 3 weeks, *n*=9	After 12 weeks, *n*=13	After 20/28 weeks, *n*=12							
Firmicutes, Tenericutes, Bacteroidetes		244±29	128±29	143±32	179±50	228±67	195±64	221±38	0.4	>0.9	>0.9	>0.9	>0.9	0.6
	Ruminococcus gnavus	4.6±1.1	40±15.6	8±2.3	15.3±9	25±17	8.1±1.8	8.8±2	**0.015**	>0.9	0.15	0.44	>0.9	0.19
	Bacteroides	27.2±4.1	38.9±8.3	47.1±16	30.9±3.7	55.4±12	49.3±11	42±5.2	>0.9	>0.9	>0.9	**0.02**	0.1	0.097
	Bacteroides, Prevotella	483±51.4	634±28	599±25.8	551±64	783±49	731±52	788±58	0.7	>0.9	>0.9	**0.005**	**0.02**	**0.009**
	Alistipes	100.5±17	100.5±2	119±17.4	140±27	186±11	208±9.6	188±15.5	>0.9	>0.9	0.9	**0.011**	**0.0006**	**0.03**
	Parabacteroides	7.6±1.8	7.9±2.9	8.3±2	14.6±5.4	11.8±1.8	15.7±3.5	19.5±3.8	>0.9	>0.9	>0.9	0.4	0.3	**0.03**
Actinobacteria		287±45	66.6±13	95±23	197±54	138±29	92±23	204±57	**0.0010**	**0.007**	0.7	0.2	**0.003**	**0.018**
	Bifidobacterium	324c57	65±13	97±25	205±57	150±34	92.5±25	241±72	**0.0011**	**0.008**	0.6	0.2	**0.004**	**0.017**
Proteobacteria		27±7.8	195±121	565±162	63.8±29	105±70	56.5±40	17±1.9	>0.9	**0.03**	>0.9	0.9	>0.9	0.5
	Shigella, Escherichia	62±23	260±124	578±128	116±45	188±77	90.9±65	41±14	>0.9	**0.002**	0.8	>0.9	>0.9	0.8

Data are presented as the mean±SEM. Comparison: Kruskal-Wallis multiple comparisons test with Dunn's post test: *Donors at the beginning of the study vs. patients on FMT day before faecal installation, **Donors at the beginning of the study vs. patients 1 week after FMT, *** Donors at the beginning of the study vs. patients 3 weeks after FMT, ****Donors at the beginning of the study vs. patients 12 weeks after FMT, *****Donors at the beginning of the study vs. patients 20/28 weeks after FMT; and Paired *t* test: ******Donors at the beginning vs. end of the study. FMT: faecal microbiota transplantation.

Results: The bacterial strain signals of Actinobacteria and Bifidobacteria were significantly reduced in IBS patients before receiving FMT compared to their donors (*P*=.0010 and .0011, respectively). These changes became non-significant after 3 weeks. Bacterial strain signals of Bacteroides/Prevotella, Alistipes and Parabacteroides significantly increased (*P*=.009, .03 and .03, respectively) at 20/28 weeks after FMT compared to the donors at the beginning of the study. The respective signals for Actinobacteria and Bifidobacteria were again significantly reduced (*P*=.003 and .004, respectively). Using RM-ANOVA, the scores of IBS-SQ were significantly reduced during the first 3 weeks after FMT; total (*P*<.0001), nausea (*P*=.001), bloating (*P*<.0001), abdominal pain (*P*=.0005), constipation (*P*=.027), diarrhea (*P*<.0001), but not for anorexia (*P*=.09). The total scores of IBS-SSS, SF-NDI and Bristol stool scale during the whole study were also significantly reduced after receiving FMT (*P*=.008, .0045 and .07, respectively). No adverse effects were reported.

Conclusion: This study shows that FMT helps in restoring the alterations in the gut microbiota in IBS patients during the first 3 weeks and improves their symptoms and quality of life for up to 28 weeks after FMT.

Policy of full disclosure: None.

146 | Parasympathomimetic agents inhibit pancreatic cancer growth by suppression of the p44/42 MAPK signalling pathway and increase overall survival

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Objective/Introduction: The vagus nerve and parasympathetic nervous system feature a suppressive effect on acute and chronic inflammation. It is therefore conceivable that the parasympathetic nervous system may also play a role in pancreatic carcinogenesis due to the known link between chronic inflammation and carcinogenesis. Therefore we investigated the potential effect of the parasympathetic nervous system on both proliferation and invasion of pancreatic cancer (PCa) and enlighten its linked intercellular signaling pathways in an in vitro and in vivo model.

Methods: Human PCa cell lines were exposed to direct and indirect parasympathomimetic agents and proliferation was quantified via the MTT-proliferation assay. Cell invasion was investigated via the matrigel invasion assay with subsequent fluorescence staining. Phosphorylation of p44/42 MAPK was determined and quantified

by immuno-blot analysis. Human tissues were immuno-stained for Acetylcholine Esterase and correlated with histopathological grading of specimen. In an invivo xenograft model, PCa cells were injected subcutaneously into Crl:NMRI-Foxn1nu nude mice and tumor area, metastasis as well as infiltration of immune cells were assessed after operative tumor extirpation. Additionally, pyridostigmine was administered during adjuvant and palliative chemotherapy in a resectable, mutated Kras-oncogene-based mouse model of PCa.

Results: The MTT proliferation assay showed a dose dependent suppression of PCa cell proliferation after treatment. The matrigel invasion assay experiments revealed a dose dependent inhibition of PCa cell invasiveness. These results were associated with a lower intracellular phosphorylation of p44/42 MAPK and corresponded with the results obtained in the in vivo experiments, in which both tumor growth and invasiveness were inhibited subsequent to prophylactic and therapeutic treatment in the xenograft model, where lower amounts of tumor-infiltrating macrophages found in the extirpated tissues, Moreover, administration of pyridostigmine as an adjunct to chemotherapy during adjuvant or palliative chemotherapy enabled longer overall survival of mice in a novel (and only) resectable model of PCa.

Conclusion: The systemic administration of activators of the parasympathetic nervous system restrains PCa proliferation and invasiveness via suppressing the intracellular phosphorylation of the p44/42 MAPK signaling pathway. Furthermore, parasympathetic activation modulates and inhibits immunological responses and increases overall survival in pancreatic cancer, opening a novel opportunity for treating gastrointestinal cancers.

Policy of full disclosure: None.

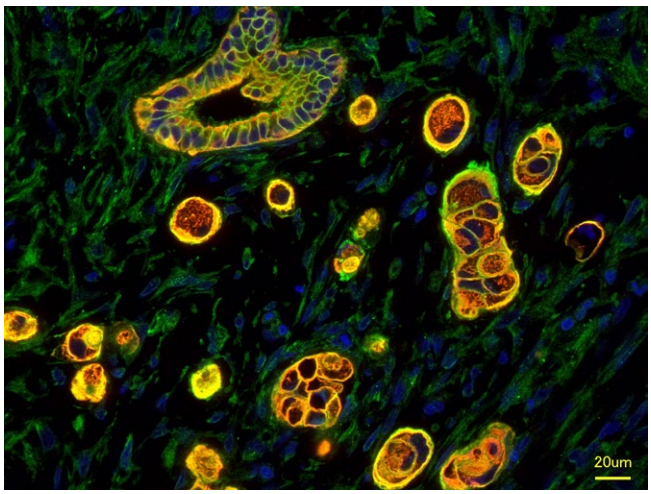

FIGURE 1 AChE, CK19 and DAPI stained anaplastic pancreatic cancer cells

147 | Wireless optogenetics: Development of simultaneous activation of multiple light emitting diodes (green and blue) for activation or inhibition of multiple different neural pathways

N. Spencer[1]

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Objective: A major recent advance in optogenetics is the ability to provide untethered (wireless) activation of miniature light emitting diodes (LEDs) to activate opsins. Our aim was to determine whether multiple LEDs can be simultaneously illuminated wirelessly, using both green and blue LEDs.

Methods: We used a 1.5 GHz resonant cavity to power miniature LEDs. Light power output was measured by affixing a 1 mm diameter optical fibre to form a measurement probe. Extra receivers(with LEDs) were placed in line along the axis of their receiving coils at 4.5 mm spacing, first one to the left, then one to the right, then a second one on the right to give four receivers in a row. Light power output was recorded for each configuration.

Results: mean values of the percentage drop in original light output were plotted, as extra receivers (with LEDs) were added. With 2 LEDs activated, there was no significant drop in light intensity (2.4% decrease; P=.16; N=3) from a mean of 41.9 μW to a mean of 40.9 μW. With 4 LEDs activated there was a significant drop in light output by 10.3%; P=.003; N=3). Green and blue LEDs could be activated simultaneously.

Conclusion: Wireless optogenetics can be used to activate multiple LEDs (green and blue) simultaneously, which opens the potential to inhibit and/or excite multiple neural pathways at the same time and at different locations within the same animal, in conscious untethered animals (eg, along the intestine in vivo). This is the first demonstration that green LEDs can be activated wirelessly.

Policy of full disclosure: None.

148 | Innervation pattern of the distal mucosal squamous epithelium may underlie hyposensitivity to acid reflux in patients with Barrett's oesophagus

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Introduction: Many patients with Barrett's oesophagus have little history of heartburn. Patients with Barrett's oesophagus are hyposensitive to experimental acid exposure compared to patients with NERD. We have recently proposed that the location of oesophageal mucosal afferent nerves may be important in oesophageal acid sensitivity We have demonstrated that distal oesophageal nerves are more superficial (ie, closer to the lumen) in patients with NERD than in healthy

volunteers (Woodland et al. 2015, 2016). We hypothesised that reflux hyposensitivity in Barrett's oesophagus may be due to a deeper location of mucosal afferent nerves in the squamous epithelium of the distal oesophagus, proximal to the Barrett's mucosa. We compared the location of oesophageal afferent mucosal nerves in the distal squamous mucosa of healthy volunteers, patients with Barrett's oesophagus and patients with NERD.

Subjects: 18 patients with Barrett's oesophagus (C0-5, M2-6) and 15 patients with NERD (typical reflux symptoms, normal endoscopy, and pathological oesophageal acid exposure). In Barrett's patients, endoscopic mucosal biopsies were taken from within the most distal 2 cm of squamous mucosa. In NERD patients, biopsies were taken from 3 cm above the gastro-oesophageal junction. Biopsies were fixed and prepared in 10 µm. Slides were examined immunohistochemically for presence of calcitonin gene-related peptide (CGRP)- and protein gene product (PGP) 9.5 immunoreactive nerve fibres. Where fibres were identified, their location in the mucosa was recorded in terms of cell layers from luminal surface. Results were compared to previous data from distal oesophageal (3 cm above GOJ) biopsies from asymptomatic volunteers. Results In the distal squamous mucosa of patients with Barrett's oesophagus afferent nerves were found a mean of 19.8±1.6 cell layers from the luminal surface. This was significantly deeper than seen in patients with NERD (8.7±2.0, $P<.01$), but no different to healthy volunteers (23.8±1.6).

Conclusion: The distal oesophageal squamous mucosal afferent nerve distribution in patients with Barrett's oesophagus resembles that of healthy volunteers, in contrast to the superficial nerve location in patients with NERD. Deeper mucosal innervation may underlie hyposensitivity to reflux perception in Barrett's oesophagus.

Policy of full disclosure: None.

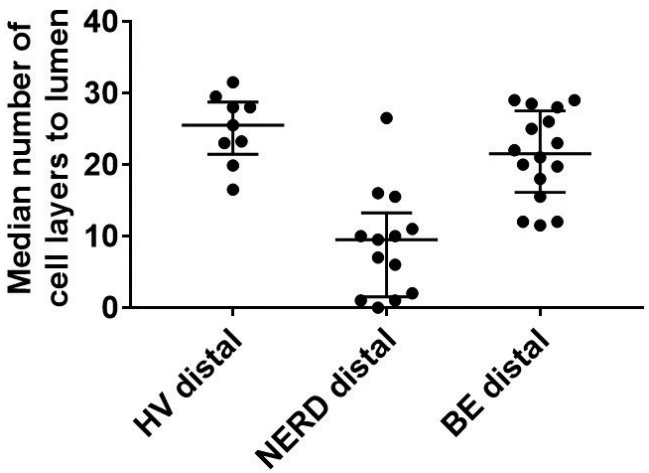

FIGURE 1 Relative distributions of distal esophageal nerves in HV, NERD and BE

149 | Chronic gut leakiness induces gender-specific neurobehavioral alterations in transgenic CA-MLCK mice

M. Rincel[1]; L. Xia[2]; C. Monchaux deOliveira[2]; J. Thomas[2]; V. Bacquie[3]; L. Gros[2]; A. Dinca[2]; S. Barnett Burns[4]; Y. Matime[5]; J. Turner[6]; L. Capuron[5]; V. Théodorou[7]; L. Ferrier[8]; M. Darnaudéry[2]

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There is a bidirectional gut-brain communication that plays a major role in neurodigestive functions but also in the regulation of behavioral processes. To date, most of the research has been focusing on the gut microbiota, and the intrinsic role of gut permeability in the regulation of behavior remains unexplored. Gut leakiness is regulated by epithelial tight junction opening, under the control of the myosin light chain kinase (MLCK). A previous study showed that transgenic mice expressing a constitutively active form of the MLCK specifically in the gut (CA-MLCK mice) develop mucosal immune activation and are more sensitive to experimental colitis. Here, using CA-MLCK mice, we examined the consequences of chronic gut leakiness on brain and behavior. Adult naive male and female CA-MLCK mice were submitted to a battery of behavioral tests for memory, sociability, emotional behavior, anhedonia, and endocrine response to acute stress. Furthermore, in order to identify brain regions potentially underlying the behavioral phenotypes, we conducted brain-wide immunomapping of c-Fos induction in response to stress. Finally, we measured cytokine mRNA levels in different brain regions to test the hypothesis that intestinal hyper-permeability leads to neuroinflammation. Our preliminary results reveal that gut leakiness induces spatial memory impairment in males and exacerbated anxiety-like behavior in females. In addition, both sexes display anhedonia and altered responsiveness of the hypothalamic-pituitary-adrenal axis to stress compared with wild-type controls. These findings provide evidence that gut barrier dysfunction could play a critical role in the regulation of complex behaviors. Further investigation will shed new light on the molecular mechanisms involved in the brain-gut alterations reported here.

Policy of full disclosure: None.

150 | Influence of dimethyl fumarate on murine postoperative ileus

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Dimethyl fumarate (DMF) is used to treat multiple sclerosis and preclinical studies suggest that its immunosuppressive and neuroprotective effects are related to induction of heme oxygenase (HO)-1; this enzyme degrades heme to ferrous iron, CO and biliverdin. Induction of HO-1 is partially responsible for the anti-inflammatory effect of CO-releasing compounds in murine postoperative ileus (POI). The effect of DMF on the intestinal inflammation and on the delay in gastrointestinal transit caused by POI was therefore studied. C57Bl6J mice were anesthetized and POI was induced by compressing the small intestine (intestinal manipulation; IM) for 5 minutes. DMF was administered intragastrically (i.g.; 100 mg/kg) or intraperitoneally (i.p.; 30 mg/kg) 24 hours before IM. Intestinal transit was measured via fluorescence imaging 24 hours after IM; after removal of the mucosa, small intestinal muscular segments were stored for later biochemical analyses. Pre-treatment with DMF via both i.g. (Geometric centre [GC] of transit: 7.4±0.4; mean±SEM of n=6) and i.p. (GC: 7.7±0.9) administration prevented the delayed transit seen after IM (GC: 4.6±0.7; in controls 7.5±0.3). I.g. and i.p. DMF significantly reduced the increased interleukin-6 levels in the intestinal muscularis caused by IM, but the leukocyte infiltration (myeloperoxidase activity) was only significantly reduced with i.p. DMF. IM per se caused a significant increase in intestinal HO-1 protein expression as previously shown; this effect was not enhanced with DMF. Nuclear factor E2-related factor-2 (Nrf2), the key transcriptional regulator of anti-oxidant mechanisms including HO-1, was not increased within the nuclear fraction of the intestinal muscularis from operated mice. Also at 12 and 24 hours after administering DMF in non-operated animals, no increase in HO-1 levels was measured. The present study indicates that both i.g. and i.p. administration of DMF prevent delayed intestinal transit and reduce inflammation upon IM, independently of intestinal HO-1 induction. The possible role of Nrf2/HO-1-independent inhibition by DMF of the proinflammatory NF-κB pathway is currently under study.

Policy of full disclosure:

None.:

151 | Investigation to prevent postoperative ileus via peroral CO

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Inhaled CO and intraperitoneal injection of CO-releasing molecules prevent postoperative ileus (POI). Steiger et al. developed Oral CO Releasing System (OCORS) tablets for oral delivery of CO (J. Contr. Release 2014). The gastrointestinal distribution and the effect on murine POI of OCORS tablets with 0.5 mm diameter was studied. Gastric emptying was studied in C57Bl6J mice fastened for 1 or 6 hours before gavaging 4 or 20 tablets. One or 2 hours after application, blood COHb was measured and the gastrointestinal tract was explanted to locate the tablets and to assess their in vitro CO release profile. POI was induced by small intestinal manipulation (IM), 1 hours after gavaging 20 OCORS tablets in mice fasting for 6 hours. Transit was measured via fluorescence imaging 24 hours after IM; intestinal muscular segments were stored for biochemical analyses. When administering 4 OCORS tablets after 6 hours of fasting, they had all left the stomach within 1 hours, which was not the case after 1 hours of fasting. The retrieved tablets had released the majority of their CO content, but blood COHb levels did not increase. When administering 20 OCORS tablets after 6 hours of fasting, a mean of 11 tablets was present in the small intestine 1 hours after application; COHb blood levels increased to 5.2±0.3% (mean±SEM of n=6). Pre-treatment with 20 OCORS tablets did not prevent the delayed transit seen after IM (Transit geometric centre: 8.2±0.3 for non-operated controls; 3.4±0.3 for IM; 4.1±0.7 for OCORS and IM; n=7). The increase in intestinal myeloperoxidase activity and IL-6 levels by IM was also not influenced. Intravenous sodium nitrite, used as positive control, prevented the delay in transit and reduced the intestinal inflammation. The present study indicates that the amount of CO released from 20 gavaged OCORS tablets was not able to influence murine POI.

Policy of full disclosure: None.

152 | Inflammatory conditions favor the interactions between T cells and enteric glial cells

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Control of the gastrointestinal homeostasis is a tremendous task in which glial cells of the enteric nervous system (ENS) are involved. Present in the intestinal wall, and notably, in the ganglions of the myenteric and submucosal plexus, enteric glial cells (EGC) secrete cytokines and chemokines that could impact the immune cells in physiological and pathological conditions. In particular, nothing is known about their implication in the formation of plexitis in chronic inflammatory digestive diseases. However, such information is important as the accumulation of immune cells inside and around the enteric neuronal ganglia of patients affected by Crohn's disease is predictive of disease recurrence after post-operative resection. As a first approach to answer this interrogation, the interactions between immune and enteric neural cells were analyzed in vitro using primary cultures. Immunocytochemistry analysis showed that non-activated T lymphocytes were capable of interacting with EGC. They also showed that activation of T cells with anti-CD3/anti-CD28 antibodies increased the number of lymphocytes interacting with EGC. To determine whether inflammatory conditions favored the interactions between glial and immune cells, EGC were treated with LPS or TNFaplha/IL1beta prior their exposition to T cells. We observed an increased number of T lymphocytes interacting with EGC pretreated with inflammatory

stimuli. This phenomenon was also noted with activated T cells. Taken together, these observations indicate that T cells interact with EGC. Their interactions are favored by T cell activation or if ECG are treated with inflammatory cytokines. Further experimentations are required to characterize the T cells that interact with EGC and the molecular mechanisms implicated in these interactions. This work is supported by the Association François Aupetit.

Policy of full disclosure: None.

153 | Acute tryptophan depletion increases esophageal sensitivity to acid perfusion in health

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Introduction & objective: Esophageal hypersensitivity is considered to be an important pathophysiological mechanism in patients with refractory gastro-esophageal reflux disease (rGERD). Since serotonin (5-HT) plays a major role in the regulation of GI motility and sensitivity, our aim was to study the effect of acute tryptophan depletion (ATD) on esophageal sensitivity in healthy volunteers (HV). ATD temporarily reduces the availability of tryptophan (TRP), thereby decreasing central and peripheral 5-HT synthesis.

Methods: Esophageal sensitivity was assessed after intragastric infusion of an amino-acid mixture (AA-mix) containing 15 AAs with TRP (control) or without TRP (ATD). After a 5 hours incubation period, a probe with a polyurethane balloon was positioned in the distal esophagus. Thermal (recirculating a heated saline solution through the balloon), mechanical (balloon distention), electrical (stimulation electrodes) and chemical sensitivity (modified Bernstein) were tested. Stimuli were evaluated for first perception, pain perception threshold (PPT) and pain toleration threshold (PTT). At 3 time points blood samples were collected. General mood was assessed by Positive and Negative Affect Schedule (PANAS) and State-Trait Anxiety Inventory (STAI) questionnaires. Results were analyzed using paired *t*-tests (or Wilcoxon matched-pairs signed-rank test) and two-way ANOVA repeated measures.

Results: In 15 HV (7 m/8f, mean age 24 year [21 year-33 year]), ATD reduced plasma levels of TRP 5 hours and 7 hours after administration of the AA-mix (P=.0005, P<.0001). ATD decreased PPT during chemical stimulation (P=.0172) with a pronounced effect size (Cohen's d+=0.67) (Table 1). No effect on sensitivity to the other stimulation modalities was found and no differences in PANAS and STAI-State scores were demonstrated.

Conclusion: To our knowledge, this is the first study to address the effect of ATD on esophageal sensitivity in health. ATD significantly decreased pain perception threshold during chemical stimulation, without affecting sensitivity to thermal, mechanical or electrical stimulation. These findings may have implications for the understanding and treatment of rGERD or functional heartburn.

Table 1: Results of esophageal multimodal stimulation for control condition and acute tryptophan depletion (ATD) in healthy volunteers.

	Control	ATD	p-value $_{uncorrected}$	Cohen's d^{+}
Temperature (°C)				
PPT	42.66 [40.56-44.91]	43.71 [41.22-49.57]	0.19	0.28
PTT	46.13 [42.33-48.55]	46.32 [45.08-51.93]	0.08	0.46
Mechanical (ml)				
PPT	17.15 [16.00-19.40]	17.05 [11.85-21.50]	0.71	0.07
PTT (n=13)	21.46 [19.55-24.35]	25.00 [17.90-30.80]	0.05	0.38
Electrical (mA)				
1st perception	4.67 [4.00-6.50]	5.17 [3.83-11.83]	0.50	0.48
PPT	9.17 [7.50-14.33]	10.00 [6.83-19.50]	0.39	0.37
Chemical (ml)				
1st perception	8.00 [7.00-15.00]	8.00 [6.00-11.00]	0.21	0.30
PPT	25.00 [14.00-29.00]	16.00 [13.00-24.00]	**0.0172***	**0.67**
PTT	30.00 [24.00-40.00]	29.00 [18.00-36.00]	0.36	0.26

Results are presented as median [25th -75th percentile]. n=15, unless indicated otherwise since only HV reaching the sensitivity threshold were taken into account for analysis. A p-value of <0.05 was considered significant. Effect size expressed as Cohen's d+ (0.2=small effect, 0.5=medium effect, >0.8 large effect). *: survives Bonferroni correction. ATD=acute tryptophan depletion, PPT=pain perception threshold, PTT=pain tolerance threshold.

Policy of full disclosure: None.

154 | Cost effective of IBDoc as a surrogate marker of mucosal healing in IBD patients post induction of biological agents

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To access the cost effectiveness of using IBDoc faecal calprotectin as a surrogate marker of mucosal healing in IBD patients post induction of biological agents Methods: Retrospective study of 131 patients with IBD commended on biological agents. IBDoc calprotectin measured at 3 month and at 6 month using home IBDoc kit.

Results: 40% had normal IBDoc at 3 month leading to avoidance of 53 clinic follow up visits of these 75% had normal IBDoc at 6 month resulting in avoidance of 40 follow up ileocolonoscopy 60% of patients had abnormal IBDoc at 3 month of these 28% had normal IBDoc at 6 month after treatment escalation resulting in avoidance of 28 colonoscopy Conclusion:IBDoc is a new technology easy to use at home and cost effective ,resulted in saving over €40.000 over 6 month in this study.

Policy of full disclosure: None.

155 | Validation of the Korean version of the GerdQ questionnaire for diagnosis of gastro-esophageal reflux disease

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Background: The incidence of gastro-esophageal reflux disease (GERD) is rapidly increasing in Asian countries including Korea. However, no single ideal test is available to confirm the diagnosis. GerdQ has been used in the diagnosis of GERD in Western countries. However, its Korean version has not yet been validated. Moreover, its ideal cut-off value in determining GERD in the Korean population has not been clearly defined. Therefore, we aimed to assess the diagnostic accuracy of the Korean version of GerdQ, and to its reproducibility and concurrent validity.

Methods: After repeated translations and backward translations, the Korean version of GerdQ was prepared. Patients with symptoms suggestive of GERD were asked to answer to the GerdQ at their first visit. A second GerdQ questionnaire was then administered to the patients when they visited for their subsequent appointment for upper endoscopy, which was at least 2 weeks after the first visit. The final questionnaire was provided after proton pump inhibitor (PPI) treatment. Reflux esophagitis or pathological acid exposure was used as diagnostic references for GERD. The diagnostic accuracy of the GerdQ for GERD with regard to symptom response to PPI therapy was assessed.

Results: A total of 149 patients (52 male, 97 female) with a mean age of 52.6±14.6 years were enrolled. A GerdQ cutoff of 7 was found to the best balance with regard to sensitivity (64.6% [95% CI: 55.0-73.2], and specificity (69.4% [95% CI: 51.7-83.1)]. The intraclass correlation coefficient of two subsequently measured GerdQ scores was 0.65 (95% CI 0.52-0.75). Moreover, GerdQ had a high positive predictive value (86.9% [95% CI: 77.4-93.0]), but a low negative predictive value (38.5% [95% CI: 26.9-51.4)) for GERD.

Conclusions: The Korean version of GerdQ is a useful complementary tool for the diagnosis of GERD in primary care of Korea. Moreover, the relatively lower cut-off represents milder GERD symptoms in Korean patients than those in patients in Western countries.

Policy of full disclosure: Sponsored by Astra-Zeneca.

156 | Pathogenic mechanisms of esophageal peristaltic dysfunction by high resolution manometry in patients with systemic sclerosis

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Backgrounds/Aims: Our hypothesis is ineffective esophageal motility (IEM) of esophageal high resolution manometry (HRM) finding in systemic sclerosis (SSc) patients may reflect neural dysfunction with/without smooth muscle (SM) atrophy and absent contractility (AC) may reflect severe SM atrophy or extensive fibrosis. Aims of our study are to evaluate the esophageal reserved function of neuronal reflexes and muscle contractility in these three groups of SSc.

Methods: Patients diagnosed with SSc who underwent HRM during recent 3 years were retrospectively included (22 female, ages 25-75). Seventeen patients of them were underwent multichannel intraluminal impedance and pH (Imp-pH) study also. We analyzed LES pressure, HRM metrics according to the Chicago classification version 3, including distal contractile integral (DCI), DCI ratio of single swallow and multiple rapid swallow (MRS) test, and reflux parameters and the post-reflux swallow-induced peristaltic wave (PSPW) index by Imp-pH test. According to the HRM findings, patients were classified as 3 groups (normal HRM, n=5; IEM, n=8, and AC, n=9). We compared SM contractility by DCI, neuronal peristaltic reserve function by MRS test using HRM and the PSPW index using Imp-pH study in normal, IEM, and AC groups. Parameters among 3 groups were analyzed by ANOVA on ranks.

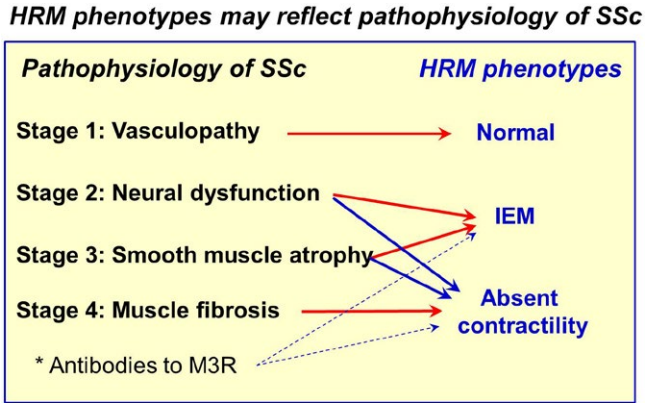

FIGURE 1

Results: DCI was significantly lower in AC and IEM group than normal group [median 0.00 (IQR 0.00-35.23), 172.80 (77.05-522.35), and 834.30 (789.85-2294.63) mmHg-s-cm respectively, $P<.001$]. DCI ratio of MRS/single swallow was lower in AC group than normal group [0.00 (0.00-0.08) vs 1.00 (0.45-1.19), $P=.030$]. Other HRM parameters including LES pressure were not different among three groups. PSPW index was lower in AC group than IEM and normal groups [0.40 (0.00-1.90), 8.95 (3.20-16.70), and 17.60 (10.0-35.40)% respectively, $P=.023$]. PSPW appearance time until 120 seconds were tended to variable in IEM group and AC group than normal group [SD 34.28 (32.29-44.89), 27.60 (16.97-32.03) and 19.60 (17.95-28.02) seconds respectively, $P=.079$]. Other reflux parameters were not different among 3 groups.

Conclusions: The AC using HRM may reflect all of SM atrophy, fibrosis and neuronal dysfunction and the IEM may reflect SM atrophy and mild to moderate neuronal dysfunction in patients with SSc (Figure 1).

Policy of full disclosure: None.

157 | The levels of pantoprazole in human breast milk and plasma: Two compartment model

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Objective: Proton pump inhibitors are the most widely used medications and their safety in the lactation is not known. Only one case report is available about PPI (omeprazole) concentrations in the human

breast milk. We aimed to evaluate the amounts of pantoprazole in human milk and plasma after oral administration to breast-feeding women and to estimate exposure of the fetus.

Methods: Twelve women who decided to stop breastfeeding were taken 40 mg pantoprazole once a day of for 7 days. Blood and milk samples were collected at day 1 and day 7 at 0-1.5-3-4.5-6-9-12th hours. A selective and rapid HPLC method was developed and validated for quantification of pantoprazole in plasma and breast milk samples using Omeprazole as internal standard. Pantoprazole was extracted from biological matrix by using Liquid-liquid extraction process. The method was validated over a linear concentration rage of 0.03-1 µg/mL and the limit of quantification (LLOQ) was 0.03 µg/mL.

Results: The plasma level was 1229.9±1160.2 (61.6-4255.6) ng/mL at day 1 and 1248.9±1448.7 (86.4-5475.8) ng/mL at day 7. The mean concentration in the milk was 61.9±36.9 (32.7-141) ng/mL for day 1 and 152.5±217.7 (32.5-762.5) ng/mL for day 7. 21 out of 97 milk samples and 47 out of 98 plasma samples were contained pantoprazole in all time points. The frequency of pantoprazole at day 1 was 20.8% in the milk and 43.8% in the plasma while they were 22.4% in the milk and 54.2% in the plasma at day 7.

Conclusions: The two compartment model was proposed to describe time profiles of pantoprazole in plasma and milk (plasma as central, milk as peripheral compartment). Pantoprazole's level of milk compartment was far less than plasma compartment. Since, the uncoated pantoprazole is unstable in acidic pH, the systemic dose received by the infant from the breast milk might be even lower. Our limited data implicate that women might not stop breastfeeding when taking pantoprazole.

Policy of full disclosure: None.

158 | Anti-inflammatory mechanisms of action in FD and IBS: The example of STW 5

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Introduction: Inflammation is involved in the etiology of Functional Dyspspsia (FD) and Irritable Bowel Syndrome (IBS) as a potential cause. The question is how it can be addressed by therapy, as eg, the NSAIDs, used as anti-inflammatory drugs, can cause inflammations in the GI tract. As an example for an anti-inflammatory drug not causing GI side effects, data for a drug used in FD and IBS, STW 5 were analyzed. Methods: Data from in vitro studies were revealed and analyzed for elucidating mechanisms of action underlying anti-inflammatory effects.

Results: STW 5 activated COX-1, but not COX-2 mRNA expression, which was in contrast to the control substances, like ASS and diclofenac, which inhibited COX-1 and COX-2 mRNA Expression (1). STW 5 inhibited the increased gene expression and reduced significantly the release of TNF-alpha by activation of adenosine A2A receptors in LPS (100 ng/mL)-stimulated human monocytes, while having no effect in untreated cells (2). Radioligand binding assays confirmed the affinity of STW 5 to adenosine A2A receptors.

Conclusion: The mechanism of action of STW 5 is anti-inflammatory, despite not involving COX-1 or COX-2 inhibitory properties. This is a base for the very good tolerability of this medicinal product.

Policy of full disclosure: O. Kelber is employee of Innovation and Development, Phytomedicines Supply and Development Center, Bayer Consumer Health, Darmstadt, Germany. K. Nieber has received honoraries and travel grants from Innovation and Development, Phytomedicines Supply and Development Center, Bayer Consumer Health, Darmstadt, Germany.

References:

1. Michael, S., et al. 2012. Inflammatory Bowel Disease 3: 41;
2. Bonaterra, G. A., et al. 2008. Z. Phytotherapie 29: S22.

159 | Melanin-concentrating hormone receptor 1 expression in colon: A multiplex immunohistochemical study with colon from "normal" donors and patients with Inflammatory Bowel Disease (IBD)

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The neuropeptide melanin-concentrating hormone (MCH) regulates appetite but there is increasing evidence that MCH may contribute to the inflammatory pathology associated with inflammatory bowel disease (IBD) via activation of the G-protein coupled receptor, MCHR1; thus mRNA levels for both MCH and MCHR1 are elevated in human inflamed colonic mucosa. To investigate this further, the present study used multiplex immunohistochemistry to investigate the expression of MCHR1 at the protein level in resected colon samples from patients with IBD in comparison to 'normal' colon (resected colon tissue at least 15 cm away from the borders of a tumour) along with phenotypic markers to identify expressing cells. Specific MCHR1 immunoreactivity (rabbit polyclonal primary anti-MCHR1 affinity purified vs isotope control) was evident in colon from either 'control' donors or patients with IBD. MCHR1 immunoreactivity was evident in the outer regions of cells consistent with a cell membrane expression. Infiltration by immune cell subsets was evident in colon samples from either 'control' donors or patients with IBD although immune cell infiltration was particularly evident in the latter samples when using antibodies to phenotypic markers such as CD3 (T cells), CD11c (eg, dendritic cells, monocytes, macrophages) and CD14 (monocytes, macrophages). Multiplex immunohistochemistry with the phenotypic markers allowed demonstration of MCHR1 immunoreactivity co-localisation to often be evident. MCHR1 immunoreactivity was also evident in epithelia identified by their location and morphology. The present study has demonstrated the expression of MCHR1 immunoreactivity in the colon from 'control' donors

and also patients with IBD. In addition to expression by epithelia, the evident MCHR1 expression by immune cell subsets offers a further potential cellular target for MCH to mediate pro-inflammatory responses that may contribute to the pathology of IBD. Such findings indicate that MCHR1 receptor antagonists may offer benefits to patients with IBD.

Policy of full disclosure: None.

160 | Tickling the 5-HT3 receptor: Potential therapeutic opportunities for patients with diarrhea-predominant Irritable Bowel Syndrome (IBS) from the selective 5-HT3 receptor partial agonist, CSTI-300

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Objective: Whilst the 5-HT3 receptor antagonists display considerable therapeutic efficacy to reduce the symptoms of diarrhea-predominant irritable bowel syndrome (IBS-d) and cancer treatment-induced emesis, 'on-target' mediated adverse effects can limit their use (eg, constipation and ischemic colitis). As these adverse effects result from high levels of 5-HT3 receptor inhibition, an alternative strategy would be to use 5-HT3 receptor partial agonists that behave as pharmacological antagonists in an environment where 5-HT tone is high—as occurs in IBS-d and emesis—yet are incapable of complete 5-HT3 receptor inhibition, so avoiding the adverse effects. The present study evaluated the potential of a selective 5-HT3 receptor partial agonist with desirable pharmaceutical characteristics, CSTI-300, to treat IBS-d using in vitro and in vivo models using the 5-HT3 receptor antagonist, alosetron, as a comparator. In comparison to 5-hydroxytryptamine (5-HT), CSTI-300 displayed partial agonist activity at the human 5-HT3A receptor stably expressed in HEK293 cells assessed by $[Ca^{2+}]I$ (intrinsic activity approximately 50%), whilst alosetron failed to display intrinsic activity. Co-application of alosetron with 5-HT resulted in complete blockade of 5-HT3 receptor activity, yet co-application of 5-HT with a maximal concentration of CSTI-300 resulted in reduced receptor activity but not complete blockade (around 40%-50% activity remaining). Patients with IBS-d display colonic hypersensitivity and the rat colon distension model in vivo, offers an established assay that translates to the clinic. CSTI-300 and alosetron displayed a dose-related ability to reduce colon sensitivity with comparable efficacy. In summary, CSTI-300 displays therapeutic potential to reduce the symptoms of patients with IBS-d with the added benefit of likely reduced adverse effects mediated by high level inhibition of the 5-HT3 receptor.

Policy of full disclosure: Conflict of interest declaration; Consynance Therapeutics Inc. has exclusive rights to commercialise CSTI-300.

161 | Radiocontrast media guided interpretation for lactulose hydrogen breath test

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Hydrogen breath test (HBT) is the simplest noninvasive diagnostic methods for suspected small intestinal bacterial overgrowth (SIBO) even though the interpretation of results may be dependent ojn orocecal transit time. We aimed to evaluate the efficacy of radiocontrast-guided interpretation of HBT in patients with suspected SIBO.

Methods: We gathered data from lactulose breath tests performed in 35 patients with suspected SIBO. Patients ingested the radiocontrast media (Omnipaque®) of 20 mL and 10 g lactulose. Breath hydrogen levels were obtained every 10 minutes for 3 hour. A rise of ≥20 ppm above baseline by 90 minutes was defined as a positive test for SIBO. We identified the location of radiocontrast media on plain abdomen X-ray at 90 minutes and 180 minutes after ingestion of lactulose and radiocontrast media to determine whether lactulose substrate arrived at the cecum.

Results: Mean age was 53.5±14.1 and 54.3% were female. Most common presenting symptoms were abdominal bloating, loose stool and borborygm. (i) There are 4 patients with elevated baseline H2>20 ppm. (ii) Six patients showed positive results for SIBO (a rise of ≥20 ppm from baseline in hydrogen by 90 minutes). Among them, radiocontrast was observed in entire colon for 3 of 6 patients and in small intestine and colon for remaining 3 of 6 patients at 90 minutes. (iii) Twenty-five patients showed negative for SIBO. Among them, 11 patients had radiocontrast media in only colon and 13 patients had radiocontrast in small intestine and colon at the end of the test. One patient had radiocontrast media in small intestine.

Conclusion: HBT need to be interpreted considering the arrival of substrate to the cecum (orocecal transit). HBT with concomitant radiocontrast media ingestion is simple and useful method to interpret the results giving the hint for the location of substrate.

Policy of full disclosure: None.

162 | NDRG4 is an enteric neuronal protein which attenuates intestinal tumor progression and protects against colitis-induced injury

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Background: We have identified promotor CpG island methylation of N-Myc-Downstream-Regulated Gene 4 (NDRG4) as a promising early detection marker for colorectal cancer (CRC) (Melotte et al. JNCI,

2009) which is incorporated in the FDA-approved multi-marker stool assay (Cologuard®) and currently used in the US as a screening modality for CRC (Imperiale et al. NEJM, 2014). Studying the role of NDRG4 in the gut we found that NDRG4 immunoreactivity was restricted to the enteric nervous system (ENS), where it labeled cell bodies of the myenteric and submucosal plexuses and inter-connecting nerve fibers (Vaes et al. NGM, 2017).

Methods: We generated NDRG4 knockout (KO) mice to explore the effect of NDRG4 deletion on the normal gut and CRC. CRC was modeled in NDRG4KO and NDRG4 wildtype (WT) mice by (i) crossing them with APCmin/+ mice, (ii) treatment with azoxymethane (AOM) and (iii) treatment with AOM combined with dextran sodium sulfate (DSS).

Results: NDRG4 deletion did not lead to morphological abnormalities in the gut nor to increased numbers of polyps during CRC development (APCmin/+, *P*=.7637; AOM, *P*=.7551). However, polyps of NDRG4KO mice tend to be enlarged (APCmin/+, *P*=.0451; AOM, *P*=.0810) and more aggressive as shown by the higher nuclear β-catenin content (APCmin/+, *P*=.0637; AOM, *P*=.0917) compared to NDRG4WT mice. In the AOM/DSS model significantly more NDRG4KO mice died before the end of the protocol (*P*=.0488) and exhibited higher colonic inflammation indicated by the increased histologic inflammatory score (*P*=.0328) compared to NDRG4WT mice.

Conclusion: NDRG4 tends to repress intestinal tumor progression and protects against inflammation-induced gut injury. These data suggests that the ENS might be a potential, so far neglected, member of the tumor microenvironment.

Policy of full disclosure: None.

163 | Prevalence of joint hypermobility varies between subtypes of Irritable Bowel Syndrome (IBS)

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Introduction: Joint hypermobility (JH) is a common condition in the general population with a prevalence of 10%-20%, varying widely according to age, gender and ethnicity. Previous studies have shown an association between JH and gastrointestinal symptoms such as abdominal pain, bloating, nausea, reflux, vomiting, constipation and diarrhea. A previous study assumed an association between JH and gastrointestinal dysmotility, with some patients showing small bowel dysmotility or colon transit delay. The current study focuses on the prevalence of Joint Hypermobility (JH) in patients with disorders of gut-brain interaction (FGID) according to subtypes of irritable bowel syndrome (IBS).

Methods: Between January 2015 and July 2016 patients with FGID referred to our tertiary ambulatory functional bowel clinic were screened for JH. JH was assessed using the Beighton score and rated positive for scores ≥4/9 points. IBS was diagnosed according to Rome III criteria. Further, IBS was subtyped into IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS (IBS-M) and unsubtyped IBS (IBS-U).

Results: Of 223 patients with FGID screened for JH, 157 (70.4%) were female and 66 (29.6%) male. Median age was 35 [range 18-79] years with JH positive patients being younger than JH negative patients (31 [18-66] vs 38.5 [18-79] years; *P*<.001). A total of 111 (49.8%) patients were screened positive for JH. Women were significantly more often JH positive than men (97 [61.8%] vs 14 [21.2%]; *P*<.001). Among those 223 patients, 55 (24.6%) suffered from IBS-C, 68 (30.5%) from IBS-D, 33 (14.8%) from IBS-M, 21 (9.4%) from IBS-U and 46 (20.6%) from a FGID other than IBS. Prevalence of JH was significantly different between IBS subtypes (*P*=.016) Table 1, with JH prevalence being significantly higher in IBS-C than IBS-D (65.5% vs 39.7%, *P*=.005).

Discussion: Our data show significant differences of JH prevalence among IBS subtypes, with IBS-C having the highest JH prevalence. We consider connective tissue alterations, as previously described, a possible cause of disturbed bowel motility, contributing to constipation in IBS patients with JH. These findings demonstrate a link between JH and IBS and might provide a next step towards underlying pathophysiology in different IBS subtypes.

Policy of full disclosure: None.

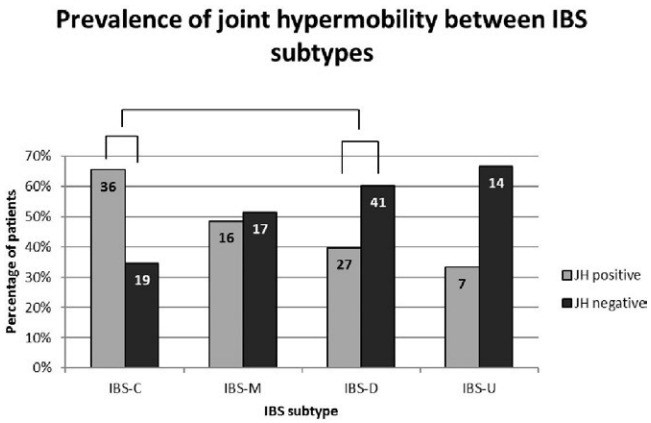

FIGURE 1

164 | Attentional and physiological processing of food images in functional dyspepsia patients

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Objective: Gastrointestinal symptoms of functional dyspepsia (FD) patients may be determined by psychophysiological and food related processes. This study aims to provide basic evidence of the physiological, emotional, and attentional aspects of food processing in FD patients.

Methods: During presentation of food and non-food images (images from international affective picture system and food image database) we measured activity of the autonomic nervous system using skin conductance response (SCR), heart rate variability (HRV), emotional response using facial electromyography, and visual attention using eye-tracking. The study was performed in 15 FD patients and 17 age- and BMI-matched healthy controls (12 females in each group) after a standard breakfast (402.09 kcal, fat 14.52 g, carbohydrate 53.61 g, protein 12.98 g).

Results: Compared to healthy controls, FD patients showed increased food craving, decreased food intake ($P<.05$), food-induced aggravation of FD symptoms, increased sympathetic activation (low frequency/high frequency ratio of HRV, $P<.01$), low pleasantness rating of food images (especially of high fat food, $P<.05$), and reduced total fixation time of food images ($P<.05$). There were no significant differences of SCR and facial electromyography data between groups. Pearson correlation analysis showed significant negative correlations between fat intake and BDI-II (depression, $r=-.88$), fat intake and fat preference questionnaire DIFF score (how much they suppress high fat food consumption, $r=-.93$), energy intake and fat preference questionnaire DIFF score ($r=-.95$), and STAI_state (anxiety) and FCQ_state score (food craving, $r=-.91$) (all $P<.05$) in FD patients.

Conclusion: The results suggest that high level cognitive functions rather than autonomic and emotional mechanisms are more likely to operate differently in FD patients. Additional to the somatic symptoms from gastrointestinal tract, abnormal dietary behavior, reduced subjective rating of pleasantness and visual attention to food should be considered as important pathophysiological characteristics in FD patients. Supported by the People Programme of the European Union's Seventh Framework Programme under REA grant agreement no. 607652 (NeuroGUT), the European Union Seventh Framework Programme (FP7/2007-2013) under Grant Agreement 607310 (Nudge-it). Key words: functional dyspepsia, eye-tracking, food images, fat.

Policy of full disclosure: None.

165 | Neural processing of fat and fat information in food: An fMRI study in healthy subjects and functional dyspepsia (FD) patients

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Objective: Especially high fat meals are associated with dyspeptic symptoms in functional dyspepsia (FD) patients. However it is unclear which neural processes are involved and how psychological factors like expectation modulate this. We aimed to investigate brain activity by functional magnetic resonance imaging (fMRI) after the ingestion of high or low fat food with correct or incorrect fat label.

Methods: We included 12 FD patients and 14 age- and BMI-matched healthy controls (5 males in each group). We recorded resting state fMRI on four different days after an overnight fast before and after ingestion of one of four yogurts (200 mL, either 10% or 1% percent fat, with low or high fat label (2×2 factorial design), sequence randomized across subjects). The statistical significance level was set at $\alpha=0.05$ and FWE correction for fMRI.

Results: FD patients showed increased activity in occipital areas before and after ingestion independent of fat content and label and increased activity in the prefrontal cortex before ingestion. In addition functional connectivity (FC) changes from the insula to occipital cortex (I-O) was increased after high fat and decreased after low fat content in FD patients and to the precuneus (I-P) increased in FD patients compared to HC after low fat label. In FD patients, I-O functional connectivity was negatively correlated with nausea and I-P functional connectivity with FD symptom intensity, food craving, and depression. On the behavioral level, FD patients showed increased dyspeptic symptoms after high fat label compared to low fat label independent of fat content (nocebo effect). In summary, our finding supports the importance of psychological factors on the occurrence of dyspeptic symptoms and on the altered brain activities. Taken together, these findings provide further evidence for the importance of cognitive components in perception of fat, food craving, depression, and brain functions for pathophysiological mechanisms of FD. The research leading to these results received funding from the People Programme of the European Union's Seventh Framework Programme under REA grant agreement no. 607652 (NeuroGUT) and under Grant Agreement 607310 (Nudge-it).

Key words: functional dyspepsia, resting state fMRI, functional connectivity, fat.

Policy of full disclosure: None.

DIETARY INTERVENTIONS INCLUDING PROBIOTICS, PREBIOTICS AND SYNBIOTICS

166 | Effects of rifaximin on neural responses to social stress: A pilot experiment

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Background & Aims: Probiotics that promote the gut microbiota have shown positive effects on central functions, eg, on the stress response in animals and humans. Whether and how antibiotics that eliminate or inhibit pathogenic and commensal gut bacteria also affect these functions is so far unknown.

Methods: In a double-blinded study, 16 healthy volunteers (27 years; nine males) received rifaximin (600 mg/day) (a poorly absorbable antibiotic) or placebo for 7 days. Before and afterwards, brain activity during rest and during a social stressor (Cyberball Game with

social inclusion/exclusion) was measured by magnetoencephalography (MEG).

Results: Social exclusion decreased (P<.001) on the Need Threat Scale score and mood questionnaire. MEG showed brain regions with higher activations during the exclusion compared to inclusion, depending on the frequency band: left inferior and middle temporal and fusiform cortex at 6 Hz (P=.006) and at 11 Hz (P=.004), bilateral inferior and middle temporal and fusiform, left hippocampal, and right angular cortex at 16 Hz (P=.06), left inferior and middle temporal cortex and right thalamus at 21 Hz (P=.01), and bilateral inferior and middle temporal and fusiform, parahippocampal, cerebellum and the right thalamus at 26 Hz (P=.006). 6 Hz and 11 Hz activations were correlated (P<.05) to subjective exclusion measures. Seven days of rifaximin increased frontal alpha power (11 Hz) during resting state and decreased frontal beta power (16 Hz) during social stress, compared to placebo (P=.04); this was unrelated to subjective measures. The 16 Hz frequency band showed an interaction of drug×condition (inclusion, exclusion) in the bilateral superior and middle frontal cortex extending to the left insula (P=.04). Only in the rifaximin group, a decrease (P=.002) in power was seen for exclusion as compared to inclusion; however, this was not correlated to subjective measures of mood and stress.

Conclusion: Social stress affects brain functioning in a specific manner, and rifaximin modulates neural responses to social stress. Contrary to our hypothesis, the antibiotic exhibited stress-reducing effects, similar to reported effects of probiotics (Supported by NeuroGUT, a EU 7th Framework Programme ITN no. 607652).

Policy of full disclosure: None.

167 | Deep brain stimulation of the nucleus accumbens shell augments body weight without affecting food intake in rats

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The treatment of eating disorders like obesity or anorexia is still challenging. One promising approach is the application of deep brain stimulation (DBS), which is a minimally invasive and reversible method to modulate the central neuronal network. The nucleus accumbens (NAcc) is part of the food reward system and divided into the NAcc core and shell. A pilot study reported that DBS of the NAcc shell modulates food intake and body weight in rats. Here, we established DBS for the use in different rat models of eating disorders, eg, activity-based anorexia. Normal weight female Sprague-Dawley rats were anesthetized and chronically equipped with a custom-made unilateral DBS electrode in the NAcc shell. A stimulator was connected to the electrode and subcutaneously placed on the back of the rats. After recovery, biphasic stimulation was performed for seven days with 100 µA and 130 Hz. Body weight and food intake were measured every day. Behavior was monitored manually during the pre-dark phase activity period on days two and three. Correct operation of the stimulator was assessed on day eight by measurement of current flow in vivo and ex vivo, correct placement of the electrode was assessed immunohistochemically. Data were assessed by two-way ANOVA or t-tests. DBS (n=6) increased body weight gain (expressed as %, 13.1±2.1) compared to sham-stimulated controls (n=6, 9.0±1.8, $F(1,95)=12.0$, P<.001) without affecting daily food intake (DBS: 16.7±0.3 vs sham: 16.9±0.4 g/200 g body weight, $F(1,95)=3.0$, P>.05). Behavior including eating, drinking, grooming and locomotion did not differ compared to controls (P>.05). Further analyses showed that light phase food intake was increased in DBS rats (+56%, P<.01), whereas dark phase food intake was reduced (−10%, P<.05) without differences in overall 24-hour food intake (P>.05). Furthermore, light phase food intake microstructure showed that meal duration (+71%) and the time spent in meals (+92%) were increased in DBS compared to sham-treated animals (P<.05). Although food intake was not changed, DBS resulted in a modest body weight gain compared to sham-treated controls. Since locomotor activity was not altered in DBS rats, these results might point towards a reduction of energy expenditure by DBS.

Policy of full disclosure: None.

168 | Alterations of gut microbiota composition in patients with chronic alcohol overconsumption

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Background: The gut microbiota may play a role in the pathophysiology of several gastrointestinal and extra-intestinal disorders, including alcohol-associated diseases. Aim To explore gut microbiota composition in patients with chronic alcohol overconsumption.

Materials and methods: Consecutive patients admitted to Lovisenberg Diaconal Hospital (Oslo, Norway) with a confirmed history of ongoing or recent chronic (>10 years) alcohol overconsumption (>20 or 40 g/day for women and men, respectively), including individuals with a binge drinking pattern, were included in the study (n=33). Patients with no history of alcohol overconsumption (n=18) were included as controls. Faecal samples were collected and DNA isolated. The V3-V4 regions of the 16S rRNA gene were amplified and sequenced on an Illumina HiSeq 2500 platform, generating 250 bp paired-end reads. Sequences with ≥97% similarity were assigned to the same OTU and annotated using the GreenGene Database (version 2.2.25).

Results: We obtained saturated sequencing depths of ~60 000 reads per sample. There was no significant difference in species diversity (Shannon alpha-diversity) between patients and controls. Testing for differential abundance at phylum level showed that patients had a significantly higher relative abundance of Proteobacteria, and a lower Firmicutes/Bacteriodetes ratio than controls. Testing for differential

abundance at genus level, we found that Faecalibacterium were less abundant, while Sutterella, Clostridium and Holdemania were more abundant in patients vs controls. A method for functional profiling of the 16S microbial gene data (denoted PICRUSt [(1)]) showed that genes related to invasion of epithelial cells, glycosaminoglycan and styrene degradation, and lipoic acid metabolism were more common in the patient group compared to the control group.

Conclusions: Our findings suggest that chronic alcohol overconsumption is associated with altered composition of the gut microbiota, skewed towards phyla and genera that may possess pro-inflammatory properties. Disturbances of microbial functions may play a role in development of alcohol-associated diseases, and should be further examined.

Policy of full disclosure: None.

Reference: 1. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology. 2013;31(9):814-21.

169 | Alteration of gut microbiota composition by the administration of probioticsin healthy individuals

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Background/Aims: Probiotics may affect the composition of gut microbiota. The purpose of the present study was to investigate whether gut microbiota composition is altered by the administration of probiotics in healthy individuals.

Methods: Twelve healthy volunteers (30-34 years-old) participated in the study and provided baseline fecal samples (Sample 1). Subsequently, they took commercially available probiotics (Bifidobacterium, Lactobacillus and Enterococcus) for 4 weeks, and fecal samples were collected at week 2 (Sample 2) and 4 (Sample 3). After 4 week-administration of probiotics, additional fecal samples were collected from all subjects at week 5 (Sample 4) and 6 (Sample 5). Gut microbiota in fecal samples were analyzed by 16S rRNA gene sequencing.

Results: There was no significant change in the mean Shannon Index in overall taxa between Sample 1 and 3 (4.365 vs 4.556, *P*>.05), and between Sample 1 and 5 (4.365 vs 4.201, *P*>.05). There was no significant change in the mean inversed Simpson Index in overall taxa between Sample 1 and 3 (0.903 vs 0.920, *P*>.05), and between Sample 1 and 5 (0.903 vs 0.874, *P*>.05). At the Phylum level, major four taxa were Bacteroidetes, Actinobacteria, Firmicutes and Proteobacteria. There was no significant change in their proportion between Sample 1 and 3. The proportion of Actinobacteria (10.2% vs 2.5%, *P*=.001) and Proteobacteria (2.7% vs 5.1%, *P*=.010) in Sample 1 was altered significantly, compared with Sample 5. There was no significant change in the proportion of Bifidobacterium between Sample 1 and Sample 3. The proportion of Lactobacillus (2.1% vs 2.8%, *P*=.030) and Enterococcus (0.002% vs 2.8%, *P*=.004) were significantly increased in Sample 3, compared with Sample 1. The proportion of Lactobacillus and Enteroccocus was not significantly changed between Sample 1 and Sample 5. The Proportion of Bifidobacterium was decreased in Sample 5, compared with Sample 1 (9.4% vs 2.2%, *P*=.001).

Conclusion: The diversity of fecal microbiota is not significantly altered by the administration of probiotics. However, the proportion of fecal microbiota at the phylum level or the genus level is significantly changed during or after the administration of probiotics.

Policy of full disclosure: None.

170 | The impact of vancomycin on the jejunal myoelectric activity and intestinal bacterial overgrowth in hypochlorhydria: Experimental study

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Objective: This experimental study investigates the impact of vancomycin on the jejunal myoelectric activity and microflora in hypochlorhydria.

Methods: 14 male Wistar rats weighing 400-450 g were used. Three stainless steel electrodes were implanted into the muscular wall of the small intestine at 5, 10 and 15 cm distally to the ligament of Treitz. An infusion cannula for drug administration was inserted into the stomach. After a 10-day recovery period, the fasting intestinal motility was recorded. Groups of seven rats received intragastrically either normal (sterile) drinking water or sterile water containing rabeprazole (0.56 mg/mL) during 9 consecutive days and then rabeprazole (0.56 mg/mL) and vancomycin (80 mg/mL) during following 7 days. On day 17 of the treatment, the fasting intestinal motility was recorded. Body weight was assessed before and after treatment, and the cecum weight was determined after sacrifice. Jejunum and cecum samples were collected for bacterial studies.

Results: Myoelectric recordings in the treatment group showed disruption of the migrating myoelectric complex (MMC) compared with background recordings. Phase III was absent. The duration of phase II was longer, and the duration of phase I was shorter than that in control animals. Cecum weight was increased significantly in the treatment group. The bacterial studies showed the appearance of Klebsiella spp. in the jejunum and in the cecum in the treatment group. Moreover, we identified a decrease in the population of normal microflora in the cecum and the appearance of significant numbers of *E. coli* (10.6 CFU/mL) in the proximal jejunum.

Conclusion: Administration vancomycin in hypochlorhydria conditions in causes the MMC disruption that results in the bacterial overgrowth possibly due to the fecal microflora migration to the proximal jejunum.

Policy of full disclosure: None.

171 | Peptide YY is a critical regulator of gut microbiota composition specifically under conditions of sucralose or high fat diet exposure

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Objective: The gut hormone peptide YY (PYY) is is a member of the neuropeptide Y (NPY) family and is being expressed by endocrine L cells of the lower gastrointestinal tract. Among other functions, PYY has been demonstrated to slow the transit of food through the gastrointestinal tract and inhibit food intake. PYY levels increase upon ingestion of a meal, with dietary fat being among the most potent inducers of PYY release. *E. coli* proteins have also been demonstrated to induce PYY release, suggesting an inductive role of gut bacteria in meal-induced signalling of intestinal satiety. However, while these findings suggest a signalling pathway between the intestinal microbiota and PYY, the effects of gut-derived PYY on gut microbiota composition and its metabolic consequences have not been investigated in detail. This work therefore aimed at exploring the differences of intestinal microbiota composition of wild-type (WT) and PYY-knockout (KO) mice under basal conditions, as well as in response to dietary interventions, which affect intestinal microbiota such as artificial sweeteners or a high fat diet (HFD).

Methods: Male WT and PYY-KO mice, derived from the same breeding pairs, were housed in mixed-genotype cages. The artificial sweetener sucralose was added to the drinking water at a concentration of 1% for a treatment period of 1 week, while HFD was given for a period of 3 weeks. Faecal samples were collected at baseline, as well as after sucralose and HFD treatment.

Results: While, at baseline no genotype differences in microbial composition were observed at the phylum level, both sucralose- and HFD-induced decreases in the phylum Bacteroidetes were attenuated in PYY-KO mice. In addition, the HFD-induced increase in the phylum Firmicutes was attenuated in PYY-KO mice. Furthermore, these changes were associated with distinct genotype as well as diet-dependent changes of gut microbiota composition at the species level.

Conclusion: Together these results highlight a critical role of gut-derived PYY in the control of gut microbiota composition, specifically in response to dietary interventions such as sucralose and HFD.

Policy of full disclosure: None.

172 | Intermittent fasting does not protect, but exacerbates sickness behavior due to the viral mimic Poly(I:C)

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Objective: Restricted feeding was shown to attenuate lipopolysaccharide (LPS)-induced fever and dampen associated pro-inflammatory effects in mice. We investigated whether intermittent fasting (IF) every other day affects sickness behavior observed 4 hours after intraperitoneal (i.p.) injection of the dsRNA-like molecule polyinosinic:polycytidylic acid (Poly(I:C)), which acts via Toll-like receptor 3 (TLR3) and mimics viral infection.

Methods: Male C57BL/6N mice (aged 9 weeks) were randomly assigned to two groups: a control group fed standard rodent chow ad libitum (AL, n=29) and an IF group (n=17) which was fasted for 24 hours every other day, with intermittent 24-hour periods of free access to food. Mice in the AL and IF group were divided into two cohorts each including 8-15 animals that were injected i.p. either with vehicle (VEH) or Poly(I:C) [12 mg/kg] on day 10 of the fasting protocol. Injections were performed after fasting of the IF group for 5 hours. Mice were submitted to the open field test (OFT) 4 hours after injections, when locomotor activity was determined to assess sickness behavior. Statistical analysis was performed using two-way ANOVA.

Results: Poly(I:C)-treated mice of both the AL ($P<.05$) and IF ($P<.001$) groups displayed symptoms of sickness as they moved significantly less than the respective vehicle-treated controls. In addition, Poly(I:C)-treated animals of the IF group were affected to a greater extent than mice of the AL group ($P<.001$). Moreover, IF had a significant influence on the explorative behavior in the OFT, as both the VEH- and Poly(I:C)-treated animals were less active in entering the central area of the open field than the AL group.

Conclusions: The current data indicate that, unlike LPS-induced sickness behavior which is blunted by restricted food intake, the sickness behavior induced by the viral mimic Poly(I:C) is exaggerated by IF. Ongoing work analyses the molecular mechanisms of the impact of IF on the immune-brain interaction evoked by TLR3 stimulation.

Policy of full disclosure: None.

173 | Effects of dietary intake of Lactobacillus gasseri CP2305 (CP2305) on the depressive-like behaviours in subchronic mild social defeat stress (sCSDS) model of mice

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Recently, the number of depressive patients has grown rapidly (WHO 2017), therefore the method for dietary intervention to prevent depression should be developed. Our research group has tried to discover the food and farm products which have significant potentials for preventing depression using a mouse model. In this study, we tried to evaluate the potentials of the heat-inactivated preparation

of Lactobacillus gasseri CP2305 (CP2305) for depressive symptoms. C57BL/6J mice (B6, male) were fed an AIN93G diet containing heat-inactivated CP2305 cells (1% w/w) for 4 weeks. After that, B6 was subjected to subchronic mild social defeat stress (sCDSD) by ICR male mice as previously described (Goto et al., 2014). Healthy control or stressed B6 were fed an AIN93G diet without or with sCSDS, respectively. Finally, the behavioural tests for evaluating depressive symptoms including social interaction (SIT), sucrose preference (SPT), tail suspension (TST), and forced swimming (FST) were conducted. sCDSD mice showed depressive-like symptoms because of their extended immobility time in FST compared with control mice, although no significant changes were occurred in other tests. Possibly, chronic supplementation of CP2305 reduced immobility of FST in sCDSD mice, therefore CP2305 may have a potential function for preventing depression. In the future, we should confirm the reproducibility of the effect of CP2305 using a larger cohort of sCSDS mice and also elucidate underlying molecular mechanism in the brain and peripheral tissues.

Policy of full disclosure: This research was supported in part by the grant from Asahi Group Holdings, Ltd.. Dr. Fujiwara is a researcher in Asahi Group Holdings, Ltd. Other members have no financial conflict of interest.

174 | Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 affect one-carbon metabolism in depressive-like rats

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Objective: Probiotics exert beneficial health effects, but the underlying mechanisms remain largely elusive. Depression and liver dysfunctions are believed to be associated with deficiency of methyl donors (such as S-adenosylmethionine [SAM]). SAM can be produced by lactic acid bacteria in vitro, but it is not known whether supplementation of lactic acid bacteria could increase SAM in vivo and thereby influence biological processes. We therefore investigated whether probiotics can alter one-carbon metabolism that is responsible for SAM production in depressive-like rats.

Methods: Probiotics containing Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 at two doses (10^9 vs 10^{10} colony-forming units/d) or vehicle were orally administered to Flinders Sensitive Line (FSL) rats, an animal model of genetic predisposition to depression (n=22). Control rats, Flinders Resistant Line (FRL) rats, only received vehicle treatment (n=8). After 6 weeks, rats underwent a behavioral test battery. Following euthanization at week 10, we measured the concentrations of one-carbon- and related metabolites in several biological samples.

Results: Compared to FRL rats, vehicle-treated FSL rats had higher plasma concentrations of betaine (*P*=.001), choline (*P*=.070), and dimethylglycine (*P*=.021). Moreover, FSL rats tended to have lower concentrations of SAM in the liver (*P*=.055). In urine, FSL rats showed lower choline (*P*=.002) and higher dimethylglycine concentrations (*P*=.004). FSL rats treated with high-dose probiotics had lower plasma betaine (*P*=.042), a lower betaine/choline ratio (*P*=.03), and higher liver extract SAM compared to vehicle treatment (*P*=.017). No behavioral changes were observed.

Conclusions: Our results suggest that depressive-like FSL rats had enhanced flow of SAM from betaine and choline, but lower liver SAM compared to control rats, which is in line with low SAM levels observed in depressed patients. High-dose probiotics reversed the metabolic dependency on betaine and choline, and increased liver SAM to control strain levels. To our knowledge, this is the first study describing that probiotics caused significant changes in one-carbon metabolism, which may pave the way for probiotics as an additive treatment for disorders associated with changes in the transmethylation pathway.

Policy of full disclosure: The probiotic and vehicle formulations were donated by Lallemand Health Solutions Inc. (Montreal, QC, Canada). The company had no role in planning the study, data acquisition, analyses or interpretation, and writing of the abstract.

175 | Vagal nerve activity is stimulated by GABA-secreting probiotics signalling across the colonic mucosal barrier

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Objectives: The gut-Brain axis is the bidirectional neurohormonal communication system between the central nervous system, the enteric nervous system, and the gastrointestinal tract which has been extended to signals from the microbes in the external environment of the gut lumen. The vagus nerve appears to serve as a major communication pathway in this axis. The study aims were to investigate if GABA-producing *Lactobacillus brevis* DPC 6108 can signal across an intact mucosal barrier to the host nervous system.

Methods: Real-time calcium imaging experiments were conducted using a standard epifluorescence imager on distal colon preparations from adult male Sprague Dawley rats. Nerve activity was recorded using a bipolar electrode on distal colonic tissue with an intact vagus nerve. Secretions from *Lactobacillus brevis* DPC 6108 (supernatants) were applied directly to the mucosa and its effects on intrinsic submucosal neurons and extrinsic afferents were recorded.

Results: Mucosal exposure to *Lactobacillus brevis* DPC 6108 supernatants induced a large increase in intracellular calcium in submucosal neurons, which was significantly reduced but not abolished by incubation with bicuculline, a GABA A receptor antagonist (n=27, *P*<.001). Furthermore, the GABA B receptor antagonist, phaclofen, also inhibited the neuronal response to the supernatants (n=19, *P*<.05).

Application of supernatants to the distal colonic mucosa also elicited a robust increase in vagal nerve firing (n=4, $P<.001$), which was suppressed by bicuculline, ($P<.001$) and phaclofen to a lesser extent ($P<.05$).

Conclusions: These data provide evidence that GABA producing *Lactobacillus brevis* DPC 6108 have the capacity to signal across the intact epithelium of the distal colon to stimulate activation of submucosal neurons and vagal nerve firing. Activation of both GABA A and GABA B receptors are implicated in the microbiota-gut-brain signalling pathway mediated by this bacterial strain.

Policy of full disclosure: None.

176 | Oligofructose-enriched Inulin improved learning in adulthood and altered stress-induced immune priming in aged male mice

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Background: Aging is associated with increased inflammation and a decline in brain function, including cognitive impairment. The microorganisms that colonise our gut have recently emerged as key factors in communicating between the gut and the brain, and can influence brain function. Prebiotics, non-digestible fibres that are fermented by colonic bacteria, help regulate microbiota complexity and diversity and may thus influence cognitive function throughout life.

Objective: The project aims to determine if chronic administration of prebiotics can affect age-related changes in behaviour, stress response, immune function and host physiology along with alterations in gut microbiota complexity and diversity. Methods Young adult male (8 weeks) and middle-aged male mice (10 months) received chow enriched with 10% Oligofructose-enriched Inulin (OE-Inulin: mixture of 92±2% Inulin and 8±2% Fructooligosaccharide, Orafti®Synergy1; Beneo/Belgium) or control chow for a period of 12 weeks. After 3 weeks of diet, mice underwent tests to determine the effects of diet on cognition, stress and anxiety-like behaviour. Peripheral immune cell activation and microglia activation were investigated by Flow Cytometry. Fat depots were assessed post-mortem.

Results: Dietary intake of OE-Inulin decreased anxiety-like behaviour and improved learning in young adult mice only. Although OE-inulin administration did not have profound changes on behaviour in middle-aged mice, OE-Inulin counteracted stress-induced peripheral immune cell activation in aged mice, suggesting an immunoregulatory effect of prebiotics on immune cell priming upon stress. Caecum size, as an indicator for the rate of microbial fermentation was increased in the OE-Inulin-treated groups of both adult and aged mice. Furthermore, aged OE-Inulin-treated mice showed decreased visceral fat mass in line with a decreased food intake. Correlation data suggest a potential link between visceral fat mass, immune priming and cognition in male mice.

Conclusion: Our data suggest a role of prebiotics in regulating cognitive behaviour in adulthood and stress-induced peripheral immune cell priming in aging.

Policy of full disclosure: None.

177 | Maternal antibiotic/prebiotic consumption alters behavior and expression of inflammatory markers and neurotransmitters in offspring

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Background: Healthy gut microbiota is essential for the maturation and maintenance of the immune and nervous system. Gut microbiota are especially important in early life (during pregnancy and/or in the first years of life), during critical developmental windows. Antimicrobial agents and antibiotics can alter microbial diversity, leading to dysbiosis. The gut communicates with the brain via the gut-brain axis, therefore antibiotics may indirectly perturb neurotransmitters and inflammatory markers in the brain as well as behavior. Recent studies showed that early life antibiotic use is associated with increased risk of obesity, altered immunoregulation, and neuropsychiatric disorders. Prebiotics are non-digestible food that selectively stimulates the proliferation of beneficial bacteria and may ameliorate the effects of antibiotics. Our objective was to alter microbiota through antibiotic/prebiotic administration in pregnant Sprague-Dawley rats and examine changes in the brain and behavior of their offspring.

Methods: 10 week old female Sprague-Dawley rats (n=36) were mated and randomized to: (i) Control (C); (ii) Antibiotic (Ab); (iii) Antibiotic+Prebiotic (AbP). Mothers received low-dose penicillin via drinking water and a prebiotic diet (10%) through the third week of pregnancy and throughout lactation. Offspring were weaned at 21 days onto a control diet until they were switched to a high fat/sucrose (HFS) diet at week 5. Behavioral tests were conducted throughout weeks 5 to 9. Real-time PCR was used to measure expression of various neurotransmitters and inflammatory markers in the hippocampus, hypothalamus, and amygdala of offspring at 10 weeks.

Results: In the sucrose preference test, AbP offspring had significantly greater % sucrose preference compared to Ab and C ($P=.01$; $P<.01$) 1 week after HFS diet introduction. AbP offspring spent significantly more time in the open arms of the elevated plus maze compared to the Ab group ($P=.03$). In females, AbP offspring spent significantly more time in the open arms compared to C ($P=.03$). Ab expressed higher levels of CD11b, Iba-1 and TLR4 than AbP in the hypothalamus ($P<.05$).

Conclusion: Administration of antibiotic during pregnancy and lactation alters behavior and levels of inflammatory markers in offspring.

This study shows that supplementing antibiotics with prebiotics may ameliorate some of these changes.

Policy of full disclosure: None.

178 | In vitro screening of human-derived lactobacilli for novel psychobiotic potential

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Psychobiotics are live microorganisms that when consumed, positively influence the gut microbiome to confer health benefits for neuropsychiatric illnesses 1,2. Accumulating pre-clinical 3-5 and clinical 6-8 evidence point towards the effectiveness of psychobiotics as biotherapeutics to treat depression and anxiety. Commensal gut bacteria may influence the microbiota-gut-brain-axis through mechanisms such as: (i) SCFA production (ii) neurotransmitter release (iii) activation of afferent sensory fibers of the vagus nerve (iv) regulation of the immune system (v) regulation of tryptophan metabolism 9. MyNewGut is a project aimed at identifying the effects of environmental factors on the gut microbiome and its influence on brain, immune, and metabolic programming, development and function. As part of this project, a biobank of future probiotics containing ~300 putative lactobacilli isolated from healthy adult males, born via Caesarean section but not prone to stress was created. These strains will be screened for psychobiotic potential; ability to produce neurotransmitters and neuroactive compounds which could influence the microbiota-gut-brain-axis. Serotonin is of particular interest because it plays an important role in the regulation of a number of bodily functions, including gastrointestinal tract motility, secretion, sensation and mood. There is significant overlap between behaviours regulated by the serotonergic system and those that are influenced by the microbiota 10. Lactobacillus species from the biobank will be compared to the serotonin producer Lactococcus lactis subsp. cremoris 11 using a commercially available ELISA kit and HPLC. The genomes of positive producers will be sequenced to identify serotonin production machinery. In the future, positive strains will be further analysed for probiotic characteristics (acid and bile tolerance) and studies will be carried out in preclinical models of anxiety and depression.

Policy of full disclosure: None.

179 | Screening of bacterial-derived metabolites for growth hormone secretagogue receptor 1a modulation

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Background: The majority of current prescription pharmaceuticals on the market are targeting G protein coupled receptors (GPCRs), which are involved in many biological functions, including the regulation of food intake and energy expenditure. The growth hormone secretagogue receptor 1a (GHS-R1a) is a peripherally- and centrally-expressed GPCR, activated by its endogenous ligand, ghrelin, which is a gastric peptide and the first and only known peripheral hormone to have an orexigenic effect. The microorganism that inhabit our gut (microbiota) have a major influence on energy homoeostasis and food intake regulation. Specifically, Bifidobacterium strains, present in the gut and commonly used as probiotics, may play a key role in the regulation of appetite.

Objective: This novel study aimed at identifying specific Bifidobacterium strains of human gut origin, which may control energy homeostasis through GHS-R1a signalling. Method Bifidobacterium-derived supernatants were screened for GPCR modulation using HEK-GHS-R1a-EGFP cell line. Calcium mobilisation assays were measured by the FLIPR-Tetra High-Throughput Cellular Screening System, and internalisation receptor assays were measured using confocal microscopy by the IN Cell Analyzer 1000 as an index of GHS-R1a activation.

Results: Several Bifidobacterium populations from the human gut were identified and characterised for a range of probiotic properties, such as bile salt hydrolase activity, exopolysaccharide production, antimicrobial activity and antibiotic resistance. In addition, the strains were screened for GHS-R1a modulation in vitro. We found some strains that could modulate GHS-R1a receptor signalling, acting as antagonists or agonist for GHS-R1a. These bacterial strains will be validated for their effects on food intake, behaviour and anxiety in animal models of obesity. Ultimately, the specific bacterial strains that increase appetite or satiety via modulation of GHS-R1a may have potential as probiotics for the treatment of obesity or anorexia.

Policy of full disclosure: None.

ENTERIC PLASTICITY

180 | Changes of enteric neurons and glia cells in the animal model of acute and chronic ulcerative colitis

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Introduction: Neurons and glial cells are two major cell populations of the enteric nervous system, which are involved in inflammatory bowel diseases like ulcerative colitis or Crohn's. The alteration of enteric neurons and to a lesser extent glial cells is described on models of acute colitis, but is not characterized sufficiently in chronic colitis.

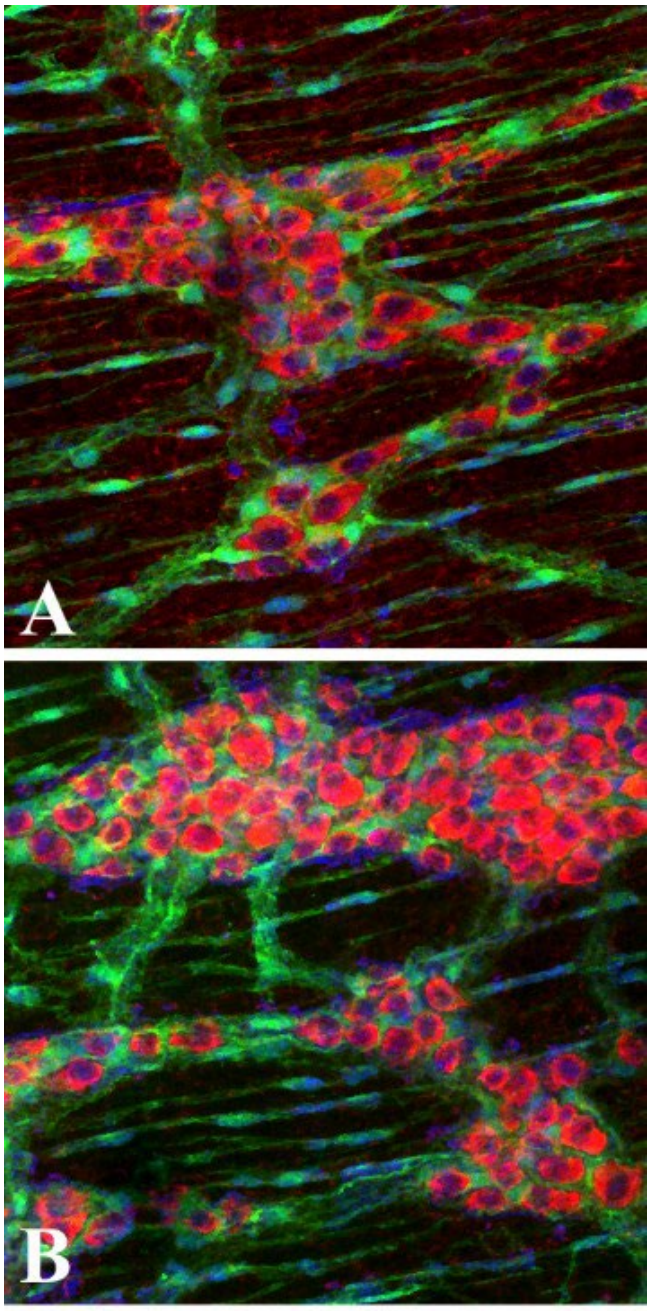

Fig. Myenteric plexus on the whole mounts of the muscular layer of the distal colon in the control mice (A) and the mice with acute colitis (B). Immunofluorescence labeling anti-HuC/D (red), anti-S100b (green) and DAPI (blue). Confocal microscopy. Field of view 212.55x212.55 mkm.

Objective: To evaluate the morphological changes of enteric nervous system in the distal colon using a murine model of acute and chronic colitis.

Methods: Adult male C57Bl/6 mice were used. Acute colitis was induced by 2.5%-5% for 5 days and chronic colitis by 1%-1.5% aquatic solution of dextran sodium sulfate salt for 3-5 days intermittently. The mice with acute colitis were sacrificed on the 7-th day and the mice with chronic colitis on 58-th day. The distal colon was used. Longitudinal histological slices from both groups were marked with the anti-βIII tubulin, anti- S100b and the muscle whole mounts from the acute group was marked with the anti-HuC/D, anti- S100b antibodies and DAPI. Digital images were obtained with Zeiss Axioplan 2 and LSM 780 and analyzed with the ImageJ (FijI). Mann-Whitney *U* test was used.

Results: The mice with acute colitis developed a severe colon inflammation with an extensive mucosal ulceration. The mice with chronic colitis had a chronic inflammation with epithelized ulcers. In acute colitis there was an increase in myenteric ganglia and neuron number per colon length. The nerve fibers density and area in both the muscular and mucosal layer increased, nerve fibers were abundant in ulcers. The number of intramuscular glial cells was unchanged, but the area of mucosal glia increased. In chronic colitis there was an increase in the area of intramuscular nerve fibers and mucosal S100b-positive cells. Epithelized ulcers contained nerve fibers as well.

Conclusion: During acute colitis the neuron cell and fiber numbers increase in both the muscular and mucosal layer, which points to their remarkable neuroplasticity during gut wall remodeling despite severe inflammation. Glial cells increase only in the mucosal layer, reflecting a glial alteration. In chronic colitis these changes persist albeit less severe, reflecting a long-term change.

Policy of full disclosure: None.

181 | Cholera toxin-evoked sustained hyperexcitability in murine cholinergic submucosal neurons

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Cholera-induced diarrhoea causes rapid and fatal dehydration if left untreated. Cholera toxin (CT) acts partly via the enteric nervous system (ENS), but the neural circuitry involved is unclear. To examine this, we incubated surgically-isolated mouse ileal loops with CT (12.5 μg/mL) or saline (control) for 3.5 hours in vivo, then analysed the loops in vitro. The mice used include Wnt1-Cre;R26R-GCaMP3 mice which express a genetically encoded calcium indicator in the ENS. Full thickness loop segments were mounted in Ussing chambers to measure electrogenic secretion. The excitability of submucosal and myenteric neurons was assessed by calcium imaging and immunohistochemical labelling of an activity dependent marker (c-Fos or pCREB) with subtype markers. CT-treated preparations had a higher basal secretion than controls ($P<.0001$; n=10-11 animals). Neither tetrodotoxin (Na+ channel blocker; n=4) nor co-incubation of CT with bupivacaine (local anesthetic; n=6) blocked the hypersecretion, indicating a major mucosal effect. However, CT-incubation

induced significant changes in submucosal neurons (defined by Hu+ stain) which displayed significantly higher pCREB expression in CT-treated tissues than controls (65±7% vs 11±5%; $P<.01$; n=3). This returned to control levels with CT and bupivacaine co-incubation ($P<.01$; n=5). There were more spontaneously active choline acetyltransferase (ChAT+) neurons in CT-incubated tissues (32/160 neurons) than controls (4/149 neurons). Spontaneous activity was not seen in ChAT-neurons. Electrically (n=4) and agonist (DMPP; nicotinic agonist, 10 µmol/L; n=3) evoked Ca^{2+} responses were also increased in ChAT+ neurons ($P<.05$). Myenteric neurons showed minor differences; CT increased c-Fos expression compared to controls (28±3% vs 6±2% Hu+ neurons; $P<.0001$; n=5), but depressed neuronal Ca^{2+} responses ($P<.05$; n=3-6). Thus, CT-exposure induced hypersecretion by acting directly on the mucosa and also induced sustained hyperexcitability in ChAT+ submucosal neurons.

Policy of full disclosure: None.

182 | Ultrastructural analysis of myenteric plexus and smooth muscular cells in colon of infected mice in prolonged infection of Chagas Disease

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Chagas Disease (CD) is an important neglected disease which remains a serious health problem, principally in Latin America. This disease presents in two phases: acute and chronic. The chronic phase is characterized by cardiac or tract gastrointestinal (TGI) involvement. The former shows megaesophagus and megacolon. Our work intended to reproduce human disease in a novel murine model of long-term infection with Trypanosoma cruzi and analyze ultrastructurally myenteric plexus and smooth muscular cells. We used 56 female Swiss mice. Ten mice composed non-infected controls, five acute phases and five chronic phases. 46 animals were inoculated intraperitoneally with blood trypomastigote forms of T. cruzi. The animals were divided into two groups: 10 for euthanasia on the 11th day after infection, 30 were treated with benznidazole and were reared until 15 months after infection. We analyzed samples of colon "roll". We observed, when comparing animals from acute and chronic control groups, changes in the last one that are possibly related to aging. In acute infected group, nucleus of neurons in the myenteric plexus were with moderate and diffuse increase of heterochromatin and irregular aspect of the nuclear membrane. There were some inflammatory cells and capillaries with activated endothelium. In chronic infected group, there are neurons with electron dense cytoplasm, scarce in organelles and nuclear profile altered. The neuronal involvement was segmentar. Compared to their control group, there was a marked increase in number and lamellar aspect of intracytoplasmic inclusions in myenteric neurons. Our ultrastructural analysis showed myenteric neuronal, and smooth muscle cells changes described in human infection, indicating enteric plasticity and being an important model to reproduce CD, especially in prolonged infection. Financial support: FAPEMIG.

Policy of full disclosure: None.

183 | Ghrelin sensitises colonic myenteric neurons to the neurostimulatory effects of Glucagon-like peptide-1

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Objective: Irritable Bowel Syndrome (IBS) is a common functional bowel disorder affecting approximately 10%-20% of the population. Although IBS pathophysiology remains elusive, dysfunctional endocrine signalling has been implicated. Ghrelin is an orexigenic hormone secreted by parietal cells lining the stomach. Ghrelin levels peak prior to food ingestion and return to basal levels upon feeding. Subsequently, after ingestion the endocrine hormone glucagon-like peptide-1 (GLP-1) is secreted by L-cells in the small and large intestine. Previous studies have shown that ghrelin directly primes L-cells to secrete GLP-1 and increases GLP-1 release (Gagnon et al., 2014). The aims of this study were to investigate if ghrelin sensitises colonic enteric neurons for GLP-1 mediated signalling and if signalling differed between two animal models; Sprague Dawley (healthy control) and Wistar Kyoto (stress model of IBS).

Methods: Cross-sections of distal colon and colonic myenteric plexus from both animal models were stimulated with ghrelin, fixed, permeabilised and blocked. Immunofluorescence was used to stain for ghrelin and GLP-1 receptors. Colonic motility was assessed in organ baths using SD and WKY colonic segments.

Results: Immunofluorescent staining of ghrelin and GLP-1 receptors showed discrete clusters of co-localisation in myenteric neurons. Ghrelin stimulation enhanced GLP-1 receptor expression in myenteric ganglia. GLP-1 stimulated contractile activity in both SD and WKY rat colons. However, this response was potentiated by prior exposure to ghrelin in both circular and longitudinal muscle in Sprague Dawley (($P=.0116$), ($P=.0093$), respectively) and Wistar Kyoto (($P=.0129$), ($P=.0006$), respectively) rat colonic segments.

Conclusions: The receptors for GLP-1 and ghrelin are highly co-localised in myenteric neurons and stimulation with ghrelin resulted in increased expression of GLP-1 receptors. Functionally, ghrelin sensitises the colon to GLP-1, which evoked an enhanced colonic contraction after exposure to ghrelin. However, no distinct differences were noted between the WKY rat model of IBS and the SD comparator.

Policy of full disclosure: None.

184 | Alterations in sigmoid interstitial cells of Cajal and their impact on colon function and clinical symptomatology in patients with diverticular disease

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Diverticular disease ranges among the most common benign diseases of the gastrointestinal tract. Colonic diverticulosis results from increase in intracolonic pressure, mainly in the sigmoid, due to colonic motility disturbance. It was reported that decrease of interstitial cells of Cajal (ICCs) density resulted in numerous motility-related disorders. We assessed ICCs in transverse sections of the sigmoid wall in patients with diverticular disease. We correlated their number and distribution with multiple clinical data (age, duration of disease, symptoms, comorbidities) along with anorectal functions recorded using manometry. A total of 101 patients were enrolled into the study (34 patients operated for colonic diverticular disease and 67 patients operated for non-obstructive colorectal cancer). ICCs were assessed in paraffin embedded specimens stained with immunohistochemistry ie, anti c-kit and anti tryptase antibodies (DAKO). Several subpopulations of ICCs were identified: ICC-SM—localized on the submucosal and circular muscle border, ICC-CM—localized in the circular muscle layer, ICC-MP—localized between circular and longitudinal muscle layers and in the neighbourhood of Auerbach's plexus and ICC-LM—localized in the longitudinal muscle layer.

The number of ICCs was statistically significantly lower in patients with diverticulosis as compared to controls (1.28 vs 1.76/1 visual field respectively; $P<.001$). The decrease of ICCs number was significantly higher in the vicinity of Auerbach's plexus and between circular and longitudinal muscle layers. Positive correlation between ICC-MP quantity and presence of flatulence and mucus in stool and negative correlation between the number of ICC-LM and pain were determined. Relationship between ICC-SM cells and presence of neurologic and thyroid gland disorders was revealed. We correlated number of ICCs localized in the sigmoid circular muscle layer (ICC-CM) and the presence of normal anorectal reflexes, as well as quantity of ICC-MP and anorectal manometry values.

In summary, a reduction of sigmoid colon ICCs might be an important etiopathogenetic factor for colonic diverticulosis. These cells also play a role in the pathophysiology of diverticular disease by modulating enteric nervous system. The results of this study may contribute to practical application of decision making regarding the extent of operative procedures, and reduction of recurrence after operative treatment.

Policy of full disclosure: None.

185 | Providing a molecular window on visceral sensitivity in intestinal schistosomiasis

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Objective: Schistosomiasis is considered a major neglected tropical disease. The mouse model of intestinal schistosomiasis, like its human counterpart, shows both in its acute and chronic phase a severe granulomatous inflammation and massive recruitment of mast cells in the gastrointestinal (GI) wall, significantly affecting the functioning of the enteric nervous system (ENS). While the disease has been extensively studied in relation to the ENS and GI motility, little is known on how schistosomiasis-associated mastocytosis affects visceral sensitivity, which is why we set out to provide a molecular window on visceral sensitivity in dorsal root ganglia (DRG) during intestinal schistosomiasis.

Methods: Intestinal schistosomiasis was induced by injecting C57BL/6 mice with 130 Schistosoma mansoni cercariae. Animals were sacrificed in the acute disease phase, ie, 8 weeks post infection. We compared the expression of multiple (hyper)sensitivity-associated mediators in T8-T13 DRG of infected (n=5) and control animals (n=5) by means of qPCR. In addition, DRG neurons that innervate the intestine were retrogradely labelled with Fluorogold (FG) and cryosections of FG-traced DRG were immunostained for pERK1/2, a phospho-protein that is commonly used as a proxy for neuronal activation.

Results: The presence of acute intestinal schistosomiasis in the GI wall of S. mansoni-infected animals was confirmed by a granulomatous inflammation surrounding the parasite eggs, as well as a substantial increase in mucosal mast cell protease-1 mRNA and protein expression. Intestinal schistosomiasis significantly increased the mRNA expression of c-fos, cgrp, tac1, trpv1 and kcnq2 in T8-T13 DRG. Moreover, the proportional expression of pERK1/2 in FG-traced neurons was significantly increased in infected animals compared to control animals.

Conclusion: The increased mRNA expression of several pain-associated mediators, as well as the increased expression of pERK1/2 in intestine-innervating DRG neurons, provide strong molecular clues to increased visceral sensitivity in infected animals. Functional studies dealing with visceral sensitivity and the role of the mastocytosis herein will further strengthen our results. This study was supported by FWO-grant G019314N to RB and JPT.

Policy of full disclosure: None.

NEUROGASTROENTEROLOGY: ACROSS THE LIFESP AN

186 | Clinical course and outcome in patients with severe dysphagia after lateral medullary syndrome

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Purpose: Although the prognosis of dysphagia in lateral medullary syndrome (LMS) is favorable, there is little information about progression of severe dysphagia over time in perspective of video fluoroscopic study (VFSS) findings, diet and/or postural modification. The purpose of this study was to verify the clinical course and outcome in patients with severe dysphagia after LMS. MATERIALS AND.

Methods: The patients with 'severe dysphagia after LMS' who admitted in rehabilitation unit from December 2013 to December 2016 were collected by retrospective medical record review. The criteria of 'severe dysphagia after LMS' was defined as: (i) acute or subacute LMS patients, (ii) initially required tube feeding, (iii) decreased pharyngeal constriction and not showing any esophageal passage in VFSS findings. Data were collected including VFSS findings, types of diet and postural modification.

Results: Eleven patients (6 men, 5 women, mean age, 59.5 years; range, 38-74 years) were identified who had 'severe dysphagia after LMS' among the 36 cases of LMS patients. The lesion side was left in 4 cases and right in 7 cases. Initial VFSS was performed at 16.1±18.0 days after the onset and serially conducted at every 2 weeks interval. Esophageal passage was begun to show at an average 32.7±18.5 days and the patients could begin partial oral diet feeding with postural modification (head rotation). There were 52.2±21.8 days required to change into full oral diet feeding. After 68.1± days, postural modification was not required any more in 7 cases.

Conclusion: Every patient with 'severe dysphagia after LMS' could start partial oral feeding approximately at about 5 weeks after the onset and they were allowed normal diet without any diet modification and limitation after 10 weeks. This clinical course and outcome might help in predicting the prognosis and planning the strategy of rehabilitation program in severe dysphagia after LMS.

Policy of full disclosure: None.

187 | The impact of naloxegol treatment on gastrointestinal transit and colonic volume

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Objective: Opioid treatment is associated with gastrointestinal (GI) side effects, known as opioid-induced bowel dysfunction (OIBD). Symptoms of OIBD are caused by opioid receptor activation in the enteric nervous system, which results in increased GI transit time and increased faecal volume in the colon. OIBD can be experimentally induced in healthy participants through oral oxycodone treatment. The aim of this study was to investigate whether administration of naloxegol, a peripherally restricted opioid antagonist, could reduce GI symptoms, GI transit time, and colorectal volume, using an experimental model of OIBD.

Methods: In a double blind crossover trial, twenty-five healthy males were randomly assigned to a 6 day treatment of oral oxycodone in combination with either oral naloxegol or placebo. At baseline and at day six, participants filled in the Patient Assessment of Constipation Symptom questionnaire, and colorectal volume was quantified with a magnetic resonance imaging method. Participants swallowed a small electromagnetic capsule, which allowed determination of total and segmental GI transit times, using the 3D-Transit system.

Results: In the established model of oxycodone induced OIBD, fewer GI symptoms were observed during naloxegol treatment, compared to placebo ($P<.01$). Naloxegol decreased median total transit time by 27% (56 vs 71 hours, $P<.05$) and decreased colorectal transit time by 33% (45 vs 59 hours, $P<.01$), compared to placebo. No difference in colorectal volume was found between the two treatments ($P>.05$).

Conclusions: In an experimental model of OIBD, GI symptoms and GI transit time were reduced during treatment with naloxegol, compared to placebo. However, naloxegol treatment did not reduce colorectal volume. These findings add information on the potential of naloxegol to be used in prevention and treatment of OIBD.

Policy of full disclosure: This study is funded (i) in part by an unrestricted grant from AstraZeneca Nordic Baltic, Sweden, and (ii) in part by the Svend Andersen Foundation. The funding sources for this work were not involved in the conduct of the study or in the development of the manuscript. The authors declare no conflicts of interests.

188 | The potential impact of mode of delivery, gestational age at birth and multiplicity on neurodevelopmental outcome in early childhood

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Background: Previous findings from the INFANTMET study have indicated that breastfed infants born by caesarean section (CS) showed a different microbiota profile to breastfed infants born by vaginal delivery (VD) over the first weeks 8 of life and also that preterm (PT) infants had a different microbiota profile to full term (FT) infants. Additionally, the microbiota of twins in the INFANTMET study was found to be more similar to one another than two unrelated infants (Hill et al., 2017). These findings indicate that mode of delivery, multiplicity of birth and gestational age have significant effects on early microbiota composition.

Purpose: The Bayley Scales of Infant Development III were conducted on the Infantmet Cohort (n=89) between the ages of two and three

years old to evaluate neurodevelopmental outcomes related to birth factors.

Findings: Children born as a result of a singleton birth showed significant differences with higher scores in relation to cognitive performance (t(54.98)=4.13, *P*=.00), cognitive composite (t(54.98)=4.13 *P*=.00), language composite (t(87)=2.63, *P*=.01), fine motor skills, t(86)=2.62, *P*=.01) and Adaptive Leisure Skills (U=117 *P*=.048) when compared to children born by multiple birth. For children born FT, mode of birth indicated significant developmental differences, with FT children born by CS showing significantly higher scores in relation to cognition (t(60.57)=2.76, *P*=.008), expressive communication, (t(63.42)=−2.3, *P*=.02) language composite (t(68)=-2.62, *P*=.01), gross motor skills (U=443, *P*=.03), motor composite, (U=404, *P*=.046) and functional academics (t(63)=−2.4, *P*=.02). These findings indicate that mode of birth and multiplicity of birth have significant effects on neurodevelopment.

Policy of full disclosure: None.

189 | Acid and weakly acidic reflux as a cause of chronic unexplained cough

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Persistent cough is a frequent problem of which gastroesophageal reflux is considered to be a significant contributing factor. Patients presumed to have reflux-induced cough are treated as such despite the limited treatment efficacy in this population. The aim of this study was to assess the yield of 24-hour ambulatory pH-impedance-pressure monitoring in finding a causal relationship between chronic cough and reflux. Twenty-four-hour ambulatory pressure-pH-impedance monitoring was used to study 192 patients with chronic cough. Patients were recruited through the outpatient clinic and through referral in three different centers. Manometric tracings were used to detect all cough bursts and pH-impedance monitoring was used to detect reflux episodes, including weakly acidic reflux. The symptom association probability (SAP) was used to determine a temporal relationship between reflux and cough. In the 192 patients studied (70.3% female, median age 57.5 years) a total of 7472 reflux episodes were detected, of which 71.5% were acidic. A total of 6442 cough burst episodes were detected manometrically, of which only 59% were registered by the patients. The majority of the patients (52.1%) did not report typical reflux symptoms. Pathological distal acid exposure time (>6%) was found in 21.4% of the patients. A total of 21.9% of all cough burst episodes were temporally associated with reflux, with 48.6% of these being reflux-cough episodes and 51.4% cough-reflux episodes. A diagnosis of reflux-induced cough (positive SAP for reflux-cough sequence) was made in 25.5% of the patients. Interestingly, if only acid reflux episodes were used, 22.4% of these patients would not have been diagnosed. Significantly more patients diagnosed with reflux-induced cough had a pathological distal acid exposure time and typical reflux symptoms in comparison to patients without the diagnosis. A diagnosis of cough-induced reflux was made in 24.0% of the patients. In approximately one quarter of the patients with chronic unexplained cough, reflux can be identified as a probable causative factor. This explains the observation that the vast majority of patients with unexplained chronic cough does not benefit from anti-reflux therapy. Ambulatory 24-hour pH-impedance-pressure monitoring provides a means to identify patients who are likely to have reflux-induced cough.

Policy of full disclosure: AJB received research funding from Endostim, Medical Measurement Systems, Danone and Given and received speaker and/or consulting fees from MMS, Astellas, AstraZeneca and Almirall; DS received a research grant from Sandhill Sc USA. TH, AP, JJ, AS and JT declare no conflicts of interest.

190 | Prolonged-release oxycodone/naloxone improves anal sphincter relaxation compared to oxycodone plus macrogol 3350

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Objective: Opioid analgesics inhibit anal sphincter function and contribute to opioid-induced bowel dysfunction (OIBD). However, it is unknown if the inhibition can be reduced by opioid antagonism with prolonged-release (PR) naloxone and how this compares to laxative treatment. The objective of this study was to compare the effects of combined PR oxycodone/naloxone or PR oxycodone plus macrogol 3350 on anal sphincter function and gastrointestinal symptoms.

Methods: A randomized, double-blind, crossover trial was conducted in 20 healthy men. Participants were treated for 5 days with combined PR oxycodone/naloxone or PR oxycodone plus macrogol 3350. Resting anal pressure, anal canal distensibility, and relaxation of the internal sphincter to rectal distension were evaluated before treatment (baseline) and on day 5. The Patient Assessment of Constipation questionnaire (PAC-SYM), stool frequency, and stool consistency were assessed daily.

Results: Both PR oxycodone/naloxone and PR oxycodone plus macrogol treatment decreased sphincter relaxation compared to baseline (−27.5%; *P*<.001 and −14.7%; *P*=.01). However, sphincter relaxation was better after the PR naloxone/oxycodone treatment compared to macrogol (difference=+17.6%; *P*<.001). Resting anal pressure and anal canal distensibility did not differ between treatments. PAC-SYM abdominal symptoms score was lower during PR naloxone compared to macrogol (0.2 vs 3.2; *P*=.002). Number of bowel movements was lower during PR naloxone vs macrogol (4.2 vs 5.4; *P*=.035).

Conclusion: Relaxation of the internal anal sphincter was significantly better after PR oxycodone/naloxone treatment compared to PR oxycodone plus macrogol 3350. These findings highlight that OIBD may require specific therapy against the complex, pan-intestinal effects of opioids.

Policy of full disclosure: Authors' declaration of personal interests (i) Asbjørn Mohr Drewes has received financial support from Mundipharma, AstraZeneca, Shire, Almirall, Grünenthal, and Pfizer. (ii) Klaus Krogh has served as an advisory board member for Coloplast, Wellspect, Nordic Health Care, Amirall, and Shire. (iii) Hans Gregersen invented the EndoFLIP technology and owns a minority share (<5%) in Crospon Ltd. Declaration of funding interests (i) The study was funded in part by Innovation Fund Denmark—Individuals, Disease and Society, grant number 10-092786 (ii), in part by The A.P. Møller Foundation for the Advancement of Medical Science, grant number 14-319, (iii) in part by Aage og Johanne Louis-Hansens Fond, (iv) in part Svend Andersen Fonden, and (v) in part by an unrestricted grant from Mundipharma Research GmbH & Co. KG.

191 | Effects of hesperetin on the gastric antral motility

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Background and aims: The purpose of the study was to investigate the effect of hesperetin, an aglycon of hesperidin, on the motility of gastric antrum, and its mechanism of action in vitro.

Methods: Adult male Sprague Dawely rats weighing 250-350 g were used. Muscle strips from rat gastric antrum were set up in organ baths in circular orientation.

Results: Both hesperidin and it's aglycon, hesperetin, decreased the amplitude of circular antral spontaneous contraction and its basal tone in a dose dependent fashion (1-100 μmol/L). However, hesperetin showed more strong effects than hesperidin. At the concentration of 100 μmol/L, hesperetin almost blocked the phasic contraction of antral strip, but hesperidin showed only about 50% inhibition. The effects of hesperetin (10 μmol/L) were significantly suppressed by L-NAME, an inhibitor of NOS and ODQ, an inhibitor of guanylyl cyclase. The action of hesperetin on the antral motility was significantly inhibited by α-adrenergic receptor antagonist, phentolamine (1 μmol/L), but not by the β-adrenergic receptor antagonist, propranolol (1 μmol/L). Doxazosin (1 μmol/L), α_1-adrenergic receptor antagonist, showed the significant inhibition on the action of hesperetin, whereas yohimbine (1 μmol/L), the α_2-adrenergic receptor antagonist, did not.

Conclusion: These results suggest that hesperetin inhibits the antral motility, and this action is mediated by the NO pathway and the α_1-adrenergic receptor.

Policy of full disclosure: None.

192 | The effects of the active metabolites of sodium picosulfate and senna on human colorectal neuromuscular activity

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Objective: Sodium picosulfate and senna are stimulant laxatives which alleviate constipation (1). In humans, their mechanisms of action are not fully understood. We investigated the effects of their active metabolites (sodium picosulphate: desacetyl bisacodyl; senna: sennoside A, sennoside B, rhein) in human isolated colon.

Methods: Mucosa-free circular muscle strips from colorectal resections for cancer (patients aged 67(28-87) years) were suspended in tissue baths (37°C; Krebs solution; isometric recording). Electrical field stimulation (EFS; 50 V, 0.5 ms, 5 Hz, 10 seconds, 1 minutes) evoked neuronally-mediated responses (abolished by tetrodotoxin 1 μmol/L; n=5). Drugs were applied non-cumulatively; n=patients. 3 parameter concentration response curves were plotted (Graphpad Prism 7.02); data from each region were similar and are pooled.

Results: EFS evoked contractions or relaxations during EFS, often followed by an after-contraction. Atropine 1 μmol/L prevented contractions during (n=5) and reduced those after EFS (n=3); L-NAME 300 μmol/L prevented relaxation or increased tension during EFS (n=6) without consistently affecting after-contractions. Desacetyl bisacodyl (0.1-100 μmol/L) had no consistent effect on responses during EFS and transiently facilitated after-contractions (Emax=90±21%, pEC50=5.3±0.6, n=4-8). However, at 10-100 μmol/L desacetyl bisacodyl caused sustained increases in muscle tension (at 100 μmol/L by 3.5±1.1 g/g; n=8). Both responses were insensitive atropine 1 μmol/L or L-NAME 300 μmol/L (n=4-6), muscle tension increases were observed in the presence of tetrodotoxin 1 μmol/L (n=5). In contrast, rhein (0.01-10 μmol/L, n=3-8), sennoside A and sennoside B (100 μmol/L; n=3-4 each) had no consistent effects on EFS or baseline tension over vehicle (1% DMSO).

Conclusion: Commonly used laxatives have different mechanisms of action in the human colon. Desacetyl bisacodyl can evoke a sustained muscle contraction. In contrast to guinea-pigs (3), senna metabolites have no consistent effects on human neuromuscular responses. Further studies of human colonic secretory mechanisms are required.

Reference:
1. Lembo & Camilleri. N Engl J Med 2003;349:1360-8.
2. Broad et al. Br J Pharmacol 2013;170:1253-61.
3. Izzo et al. Br J Pharmacol 1998;124:825-31.

Policy of full disclosure: None.

193 | Isolation of myenteric neurons from adult human colon, to investigate changes during advanced age and degenerative disorders

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Objectives: Degenerative disorders of the gastrointestinal (GI) tract occur during diabetes, advanced age, and many other disorders. To investigate mechanisms and treatments we are developing new techniques to culture primary adult human enteric neurons and glia. This has not previously been achieved for adult tissues.

Methods: Macroscopically-normal colon and rectum was obtained with consent from patients undergoing surgery for malignancy. Smooth muscle was isolated, digested and cultured in a neuron-specific medium containing serum, supplements and growth factors which discourage growth of other cell types. Culture surfaces were coated with poly-d-lysine and laminin to encourage neuronal growth and neurite formation. Decontamination steps were used to decrease bacterial and fungal contamination from the non-sterile tissue, and penicillin, streptomycin and amphotericin B were added to the media to reduce growth of contaminants. Cells were cultured for up to 10 days.

Results: The cultured primary neurons were evaluated by immunohistochemistry, and βIII-tubulin (pan-neuronal marker) was detected. It will also be used to assess the presence of specific cell markers, including ChAT (cholinergic neurons), nNOS (nitrergic neurons) GFAP (glial cells), ACTA2 (smooth muscle), and SYN1 (synaptic marker). Neurons will also be harvested for gene expression analysis, and calcium-sensitive dyes will be used to study electrical activity in the cultured neurons. Primary GI smooth muscle cell cultures were also developed from human surgical resections. A similar method of cell dissociation was used as for the neuronal cultures, but cells were cultured in DMEM with serum, and culture plates coated with collagen. These cultures will be used for both control and experimental purposes investigating nerve-muscle interactions.

Conclusion: Once characterised, these live, primary human cells will be manipulated by exposure to novel environments and pharmacological tools, evaluating the responses by techniques such as calcium imaging, RNA analysis and immunohistochemistry. In particular, certain aspects of ageing and/or degeneration will be replicated to identify important cellular and molecular pathways involved in ageing, and to develop tools for evaluation of potential preventative drugs.

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194 | The effects of ageing on gene expression in the human colon

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Objectives: The elderly are increasingly likely to experience gastrointestinal disorders, including chronic constipation, faecal incontinence and various degenerative diseases. This study aims to identify cellular pathways involved in age-related changes within the human colon.

Methods: Macroscopically-normal ascending and descending colon was obtained with consent from patients undergoing surgery for malignancy. Samples of muscle were collected for RNA extraction, and qPCR carried out using SYBR Green. For both regions of colon, two age groups were compared: patients aged between 35 and 60 years (adult) and aged 70 or more years (elderly); n=10 for each group with an equal number of males and females. Genes were chosen from cellular and molecular pathways associated with ageing, including inflammation, oxidative stress, axonal transport, autophagy, angiogenesis and senescence, as well as markers of various cell populations.

Results: Age-related gene expression changes were observed in multiple pathways, in particular oxidative stress, senescence and inflammation. Many changes were found only in the ascending, and not the descending colon. Most were small but statistically significant and could have a cumulative effect. In particular, there was a 2.4-fold increase in TNFα expression and a 1.5-fold increase in the senescence marker p16 in aged ascending colon compared to adult ascending colon. Further, many genes show an improved and striking association in expression when correlated with p16 expression, rather than age. Again, this correlation was often more pronounced in the ascending colon. Notably, TNFα (2.3-fold increase), NOX4 (1.9-fold increase), GPX3 (1.7-fold increase), TIE1 (1.6-fold increase), and VEGFA (1.5-fold increase) show significant changes in gene expression in the ascending colon associated with increased p16 expression.

Conclusions: These results suggest several cellular processes are associated with ageing in the human colon, particularly the ascending colon. They also suggest that p16 expression is a more representative measure of biological ageing than temporal age. The findings may now be used to research causes and potential treatments for age-related disorders.

Policy of full disclosure: This work was funded by Takeda.

195 | Optimal cutoff value of integrated relaxation pressure for esophagogastric junction outflow obstruction in the Sandhill high-resolution manometry system

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Background/Aims: Integrated relaxation pressure (IRP) is a key metric for diagnosing esophagogastric junction outflow obstruction (EGJOO). However, its normal value might be different according to the manufacturer of high-resolution manometry (HRM). This study aimed to investigate optimal value of IRP for segregating clinical relevant patients with EGJOO diagnosed by Sandhill HRM system and to find clinicomanetric factors associated with clinical significance.

Methods: We analyzed 262 consecutive patients who underwent HRM between June 2011 and December 2016 for esophageal symptoms and had elevated median IRP (>15 mmHg). Clinical relevant patients were defined as follows: (i) subsequent HRM met the achalasia criteria during follow-up (early achalasia); (ii) Eckardt score decreased at least two points and did not exceed a score of 3 after pneumatic dilatation (variant achalasia), and (iii) significant passage disturbance without structural abnormality on esophagogram (possible achalasia). After determining the optimal cutoff value with reference to the IRP distribution, we compared clinicomanometric characteristics between clinically relevant and non-relevant groups.

Results: Seven patients (2.7%) with elevated median IRP were clinical relevant. Two of them were early achalasia, four patients were variant achalasia, and one patient was possible achalasia. All clinically relevant patients had IRP 20 mmHg or above, and all patients with IRP of less than 20 mmHg belonged to non-clinically relevant group. Among patients with IRP 20 mmHg or more (n=122), clinically relevant group (n=7) had significantly higher rate of dysphagia (100% vs 24.3%, *P*<.001) and compartmentalized pressurization (85.7% vs 21.7%, *P*=.001) compared to non-clinically relevant group (n=115).

Conclusions: Our results suggest that IRP of 20 mmHg or higher could segregate clinically relevant patients showing EGJOO in Sandhill HRM system. Additionally, if patients had dysphagia and compartmental pressurization together, careful follow-up is essential. However, if they do not have dysphagia and/or compartmental pressurization, elevated IRP may be nonspecific.

Policy of full disclosure: None.

196 | Lactobacillus rhamnosus JB-1 reverses old-age-related reduction in murine vagal afferent firing frequency

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Background: The increased incidence of constipation in individuals over the age of 65 and a comorbidity of gastrointestinal (GI) disorders with common age-related conditions and depression is a growing concern in an aging population. Gut bacteria can regulate mood and behaviour through the vagal gut-brain axis. Previous literature suggests that there is reduced mesenteric afferent firing with old age. We hypothesize that there is a decrease in jejunal vagal afferent firing with old age and that this effect may be treated by application of beneficial bacteria. We recorded vagal background firing in aged mice and tested whether a known vagal-dependent psychoactive bacteria Lactobacillus rhamnosus JB-1™ can facilitate the background discharge.

Methods: Ex-vivo jejunal segments with attached mesentery from aged (18-24 months) and young (3 months) male CD1 mice were carefully dissected to isolate the mesenteric nerve bundle. The mesenteric nerve bundle was sucked onto by a glass micropipette attached to a patch-clamp electrode. Multi-unit electrical activity was recorded using an amplifier and signal converter. Vagal fibres were identified by response to CCK and basal vagal afferent firing in aged and young mouse controls were compared. Intraluminal application of JB-1 was applied to both young and aged tissue preparations.

Results: The mean interval between spikes for basal vagal afferent firing was increased in the aged mouse controls in comparison to the young mouse controls. This was indicative of a decreased basal firing frequency in vagal fibres from the jejunum in aged mice. Addition of JB-1 increased vagal firing frequency by 80% (*P*<.0001) in aged mesenteric nerve afferents in comparison to the aged control.

Conclusion: Luminal administration of JB-1 increased previously depressed vagal afferent nerve firing in aged mice. Oral administration of JB-1 bacteria may potentially improve management of constipation and simultaneously improve mood and depression in aged individuals.

Policy of full disclosure: None.

197 | Colonic flora and small intestine bacterial overgrowth in patients with Intestinal Bowel Syndrome (IBS) by Rome III Criteria

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Background: Lactulose hydrogen breath test (LHBT) determines the presence of small intestinal bacterial overgrowth (SIBO) and allows the characterization of colonic flora, according to production of hydrogen (H2) and methane (CH4). It has been reported that the type of microbiota and the presence of SIBO could be involved in the pathophysiology of Irritable Bowel Syndrome (IBS).

Aim: To characterize colonic flora according to production of H2 and CH4 in IBS-patients and evaluate SIBO. METHODS: 900 patients with IBS were evaluated by Rome III Criteria and LHBT standardized technique, 78% female sex, between 18 and 88 years. According to Rome III, 37% IBS-diarrhea (IBS-D), 27% IBS-constipation (IBS-C), 18% IBS-mixed (IBS-M) and 18% IBS-undetermined (IBS-U). It was characterized as H2-producing flora the presence of concentrations of H2 more than 20 ppm sustained after of 60 minutes and CH4 less than 12 ppm during study time (180 minutes); CH4-producing flora in concentrations of H2 less than 20 ppm and CH4 more than 12 ppm after 60 minutes and mixed flora concentrations of H2 greater than 20 ppm and CH4 greater than 12 ppm during study time. SIBO was diagnosed when elevation curve of H2 more than 20 ppm above baseline and elevation of curve CH4 over 12 ppm of baseline, in more than two consecutive measurements in the first 60 minutes. Statistical analysis Mann-Whitney and Chi-square.

Results: 54% presented mixed flora, 41% H2-producing flora and 5% CH4 producing flora. 34% of patients with mixed flora have IBS-D

and 30% IBS-C. 42% of patients with H2-producing flora have IBS-D. 32% of patients with CH4-producing flora have IBS-C, without differences with other phenotypes. 43% of patients with IBS have SIBO; of these 68% have mixed flora. SIBO was more frequent between 27 and 43 years.

Conclusion: Unlike other studies, the CH4-producing flora was not predominant in patients wit IBS-C. Mixed flora is the most frequent in Chilean IBS-patients. It is confirmed that SIBO is common in IBS.

Policy of full disclosure: None.

198 | Signs of leaky gut in Parkinson's disease

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Objective: Since there is a growing body of evidence indicating that increased intestinal permeability may play an important role in the pathogenesis of neurodegenerative disorders, the aim of this study was to evaluate fecal calprotectin and zonulin as biomarkers of gut inflammation and intestinal barrier dysfunction, respectively, in patients with Parkinson's disease (PD).

Methods: Thirty consecutive patients with PD (mean age 66, range 47-78 years) and 15 healthy controls (mean age 58, range 49-75 years) were included in the study. The quantitative evaluation of calprotectin and zonulin in stool samples was performed by ELISA tests. The prevalence of gastrointestinal symptoms was assessed based on a questionnaire. The Kruskal-Wallis test was used for the comparison of differences between the groups.

Results: A mean duration of PD in the studied group was 7 years (range 1-20 years). None of the patients was diagnosed with inflammatory bowel disease, neither was using nonsteroidal anti-inflammatory drugs, which may affect intestinal permeability. The most frequent gastrointestinal symptoms reported by PD patients included: constipation (63%), feeling of incomplete evacuation (60%), bloating (40%), abdominal pain (23%) and alternating bowel movement pattern (13%). The biomarker levels are presented as median and the lower quartile (25Q)—upper quartile (75Q). The fecal calprotectin level (μg/g) was significantly higher in PD patients compared to the controls 46.9 (12.2-119.7) vs 18.0 (7.1-25.4), P=.002. The fecal zonulin level (ng/mL) was also higher in PD patients compared to the controls, but the p-value did not reach statistical significance: 181.8 (95.5-276.0) vs 87.5 (47.2-239.9), P=.094.

Conclusions: PD is associated with a high prevalence of gastrointestinal symptoms. The signs of leaky gut related to colonic inflammation and increased intestinal permeability are present in a remarkable number of PD patients. The stool ELISA tests may be a useful tool in diagnosing gut immune system activation and changes in the intestinal barrier integrity in PD patients.

Policy of full disclosure: None.

199 | The gastrointestinal regional pH profile is altered in patients with type 1 diabetes and peripheral neuropathy

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Background: Gastrointestinal (GI) symptoms are common in patients with type 1 diabetes (T1DM). Autonomic dysfunction in diabetes can lead to changes in the GI secreto-motor function and hypochlorhydria and the development of symptoms. We hypothesized that the pan-enteric pH profiles in patients would be different from healthy subjects and associated with objective physiological/clinical markers.

Methods: 47 patients with T1DM and diabetic sensory peripheral neuropathy (DSPN) and 41 healthy age matched subjects underwent a standardized wireless motility capsule test. The gastroparesis cardinal symptom index (GCSI) comprising of subscales assessing nausea, bloating and fullness, the gastrointestinal symptom rating scale (GSRS) assessing abdominal pain, reflux-, diarrhoea-, ingestion- and constipation syndrome, disease duration, glycemic control, insulin usage and 24-hour heart rate variability testing was undertaken in patients.

Results: In comparison to healthy subjects, the mean gastric and large bowel pH was lower in patients (P=.001 and P=.001, respectively). The mean pH was higher in the small bowel of patients (P=.04). There was a heightened decrease in pH across the ileocaecal junction in patients (P=.0001). Mean stomach pH was associated with bloating (r=.35, P=.03) and ingestion syndrome (r=.39, P=.04) and mean small bowel pH was associated with nausea (r=.38, P=.01). The mean large bowel pH was associated with nausea (r=.39, P=.008), bloating (r=.33, P=.03) and total GCSI (r=.39, P=.008). RR-intervals were negatively associated with large bowel pH (r=−.3, P=.046). No association was found between age, disease duration or glycaemic control.

Conclusions: The GI pH profile in patients with T1DM and DSPN is different from healthy controls. These findings may have important implications for oral pharmacotherapeutic interventions as changes in pH profile can influence bioavailability.

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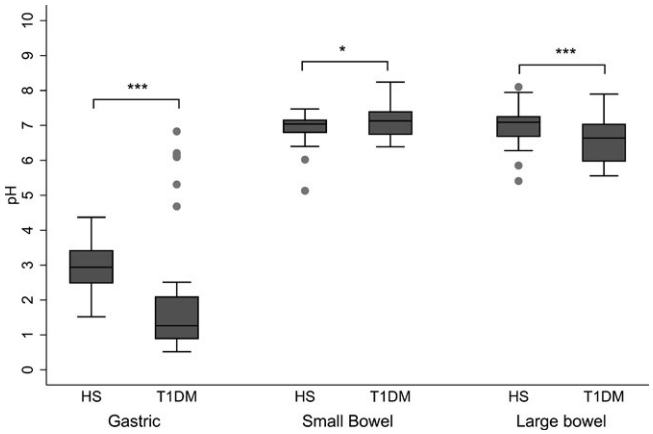

FIGURE 1 Box and whiskers plot of mean pH in the gastric, small bowel and large bowel of healthy subjects and patients with type 1 diabetes. *P<.05 and ***P<.001. HS: healthy subjects, T1DM: patients with type 1 diabetes mellitus.

200 | Chronic stress in informal dementia caregivers: Differential effects on the Brain-Gut-Microbiome axis

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Introduction: Acute stress can induce gastrointestinal distress but the impact of de novo chronic stress in human subjects is poorly understood. Caring for a relative with dementia is considered particularly stressful, and there is evidence that long-term family caregivers are more likely to develop irritable bowel syndrome, a disorder of the brain-gut-microbiome axis.

Aims/Background: The current study aimed to compare family dementia caregivers to a non-caregiver control group, and to examine the impact of interventions designed to help dementia caregivers manage stress and the caregiving role on gastrointestinal symptoms, cognitive performance and psychological well-being.

Methods: Caregiver participants were recruited via clinics at St. Finbarr's Hospital, Cork and control participants via the university community. Participants completed the irritable bowel syndrome symptom severity scale, as well as validated tests of stress, and depression. Participants also completed cognitive tasks from the CANTAB battery. A subset of caregivers completed both a carer training program and mindfulness-based stress reduction program. Each program was delivered in a group setting by an experienced instructor and lasted 6-8 weeks.

Results: Compared to controls, caregivers had higher levels of stress, marginally higher depression, and poorer cognitive performance, both in terms of visuospatial memory, and sustained attention. However, gastrointestinal symptoms were not altered compared to controls. Following both interventions, caregivers had improved cognitive performance, both in terms of memory and attention but gastrointestinal symptom severity scores were not altered following the interventions.

Conclusions: Dementia caregiving was associated with heightened stress and depression, as well as poorer cognitive performance in the absence of gastrointestinal dysfunction. Furthermore, psychological interventions improved cognitive performance in caregivers but did not affect gastrointestinal parameters. Chronic stress can produce differential effects at different nodes of the brain-gut axis and may not always be associated with gastrointestinal distress.

Policy of full disclosure: None.

201 | Prospective randomized clinical trial with three antibiotics therapies in treatment of small intestinal bacterial overgrowth

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Background: There is evidence of the importance of treatment for small intestinal bacterial overgrowth (SIBO) in patients with functional gastrointestinal disorder (FGD). AIM: to evaluate efficacy, safety and tolerability of three antibiotic treatments for SIBO and the presence of pain and bloating in patients with FGD.

Methods: 97 patients with FGD and diagnoses of SIBO according lactulose breath test (LBT) were included in this prospective, double-blind, longitudinal, randomized study with three antibiotics therapies during 10 days. Group A: 32 patients, rifaximin 400 mg twice daily; Group B: 32 patients, ciprofloxacin 500 mg twice daily; Group C, metronidazole, 500 mg three times daily. 15 days after the treatment LBT was performed in order to analyse intensity of symptoms (visual scale from 0 to 10), compliance with therapy and side effects. Statistical analysis with Fisher's test.

Results: 81 patients completed the study (27, 29 and 25 per group, respectively) (Figure 1), 90% women. SBI improvement was observed in 81% with metronidazole, 62% with rifaximin and 43% ciprofloxacin (P=.016). There was improvement of symptoms: pain and boating in three groups (P=.021). 28 patients presented side effects: 50% with metronidazole, 39% with ciprofloxacin and 11% rifaximin (P=.001).

Conclusion: Metronidazole and Rifaximin showed significant improvement in SIBO compared to ciprofloxacin. Moreover, there was improvement of symptoms with three therapies independent of SIBO. Metronidazole had a high incidence of side effects. Our results confirm that Rifaximin is effective for the treatment of SIBO with less adverse effects.

Policy of full disclosure: None.

Group (n patients started / completed therapy)	Bloating		Pain		SBI After treatment	Side effects
	1	2	1	2	%	%
A : Rifaximin (32/27)	7,2 ± 2,2	3,4 ± 2,3	6,4 ± 2,0	2,4 ± 2,0	62	11
B : Ciprofloxacin (32/29)	6,3 ± 2,0	3,6 ± 3,0	6,7 ± 2,4	2,7 ± 2,5	43	39
C : Metronidazole (33/25)	7,4 ± 2,3	3,1 ± 2,5	6,0 ± 2,8	2,3 ± 2,2	81	50

1: baseline; 2: 15 days after treatment.

TABLE

202 | Presence of non-included digestive symptoms in Rome III criteria for diagnosis of irritable intestinal syndrome in symptomatic patients

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Background: The diagnosis of Irritable Bowel Syndrome (IBS) is established according to Rome III criteria. These criteria consider abdominal pain or discomfort as the main symptom. There are symptoms not included as painful defecation, compromise of daily life activities, mucus in feces, feeling of incomplete evacuation and bloating. AIM: to evaluate the presence of non-included digestive symptoms in Rome III criteria in patients with IBS and patients with abdominal discomfort, or abdominal pain without IBS by Rome III (No-IBS). In addition, to determine the proportion of IBS patients who meet Rome IV criteria.
Methods: Rome III for IBS were completed by 963 symptomatic patients, 76% female sex. The frequency and intensity (analogous visual scale from 0 to 10) of non-included symptoms in Rome III criteria were considered. The Rome IV criteria only considers abdominal pain but not discomfort. Statistical analysis with Mann-Whitney test and Chi-square.
Results: 816 symptomatic patients (85%) presented SII according to Rome III criteria, 78% female sex. Pain interferes with daily activities 56% of patients with IBS and 29% of NO-IBS (*P*=.0004). In IBS patients, 45% reported incomplete evacuation feeling, 95% reported bloating and 35% mucus in feces vs 10%, 80% and 21% respectively in No-IBS patients (*P*<.005). The intensity of non-included symptoms in IBS patients revealed a score of: 4.4±2.6 in pain during defecation, 5.7±2.6 in feeling of incomplete evacuation and 7.5±2.3 in bloating vs 3.3±2.4; 3.7±3.0 and 6.5±2.3, respectively, in No-IBS (*P*<.005). No differences were found in the intensity of difficulty in defecating. 35% of IBS patients met criteria according to Rome IV, 82% female.
Conclusion: The non-included digestive symptoms in Rome III criteria for the diagnosis of IBS are highly frequently in symptomatic patients. These symptoms affect significantly the daily activities. The Rome IV criteria indicate a decrease in the number of patients meeting criteria for IBS, although it maintains a higher proportion of women.
Policy of full disclosure: None.

203 | The contribution of TRPM7—Channels for generating phasic contractions of gastric antrum and colon smooth muscle

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Objective: The ionic currents underlying the slow wave depolarizations of intestinal smooth muscle are not yet fully characterized. In cultured interstitial cells of Cajal (ICC) from newborn animals, the Mg^{2+}-permeable ion channel TRPM7 has been proposed to be essential for pacemaker activity. The goal of this study is to test whether it is also essential for phasic contractions in adult animals.
Methods: The expression of TRPM7 in tissues from adult rats was estimated by qRT-PCR. The organ bath technique was used to measure the in vitro activity of smooth muscle preparations from gastric antrum and colon. Frequency, amplitude and acetylcholine/potassium induced contractions were quantified over time and the effects of the TRPM7-modulators spermine, flufenamic acid (FFA) and quinidine were examined. Intravital fluorescence microscopy of cultured ICC-clusters was used to test the effects of spermine on spontaneous calcium oscillations.
Results: The expression of TRPM7 in both tissues could be confirmed in both colon and antrum. Adding of the TRPM7-modulator spermine to a concentration of 700 µmol/L reduced the amplitude of spontaneous activity in antrum and colon preparations to 26% (n=8, SD=25%, *P*<.01.) and 40% (n=8, SD=31%, *P*=.03) whereas the contraction frequency of the both tissues was only insignificantly changed (mean: 91%, SD=37% and mean: 67%, SD=39%). In contrast, FFA and Quinidine led to parallel dose dependent decreases in force and frequency with a complete cessation of both at 50 µmol/L and 300 µmol/L, respectively. In both colon and antrum, acetylcholine-induced contractions were abolished in the presence of quinidine and diminished with spermine and FFA. Potassium-induced contractions (60 mmol) were decreased in both tissues by FFA and quinidine, but not by spermine. Rhythmic calcium oscillations in ICC clusters from gastric antrum were not altered in frequency of amplitude by adding spermine up to 700 µmol/L.
Conclusions: The potent TRPM7-blocker spermine does not inhibit spontaneous contractility completely. FFA and quinidine had more pronounced inhibitory effects, especially at concentrations were they also modulate other ion channels that are involved in regular organ function (eg, connexins and L-type calcium channels). Taken together, our data suggest that TRPM7 is not essential for pacemaking in intestinal smooth muscle from adult rats.
Policy of full disclosure: None.

204 | Neurotransmission in Irritable Bowel Syndrome (IBS): Glutamate and glutamine (Glx), but not GABA+, concentrations in right anterior insula are inversely correlated to pain duration

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Objective: Anterior insula (AIns) serves a key role in interoceptive awareness and is reportedly involved in the integration of affective, cognitive and motivational aspects of pain processing (Craig 2011). Alterations in AIns function have been documented in irritable bowel syndrome (IBS) (Mayer 2015), yet the association between

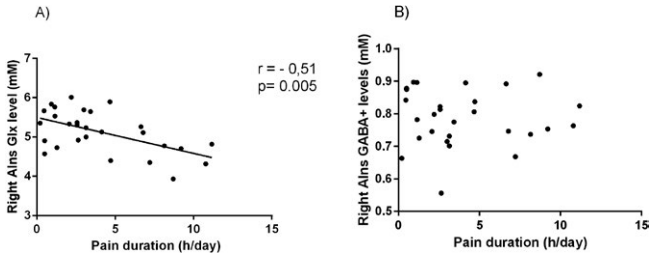

FIGURE 1 Correlation between Glx (A) and GABA+ (B) concentrations (mM) in right AIns and mean hours of abdominal pain per day recorded in the diary

neurotransmitter concentrations in AIns and abdominal pain in IBS remains unknown. We therefore aimed to elucidate the association between concentrations of both inhibitory (gammaaminobutyric acid; GABA+) and excitatory neurotransmitters (glutamate+glutamine; Glx) in AIns and abdominal pain duration in IBS.

Methods: 28 right-handed female IBS patients (mean age 31.9 years) recorded their gastrointestinal symptoms on a 14-days diary. Quantitative magnetic resonance spectroscopy (qMRS) was performed in a 3 T MRI scanner. A water-suppressed MEGA-PRESS sequence (kindly provided by R.Edden, Johns Hopkins University) was used with a voxel (4.5×2.0×3.0 cm^3) placed in bilateral AIns. Mean hours of abdominal pain per day (pain duration) were extracted from diary data and correlated with levels of AIns GABA+ and Glx.

Results: Pain duration was inversely associated with Glx concentrations in the right AIns (r=−.51; P=.005; Figure 1A), whereas no correlation was observed for the left AIns. There was no significant correlation between GABA+ levels in AIns and pain duration (Figure 1B).

Conclusion: Our findings showed a reduced concentration of excitatory neurotransmitter in right AIns which was related to increased abdominal pain experience in IBS, extending evidence on a crucial role of right AIns in the subjective awareness of visceral pain, as earlier suggested (Mayer 2015). Craig AD Significance of the insula for the evolution of human awareness of feelings from the body; Ann N Y Acad Sci. 2011 Mayer EA, Gupta A, Kilpatrick LA, Hong JY Imaging brain mechanisms in chronic visceral pain; Pain 2015.

Policy of full disclosure: None.

NEW TECHNOLOGIES IN CLINICAL NEUROGASTROENTEROLOGY

205 | Operation of patients with congenital anorectal malformations seem to impair the rectoanal inhibitory reflex and therefore increase the chance on constipation

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Objective: We inventorised the presence of the rectoanal inhibitory reflex (RAIR), type of congenital anorectal malformations (CARM) and kind of operation that these patients had undergone, in relation to objectively measured fecal incontinence and defecation problems.

Methods: We retrospectively reviewed clinical data of 47 patients who underwent operation for CARM at the University Medical Center Groningen between 1976 and 2009. We analyzed anorectal function tests, including the presence of RAIR. We classified the patients according to the Krickenbeck classification.

Main Results: We found RAIR in 49% (n=23) of CARM patients. Of the patients born without fistula 29% had RAIR, of patients with a rectoperineal fistula 75%, of patients who had either urethral-, bulbar-, vestibular, or prostatic fistula 43%, of patients who had a bladderneck fistula none had RAIR, and of patients with a cloaca 67% had RAIR. RAIR was present in all patients who were not operated (n=2), had undergone ASARP (n=4), PSARVUP (n=2), and was present in 42% (n=31) of the patients operated with PSARP. There was no association between the absence of RAIR and fecal incontinence (P=.405). Rectal volumes in patients with perineal fistula and urethral/vestibular/bulbar/prostate fistula who had a RAIR were lower than in patients without RAIR (median: 21 143 mL vs 49 000 mL and median: 30 450 mL vs 40 056 mL, respectively).

Conclusions: Patients born with CARM seem to have RAIR independently of the CARM form. RAIR however may be impaired due to operation. ASARP seems to be less destructive for the RAIR than PSARP. The increased rectal volumes in patients without RAIR indicate that they have the tendency to be more constipated. Therefore, saving the internal anal sphincter during the operation might improve the clinical results of CARM patients.

Policy of full disclosure: None.

206 | Role of esophageal intraluminal baseline impedance levels in patients with suspected gastroesophageal reflux symptoms

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Background: According to the ROME IV classification, NERD (non-erosive reflux disease) and RH (reflux hypersensitivity) are related to a combination of both acid exposure and hypersensitivity. Level of baseline impedance (BI) reflect the status of the esophageal mucosa and impairment of mucosal integrity. We aimed to evaluate differences on level of BI among the patients with suspected gastroesophageal reflux disease.

Method: A retrospective study involved 130 patients with heartburn, acid regurgitation, globus or non-cardiac chest pain who underwent endoscopy, high resolution manometry and 24-hour multichannel intraluminal impedance and pH monitoring (MII-pH) at a single tertiary

center from January 2013 to December 2016. Esophageal motility parameters, esophageal acid exposure time (AET), characteristics of acid and non-acid reflux episodes, symptom index and mean BI level at the distal esophagus (Z5-Z6) were analyzed.

Results: Sixty-seven patients of 130 enrolled patients were male (51.5%). The median age was 52.0. Of 130 patients, 32 (24.6%) were diagnosed erosive reflux disease (ERD) by endoscopy, 66 (50.8%) were NERD in impedance pH test, 7 (5.4%) were confirmed RH by symptom index and 25 (19.2%) were classified functional heartburn (FH). Although level of BI was not significant difference among each group, there was significantly higher in patients with RH than another group (P=.037). Also, there were negative correlations between level of BI and total acid percent time (r=−.390; P<.001) and number of total acid reflux episodes (r=−.188; P=.032). On subgroup analysis between NERD with abnormal AET and RH, patients with RH showed significant higher level of BI (P<.001) and distal contractile integral (P=.045).

Conclusions: Although there was no clinical impact in FH group, level of baseline impedance could be helpful for differential diagnosis among the patients with gastroesophageal reflux symptoms. Especially, the lower the level of BI may indicate the more acid exposure related to mucosal integrity. In addition, if the BI is high and the DIC is not reduced, it may indicate hypersensitivity to a small amount of acid reflux.

Policy of full disclosure: None.

207 | Defining response to the PAC-SYM questionnaire in patients with chronic constipation

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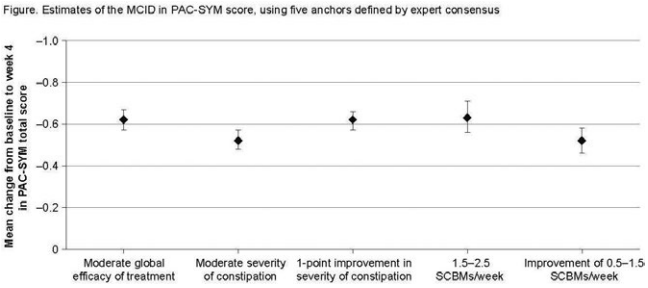
Objective: The Patient Assessment of Constipation-Symptoms (PAC-SYM) questionnaire is increasingly being used as a patient-reported outcome measure in clinical trials of constipation. It has internal consistency, test-retest reliability, concurrent validity and is responsive to change over time (1). However, the threshold for reduction in total PAC-SYM score used to define a clinical response has not undergone formal appraisal. We aimed to determine the minimal clinically important difference (MCID) in PAC-SYM score, and the optimum cut-off value for defining responders.

Methods: These analyses used Week 4 data from six phase 3/4, double-blind, randomized, placebo-controlled trials of prucalopride in chronic constipation (NCT01147926, NCT01424228, NCT01116206, NCT00485940, NCT00483886, NCT00488137). MCID analyses were performed using anchor-based methods. Anchors, defined by expert consensus, comprised: (i) moderate global efficacy of treatment; (ii) moderately severe constipation; (iii) 1 point improvement in global severity of constipation score; (iv) 1.5-2.5 spontaneous complete bowel movements (SCBMs)/week; and (v) increase of 0.5-1.5 SCBMs/week. Receiver Operating Characteristics (ROC) curve analyses were performed using the same anchors.

Figure. Estimates of the MCID in PAC-SYM score, using five anchors defined by expert consensus



Results: Data from 2884 patients were included. The MCIDs for different anchors are shown in the Figure. ROC analyses showed optimum cut-off values for discriminating responders to be slightly lower but in the same range as the MCIDs.

Conclusions: Anchor-based methods gave consistent results for the MCID, at approximately −0.6; this value was close to the optimal cut-off score for responder discrimination. This value could be considered in clinical practice. A slightly more conservative threshold, eg, −0.75, could be used in clinical trials to avoid a high placebo response rate. 1. Frank L et al. Scand J Gastroenterol 1999;34:870-7.

Policy of full disclosure: Yan Yiannakou has received speaker fees or educational grants from Allergan, Almirall, Coloplast, Kyowa Kirin, Medtronic and Shire. Jan Tack has provided scientific advice to Abide Therapeutics, Alfa Wassermann, Allergan, Chr. Hansen, Danone, Genfit, Ironwood, Janssen, Kyowa Kirin, Menarini, Mylan, Novartis, Nutricia, Ono Pharma, Rhythm, Shionogi, Shire, SK Life Science, Takeda, Theravance Biopharma, Tsumura, Yuhan, Zealand and Zeria; has received research grants or support from Abide Therapeutics, Shire and Zeria; and has served on speakers' bureaus for Abbott, Allergan, AstraZeneca, Janssen, Kyowa Kirin, Menarini, Mylan, Novartis, Shire, Takeda and Zeria. Hubert Piessevaux has received speaker and consulting fees from Shire. Dominique Dubois is a consultant to Shire. Eamonn MM Quigley has provided scientific advice to Alimentary Health, Almirall, Forest, Ironwood, Movetis, Rhythm, Salix, Shire, and Vibrant; has received honoraria for speaking from Almirall, Ironwood, Metagenics, Procter & Gamble, and Shire/Movetis; and has received research support from Rhythm and Vibrant. MeiYun Ke has received speaker fees from Janssen. Susana Da Silva is a shareholder and employee of Shire. Alain Joseph is a shareholder and employee of Shire. René Kerstens is a consultant to Shire.

208 | Provocative testing increases the diagnostic yield of high resolution oesophageal manometry in patients with oesophageal diverticula

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Objective: Oesophageal diverticula are rare diverticula of the gastrointestinal tract known to be associated with oesophageal motor disorders. Our aim was to study manometric abnormalities associated with this condition, both using wet swallows and solid swallows.

Methods: All patients with oesophageal diverticula who benefited from a high resolution oesophageal manometry (HRM) at Rouen University Hospital were included retrospectively.

Results: We retrospectively found 24 patients with oesophageal diverticula. 6 patients were excluded (5 with previous surgical history and 1 with traction diverticulum); 18 patients were selected for the analysis. HRM was performed using wet (5 mL of water) and solid (meat) swallows. Mean age of the patients was 70 and the main reported symptom was dysphagia. Normal manometry using wet swallows was found in 7 (39%) patients. 11 (61%) patients had an oesophageal motor disorder including 2 oesophagogastric junction outflow obstruction [OGJOO], 4 achalasia (subtype 2: n=2; subtype 3: n=2), 4 distal oesophageal spasms [DES] and 1 jackhammer oesophagus. In the 7 patients with normal manometry using wet swallows, solid swallows identified 4 (57%) additional motor disorder including 2 OGJOO, 1 jackhammer oesophagus and 1 DES. Provocative testing using solid swallows increased the diagnostic yield by 22% in overall patients and by 57% in patients with normal manometry using wet swallows only.

Conclusion: While more than one third of HRM using wet swallows were normal, provocative testing using chicken increased the diagnostic yield of the procedure by more than 50% in these patients.

Policy of full disclosure: None.

209 | Per oral endoscopic myotomy: A tertiary center experience in Turkey with 225 cases

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Background: In the management of achalasia recently per oral endoscopic myotomy (POEM) has become the rising treatment modality. When performed in experienced centers this minimally invasive technique is an effective and safe method with low incidence of complication rate. We present our results of POEM which are the first experiences of Turkey.

Methods: Between May 2014-February 2017, 225 patients with achalasia have undergone POEM. All the procedures were performed by a gastroenterologist experienced in endoscopic submucosal dissection and certificated for POEM, and the procedures took place in the endoscopy unit under general anesthesia given by an anaethetist. All the demographic and numeric data regarding the procedure have been recorded prospectively during and after the procedure to an electronic database specifically designed for this purpose.

Results: The median age of the patients was 41 (12-75) years. Preoperative and postoperative (3 months) median Eckardt scores were 8 (5-12) and 0 (0-2), respectively. The median total length of the procedure was 49; (24-153) minutes, tunnel length was 17; (12-27) cms and the myotomy length was 14 (8-25) cms. Postoperative oral intake started at median 1 (1-2) day, length of stay was 5 (3-7) days. Capnoperiteneum developed during the procedure in 65 patients, 59 of them were treated with veress needle. Mucosal injury developed in 3 patients which were managed successfully with endoscopic hemoclips. During follow-up, twelve and 18 months after the procedure the Eckardt scores of two patients with radiological sigmoid esophagus and dysphagia were measured as 4. In the barium swallow there were no differences in barium retention compared to third month control. As there were no severe dysphagia and weight loss re-POEM was not performed. Endoscopic follow-up was made in 153 patients, esophogitis were seen in 28 patients. All of them were treated with proton pump inhibitors. Only 3 patients had gastroesophageal reflux symptoms which resolved after treatment.

Conclusion: POEM for esophageal achalasia is a novel, safe and effective endoscopic treatment modality in centers that are experienced in advanced endoscopic techniques such as ESD.

Policy of full disclosure: None.

210 | Gastrointestinal motility and luminal pH influence in vivo dissolution and systemic drug absorption in human gastrointestinal tract under fasted and fed conditions

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Objective: In addition to drug dose, dissolution properties, and solubility, fasting and fed gastrointestinal motility and luminal pH can influence absorption kinetics and plasma concentrations of orally ingested medications. We investigated relationships of gastrointestinal contractility, luminal pH, and concentrations of ibuprofen in plasma and different gut regions under fasted vs fed conditions to characterize effects of motility on medication absorption.

Methods: Manometry catheters with 4 aspiration ports in the stomach, duodenum, and jejunum were fluoroscopically positioned in 16 healthy humans (34 procedures). Subjects were randomized to fasting or fed (Pulmocare, 710 kcal) conditions. Ibuprofen 800 mg was ingested with 250 mL water. Luminal fluid was sampled from the 4 ports×7 hours and venous blood was obtained×28 h to measure ibuprofen levels by LC-MS/MS. Luminal pH was measured and

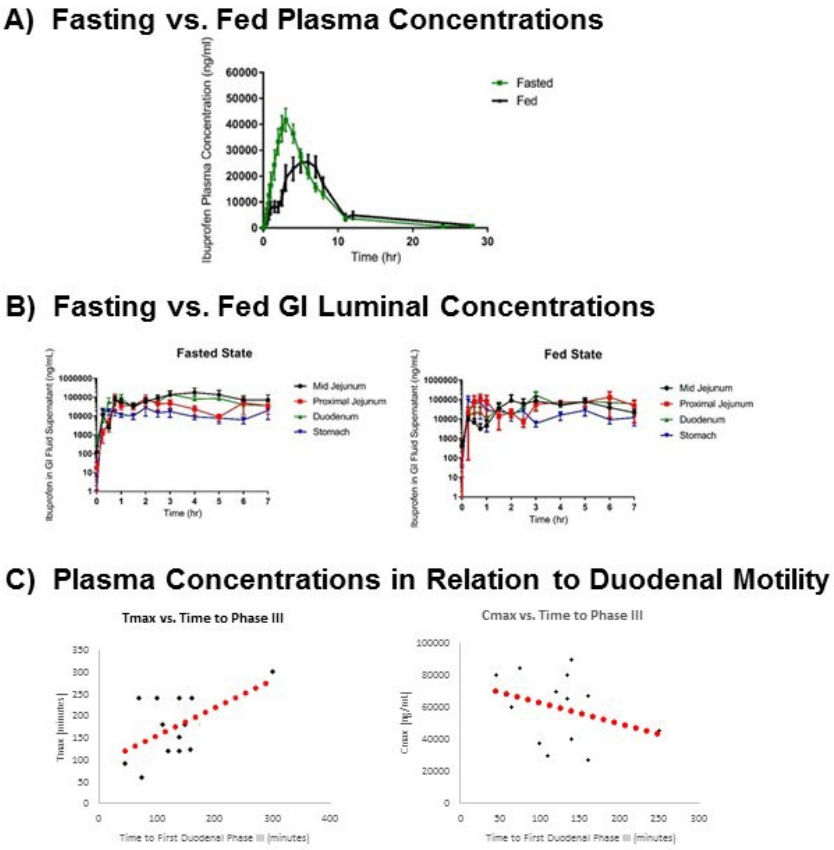

Figure 1

gastrointestinal migrating motor complex (MMC) phase III activity was recorded on manometry.

Results: Gastric ibuprofen levels increased immediately after ingestion and were higher under fed vs fasting conditions×7 hours (Figure 1A). Duodenal and jejunal ibuprofen concentrations increased after gastric levels and persisted×7 hours. Conversely, plasma ibuprofen levels were higher under fasting vs fed conditions×8 hours and remained detectable×28 hours (Figure 1B). Intragastric pH increased to near neutrality after feeding before decreasing at 7 hours. Higher gastric pH was associated with greater gastric ibuprofen levels but lower plasma ibuprofen concentrations. Times to maximal plasma ibuprofen concentrations (Tmax) correlated with times to first duodenal phase III complexes (r=.55, P=.05); maximal ibuprofen concentrations (Cmax) exhibited no relation to duodenal phase III timing (r=−.32, P=.29) (Figure 1C).

Conclusions: Fasting MMC activity and luminal pH as well as whether subjects were fasting or fed influenced gut and plasma ibuprofen concentrations and timing of medication absorption in healthy humans. This novel in vivo methodology involving multichannel manometry and multisite fluid sampling provides insight into roles of gut motor function in modifying drug distribution under fasting and postprandial conditions. Furthermore in future studies, this technique may be adaptable to help better understand abnormalities of drug absorption in patients with gut dysmotility.

Policy of full disclosure: None.

211 | High resolution anorectal manometry in multiple sclerosis patients: A pilot study

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Introduction: Fecal incontinence is a common symptom encountered in many neurological conditions. The aim of our study was to evaluate whether anorectal function obtained through high resolution anorectal manometry (HRAM) measurements, differs between multiple sclerosis (MS) patients and patients with unknown cause of fecal incontinence.

Methods: Ten patients underwent HRAM procedure. Reusable solid state catheter (MMS, The Netherlands) with 8 circumferential pressures and one balloon pressure was used. Beside epidemiological data, we analysed following tests: resting pressure, squeeze pressure, push, rectal sensations (first sensation, urge, intense urge and maximal tolerable volume (MTV)) and RAIR. Comparisons between the MS group and the group with unknown cause of incontinence (UCI) were analysed with a two sample t-test, with a significance set at P<.5. The statistical package "R" was used for analyses.

Results: 10 patients with fecal incontinence were included in the study (1M, 9F). Five of them were MS patients (4 with relapsing-remitting

course, 1 with primary progressive course; median EDSS 5), the other five were in the UCI group. The mean age for the MS group was 42 years and for the UCI group 51.6 years. We found statistically significant difference between the groups for squeeze pressure and MTV: *P*=.043 and *P*=.024, respectively; where the MS group obtained significantly smaller values for both parameters. There was no significant difference for resting pressure, push, first sensation, urge and intense urge: *P*=.341, *P*=.592, *P*=.978, *P*=.153, *P*=.101, respectively. RAIR was present in all patients.

Conclusion: Our results with the HRAM solid state catheter show a significant loss of voluntary control of external anal sphincter and rectal sensitivity in the MS group, contrary to the UCI group. Considering a very small number of patients, as well as that normative values on solid state catheter still have to be standardized, results might not be sufficient. However, to the best of our knowledge, this is the first study of MS patients performed on the solid state catheter - which offers a quick, comfortable and user-friendly approach.

Policy of full disclosure: None.

212 | Normative values of regional and sub-regional gastrointestinal motility and contractility parameters using the wireless motility capsule

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Backgroud: The wireless motility capsule (WMC) is a minimally invasive ambulatory technology and concurrently measures pressure, pH and pressure as it traverses the gastrointestinal tract. Whilst normal values for regional and pan-enteric transit and pH have been described, the pressure data has received little attention. The primary aim of the study was to describe normative values for motility/contractility parameters. We also aimed to evaluate inter-observer agreement as well as investigating the co-relationship between these and the motility index (MI).

Methods: 125 healthy participants were included in the study. All participants underwent a standardized WMC assessment following an overnight fast and consumption of a meal of known nutritional content (SmartBar). Traces were analysed using two data analysis software packages and divided into regional and sub-regional areas of interest (MotiliGI and GIMS Data Viewer). One third of traces, chosen at random, were used to evaluate inter-observer agreement by two independent investigators.

Results: We present normative data for in excess of 100 motility/contractility parameters in 107 individuals (62 male, median age 40 years, range 18-78). Inter-observer agreement was good for transit times, pH and contractility/motility parameters. MI correlated with the area under the curve, amplitude, frequency and number of contractions. Based the highest agreement, we propose that MI and frequency of contractions could be used in clinical practice.

Conclusion: Our analysis provides normative data for motility/contractility parameters. The relevance of these parameters in clinical populations warrants further investigation.

Policy of full disclosure: C Brock received funding from the Talent Programme, Aalborg University. AD Farmer was supported by the Danish Diabetes Academy founded by the Novo Nordisk Foundation and the Research and Development Department, University Hospitals of North Midlands. Dr Scott and professor Hellström have previously received grant funding from the SmartPill Corporation. Dr Semler is an employee of Medtronic. Dr Hobson was a paid instructor for Given Imaging. Some of data from healthy participants was derived from studies supported by the SmartPill Corporation. None of the other authors have any relevant conflicts of interest.

213 | Decreased esophageal wall compliance and longitudinal muscle dysfunction in esophagogastric junction outflow obstruction

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Objective: The aim of the study was to evaluate the esophageal wall compliance in patients with esophagogastric junction outflow obstruction (EGJOO) with high resolution esophageal manometry.

Methods: 15 patients diagnosed as EGJOO with HRM were classified into two groups; EGJOO with intact body peristalsis (group A) and EGJOO with ineffective body peristalsis (group B). Ineffective body peristalsis was defined as ≥50% of failed or weak peristalsis (DCI<450 mmHg s cm). Baseline esophageal muscle thickness, cross-sectional area (CSA) at peak dilatation, frequency of failed esophageal body contraction using intraluminal ultrasound (ILUS), and LES contractile integral were analyzed compared to normal control (n=5).

Results: 4 patients were classified as group B, whereas 11 patients classified as group A. Baseline muscle thickness and LES contractile integral were significantly increased in group A than that of normal control and group B. In contrary, CSA at peak distension was significantly decreased in group A than that of group B and normal control. There was no significant difference in baseline esophageal wall thickness, CSA at peak distension and LES contractile integral between group B and normal control. Failed esophageal contraction with ILUS during swallow was more frequently found in group B than normal control.

Conclusion: Decreased esophageal body compliance and defect of longitudinal muscle contraction might be the main pathophysiology for EGJOO.

Policy of full disclosure: None.

214 | Assessment of colorectal length using the electromagnetic capsule tracking system: A comparative validation study in healthy subjects

E. Bolvig Mark[1]; J. L. Poulsen[2]; A.-M. Haase[3]; J. B. Frøkjær[2]; V. Schlageter[4]; M. Scott[5]; K. Krogh[3]; A. M. Drewes[2]

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Objective: We aimed to determine colorectal length with the 3D-Transit system by describing a 'centerline' of capsule movement and compare it to known anatomy, as determined by magnetic resonance imaging (MRI). Further, we aimed to test the day-to-day variation of colorectal length assessed with the system.

Methods: The 3D-Transit system consists of electromagnetic capsules that can be tracked as they traverse the gastrointestinal tract. 25 healthy subjects were examined with both 3D-Transit and MRI. Another 21 healthy subjects were examined with 3D-Transit on two consecutive days.

Results: Computation of colorectal length from capsule passage was possible in 60 of the 67 3D-Transit recordings. Length of the colorectum measured with MRI and 3D-Transit was respectively 95 cm (75-153 cm) and 99 cm (77-147 cm), *P*=.15. Coefficient of variation (CV) between MRI and 3D-Transit was 7.8%. Apart from the cecum/ascending colon being 26% (*P*=.002) shorter on MRI, there were no other differences in total or segmental colorectal lengths between methods (all *P*>.05). Length of the colorectum measured with 3D-Transit on two consecutive days was 102 cm (73-119 cm) and 103 cm (75-123 cm), *P*=.67. CV between days was 7.3%.

Conclusions: The 3D-Transit system allows accurate and reliable determination of colorectal length compared to MRI derived colorectal length and between days. Antegrade or retrograde capsule movement relative to this centerline, as well as the length and speed of movements may be determined by future studies to allow better classification and treatment in patients with dysmotility.

Policy of full disclosure: Vincent Schlageter is co-owner of Motilis Medica SA. The other authors have no conflicts of interest.

215 | The effect of opioid treatment on colorectal motility assessed by electromagnetic capsules

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Objective: Opioid treatment often causes debilitating constipation. However, it is not well described how opioids affect colonic motor patterns, and whether opioid-induced constipation is due to dyscoordinated peristalsis or decrease in number of prolonged peristaltic contractions known as 'mass movements'. Thus, this study aimed at investigating the effect of oxycodone on colonic motility dynamics.

Methods: Twenty-five healthy males were administered oxycodone or placebo in a randomized, double-blind crossover trial. Gastrointestinal (GI) motility was assessed with a small electromagnetic capsule by tracking its progression throughout the GI tract. Segmental colonic transit times, mass movements, and retrograde movements were calculated using a custom designed MATLAB application.

Results: Median colonic transit time was increased during oxycodone treatment compared to placebo (39 vs 18 hours, *P*<.01). Fewer mass movements were observed during oxycodone treatment compared to placebo (13 vs 31, *P*<.01). No difference in retrograde movements were observed between treatments (*P*>.05).

Conclusion: Oxycodone treatment impaired colonic motility as demonstrated by increased colonic transit time and fewer mass movements. The current findings suggest that the increased transit time during opioid treatment is caused by a decrease in mass movements rather than dyscoordinated peristalsis.

Policy of full disclosure: Vincent Schlageter is co-owner of Motilis Medica SA. The other authors have no conflicts of interest.

216 | Predictive model of biofeedback therapy responsiveness based on three-dimensional integrated pressurized volume in female patients with dyssynergic defecation using high-resolution anorectal manometry

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Background/Aims: Biofeedback therapy (BFT) is effective treatment of dyssynergic defecation (DD). However, no study has examined the factors that can predict the responsiveness of BFT based on high-resolution manometry (HRAM). We previously suggested the concept of integrated pressurized volume (IPV). Thus, we aimed to construct a predictive model for BFT responders by applying IPV parameter.

Methods: Seventy-one female (age: 48-68 years) with DD were enrolled at Asan Medical Center from 2011 to 2015. All subjects underwent initial HRAM and subsequently received BFT. A responder was defined as a subject with at least a three-point improvement on the global bowel satisfaction (GBS) scale after BFT or a two-point increase if the baseline GBS was more than six points. Partial least square regression (PLSR) was conducted to develop a predictive model for BFT responders using IPV parameters, and the PLSR model was assessed using leave-one-out cross validation (LOOCV).

Results: Among 71 subjects, 55 (77.5%) showed responsiveness to BFT. There was no significant difference in conventional manometric parameters between responders and non-responders to BFT. Receiver operating characteristic curve analysis demonstrated that the IPV of the upper 1 cm of the anal canal was the best predictor of BFT

response (area under the curve [AUC]: 0.74, 95% confidence interval [CI]: 0.63-0.80, *P*<.01). The PLSR model using a linear combination of eight IPV parameters provides an AUC of 0.84 (95% CI: 0.76-0.95, *P*<.01), with a sensitivity of 85.5% and specificity of 62.1%. After LOOCV, the PLSR model was able to correctly discriminate BFT responders, with a sensitivity of 78.9% and specificity of 69.8%.

Conclusions: A combination of IPV parameters are superior to conventional parameters in the prediction of responsiveness to BFT. Therefore, IPV parameters can explain the physiology of the anorectal canal more effectively than the conventional parameters.

Policy of full disclosure: None.

BIOMARKERS IN IRRITABLE BOWEL SYNDROME/TREATMENT OF VISCERAL PAIN

217 | Is the colonoscopy mandatory in patients with irritable bowel syndrome or chronic constipation?

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Objective: Chronic constipation (CC) and irritable bowel syndrome - constipate type (IBS-C) are two disorders (ROMA IV) that should be theoretically separated but many specialists have serious doubts regarding a clear separation. However, the considerable symptom overlap between these 2 disorders has been highlighted in recent studies and similar therapeutic strategies are often utilized. From a regulatory standpoint, these conditions are treated as separate entities but in clinical practice, the symptoms of IBS-C and CC overlap significantly and often times, their distinction is quite arbitrary. The aim of this study was to investigate the relationship between IBS-C and chronic constipation and their clinical consequences.

Results: We analyzed 68 patients (predominate women—69%, mean age 42.7±3.6 years), from which 41 met criteria of the IBS-C and 27 with chronic constipation. Thus, 20.0% of all patients with CC met criteria for IBS, and 13 patients (31.7%) of all patients with IBS-C met criteria for CC. Constipation was very bothersome in 70.7% of IBS-C patients, 66.6% of CIC (*P*<.01). Gastrointestinal symptoms disrupted productivity a mean of 4.9 days per month in patients with overlap (IBS and CC) vs 3.6 IBS-C (*P*<.01), 2.9 in CIC (*P*<.001). The patients with chronic constipation were more satisfied with laxative use than subgroup IBS (*P*<.01) and overlap-patients (*P*<.01). Fiber and probiotics use were similar between patients with SII (100%) and CC (87.9%). The reevaluation (during 5±2.7 years) of the patients has included colonoscopy, thus 83% of the patients with SII haven't been detected with any intestinal mucosal injuries, at 9.7% of them had hyperplastic colonic polyps, 7.3%—adenomas and 2.4%—melanosis coli, on when those with CC were detected 11.1% (3) with melanosis coli, 14.8% (4)—rectal polyps (hyperplastic or adenomas without dysplasia), 7.4% (2)—advanced lesions (cancer and adenomas with malignancy).

Conclusion: This study confirm the difficulty in differentially identifying patients with IBS and CC, meanwhile attesting the high presence of the overlap between these diseases with meaningful consequences for clinical practices and optimal treatment in a managed care setting. Colonoscopy examination is appropriate in patients with prolonged duration of constipation or laxative use and those who have not previously had colon cancer screening.

Policy of full disclosure: None.

218 | Assotiation between small intestinal bacterial overgrowth, characteristics of anorectal motility and rectal sensitivity in patients with diarrhea-predominant irritable bowel syndrome

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Objective: To assess correlation between small intestinal bacterial overgrowth (SIBO), anorectal motility disorders and visceral hypersensitivity in patients with diarrhea-predominant IBS (IBS-D).

Methods: 31 patients with IBS-D (according to the ROME III criteria) and 15 healthy volunteers were studied. All persons were analysed by the hydrogen breath test with lactulose using Gastro+Gastrolyzer (Bedfont, UK) to determine SIBO, high-resolution anorectal manometry (HRAM) using 20 channel water-perfused catheter with a polyethylene balloon (Solar GI, MMS, the Netherlands).

Results: SIBO was found in 20 patients with IBS-D (62,5%) and was not detected in the control group. A negative correlation was revealed between SIBO and the following parameters of rectal sensitivity and function of the anal sphincter (AS): average absolute anal squeeze pressure (Kendall`s tau coefficient −0.34; *P*=.012), maximum absolute anal squeeze pressure (Kendall`s tau coefficient −0.35; *P*=.011), threshold for intense urge to defecate (Kendall`s tau coefficient −0.31; *P*=.022) and maximum tolerable volume (Kendall`s tau coefficient −0.31; *P*=.025).

Conclusions: SIBO are correlated with the parameters of anorectal motility and rectal sensitivity, causing symptoms in IBS patients.

Policy of full disclosure: None.

219 | Altered expression of membrane transporters in colonic mucosa of patients with Irritable Bowel Syndrome (IBS) and Post-infectious (PI)-IBS compared to healthy subjects

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Background: Irritable bowel syndrome (IBS) affects 5%-15% of adults in the general population, and is characterized by chronic recurrent abdominal pain and discomfort and associated with altered bowel habits. The pathophysiology of IBS is complex and not fully understood. Hence, treatment is often based on symptomatology rather than underlying physiological aberrancies.

Objective: To compare the expression of membrane transporters in mucosal biopsies of healthy subjects, IBS patients and post-infectious (PI)-IBS patients.

Methods: Mucosal biopsies were obtained from the unprepared sigmoid colon in 18 IBS patients, 9 PI-IBS patients and 10 healthy subjects. Total RNA was isolated and prepared for gene expression analyses using quantitative reverse-transcription polymerase chain reaction (qRT-PCR). We compared the expression of genes encoding membrane-spanning transporters, using GAPDH as a reference gene, and by using the comparative 2-ΔΔCt method.

Results: Colonic expression of SCL7A5 and SLC3A2 (together comprising the amino acid transporter LAT1+4F2hc) was significantly lower in IBS patients, but not in PI-IBS patients, compared to healthy controls (P<.001). The expression of SLC7A8 (LAT2) tended to be lower in IBS patients compared to controls (P=.06). Mucosal gene expression of the short chain fatty acid transporter SMCT1 (SLC5A8) was lower in both IBS-patients and PI-IBS patients compared to healthy subjects (P<.01).

Conclusions: The amino acid transporters LAT1 and LAT2 appeared to be affected in IBS patients, but not in PI-IBS patients, compared to healthy subjects, suggesting a possible alteration in amino acids transport in this patient group. Furthermore, our results suggest a lower uptake of short chain fatty acids in both IBS- and PI-IBS patients. Altered expression of these transporters may be involved in the pathophysiology of IBS as well as being a potential biomarker of this aberration, and therefore deserves further study in IBS.

Policy of full disclosure: None.

220 | A potential role for actinomycetales as a modulator of resting state functional connectivity: A pilot study

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Objectives: Alterations in gut microbiota may have a role in the pathophysiology of IBS. An inverse association between Actinomycetales and depression has been demonstrated in IBS. We conducted a preliminary investigation into the association between functional connectivity in the brain´s Default Mode Network (DMN) and gut microbiota in IBS patients and healthy controls (HC).

Methods: 28 females with IBS and 15 age and sex-matched HC underwent a 10 minute fMRI resting state scan using single-shot, gradient-echo EPI sequence. Data were preprocessed using SPM8, and the group Independent component analysis (GIFT) was used to identify the DMN component. Questionnaires used were for the IBS symptom severity (IBS-SSS), and depression (HADS-D). Stool samples were analysed using a panel of 54 probes covering the sites across the variable regions (V3 to V7) on the 16S rRNA gene. We performed a between-group covariate analysis with Actinomycetales as the covariate of interest resulting in the observed correlations.

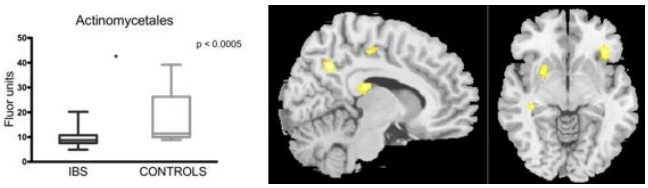

FIGURE 1 Group differences for Actinomycetales (left), and altered brain connectivity (IBS>HC) (right)

Results: Significant between-group differences were found for Actinomycetales, P=.0005 (Figure 1A), which also displayed a negative correlation with symptom severity (r=−.45, P=.002) and depression scores (r=−.35, P=.023) over all participants. Whole brain covariate of interest analysis showed group differences in DMN connectivity driven by Actinomycetales as follows: IBS>HC (fig. 1B) showed increases in connectivity in the right insula, left putamen, left precuneus, and the right superior frontal medial regions, whereas HC>IBS showed increases in the left insula, right putamen, right midcingulate and the right superior frontal regions (P(uncorr)=.001).

Conclusion: Our preliminary result shows that Actinomycetales may play a role in altered functional connectivity in the DMN of IBS patients relative to HC. Figure 1. (i) IBS and HC Between-group differences in Actinomycetales. (ii) fMRI results showing IBS>HC regions of increased connectivity associated with Actinomycetales REFS: Jeffery I. B. et al. Gut 2011.

Policy of full disclosure: None.

221 | Enhanced organization of bilateral anterior limb of internal capsule in Irritable Bowel Syndrome (IBS) relates to gastrointestinal specific anxiety

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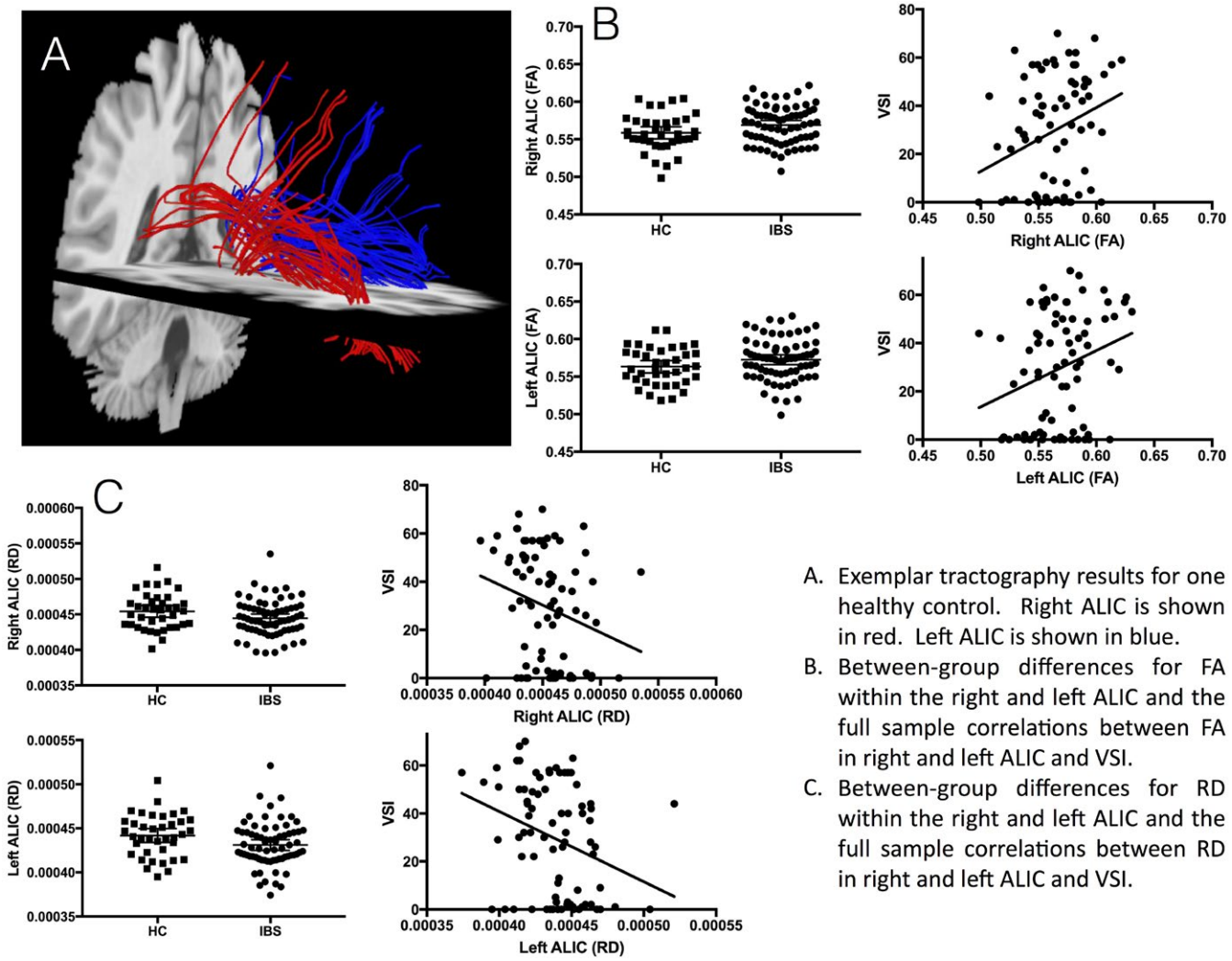
Objective: Irritable bowel syndrome (IBS) is often accompanied by gastrointestinal (GI) specific anxiety. The anterior limb of the internal capsule (ALIC) is a key white matter (WM) tract that connects the corticospinal tracts via the thalamus to the frontal cortex, making it well-positioned to facilitate communication between ascending spinal tracts and limbic and frontal brain regions previously linked to anxiety. We used diffusion tensor imaging to examine both differences in white



A. Exemplar tractography results for one healthy control. Right ALIC is shown in red. Left ALIC is shown in blue.
B. Between-group differences for FA within the right and left ALIC and the full sample correlations between FA in right and left ALIC and VSI.
C. Between-group differences for RD within the right and left ALIC and the full sample correlations between RD in right and left ALIC and VSI.

FIGURE 1

matter microstructure of bilateral ALIC between healthy women and those diagnosed with IBS and the possible relationship to GI-specific anxiety.

Methods: Diffusion weighted images were acquired for 71 women diagnosed with IBS and 38 healthy women (HCs). The images were acquired using a 64-direction sequence on a 3T MRI scanner. Prior to calculating that tensor in FSL, data for each participant were individually eddy corrected. Group-level maps of fractional anisotropy (FA) and radial diffusivity (RD) were estimated using tract based spatial statistics. Average FA and RD values for the left and right ALICs were extracted for each subject using the JHU-ICBM-labels parcellations. The Visceral Sensitivity Index (VSI) was used to measure the level of GI-specific anxiety in all participants (IBS: 44.19±14.14; HC: 3.14±6.49).

Results: The IBS patients were found to have increased FA in both left ($P<.08$) and right ($P<.038$) ALICs and decreased RD in these same two tracts (right: $P<.06$; left: $P<.034$). Across the entire sample, we found that FA in both left ($P<.02$) and right ($P<.006$) ALICs positively correlated with VSI scores. RD in both left ($P<.005$) and right ($P<.009$) ALICs negatively correlated with VSI scores. See Figure 1A-C.

Conclusion: The development and maturation of WM tracts is characterized by increased FA (fiber organization) and decreased RD (myelination). Our results would suggest that IBS patients exhibit an enhanced organization of bilateral ALICs compared with HCs that appears to be related to the level of self-reported GI-specific anxiety.

Policy of full disclosure: None.

222 | Hormonal contraceptive use is associated with increased levels lipopolysaccharide-binding protein, but not inflammation

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Background: Lipopolysaccharide-binding protein (LBP) is being increasingly used as an indirect gut permeability marker, however

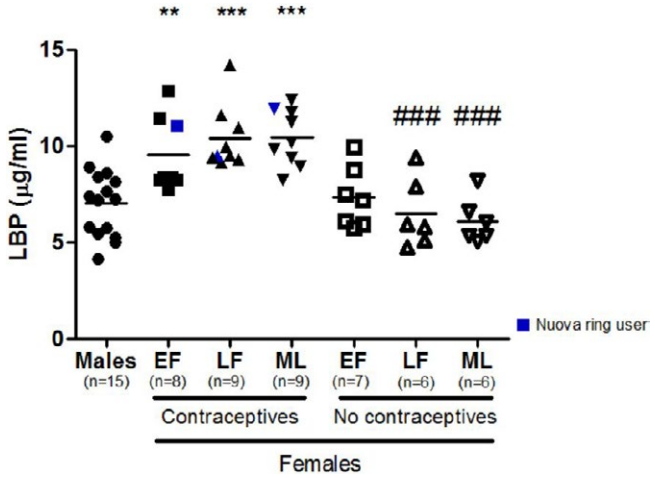

FIGURE 1 Hormonal contraceptive use is associated with increased LBP levels

recent preclinical data demonstrated that LBP is also regulated by estrogen receptor-α activation. This suggests that alterations in estrogen bioactivity may influence systemic LBP levels independently from gut permeability and associated inflammatory state.

Objective: Investigate whether gender, hormonal contraceptive use and menstrual cycle influence systemic LBP, TNF-α and IL-8 levels.

Methods: 31 non-obese subjects (15 males, 16 females) were recruited from University College Cork. Eight females used oral contraceptives and one female used a Nuova ring. Male subjects were invited for blood collection once, whereas females were invited three times across the menstrual cycle. Self-reported menstrual cycle days 2-7, 9-13, and 18-23 were defined as early follicular phase (EF), late follicular (LF) phase, and mid-luteal (ML) phase, respectively. Levels of LBP, TNF-α and IL-8 were analyzed in plasma, and Kruskal-Wallis and Friedman tests with Dunn's post-hoc test were performed.

Results: LBP levels of females using hormonal contraceptives were higher compared to males and female non-users, except for the EF phase. No differences were observed between females not using contraceptives and males. TNF-α and IL-8 levels did not differ between the three groups.

Conclusions: TThese data indicate that LBP may not be an appropriate indirect gut permeability marker in studies including hormonal contraceptive users. Since oral contraceptive users normally do not ingest contraceptives during the first days of the follicular phase, and no differences in LBP levels were observed between the EF phase of hormonal contraceptive users, EF phase of non-users, and males, we suggest that LBP level increases may be reversible on a short-term basis. Future studies should confirm these data using multi-sugar tests assessing gastrointestinal permeability. Furthermore, the impact of increased LBP levels in hormonal contraceptive users requires elucidation.

Policy of full disclosure: None.

223 | BCL-3 upregulation by mast cells in Irritable Bowel Syndrome (IBS) patients

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Objective: Irritable Bowel Syndrome (IBS) is characterised by mucosal mast cell activation in association with increased epithelial permeability. The B-cell leukemia/lymphoma-3 (Bcl-3), a co-transcriptional regulator of the NF-κB target-gene, modulates inflammatory responses and has been associated with increased intestinal epithelial permeability in an experimental model of infection. Mast cell modulation of Bcl-3 expression in epithelial cells has not been addressed. Our aim was to assess the regulation of epithelial Bcl3 expression by mast cells in IBS and to underlying mechanisms.

Methods: Colonic and ileal mucosal biopsies from IBS (IBS-D n=9; IBS-C n=6; IBS-M n=4; IBS-U n=1) patients (n=20) and healthy controls (HC n=19) were evaluated for Bcl-3 expression and mast cell number by immunoblot and immunofluorescence microscopy, respectively. Mast cell activity was assessed by measuring intestinal tryptase content (immunoblot and ELISA), and by analysing mast cell degranulation by transmission electron microscopy. In Caco-2 cells, Bcl-3 expression by tryptase was evaluated by immunoblot.

Results: The epithelium of IBS patients showed increased expression of Bcl-3 in both the ileum (IBS: 2.154±1.159; HC: 1.000±0.6844, *P*=.0147) and the colon (IBS: 2.221±1.687, HC: 1.000±0.7248, *P*=.0094) as compared to HC. The number of mast cells in the ileal (IBS: 26.00±14.82; HC: 14.64±8.10, mast cell/hpf *P*=.0050) and the colonic mucosa (IBS: 17.40±10.39; HC: 11.94±6.54 mast cell/hpf, *P*=.0474) was increased in IBS patients compared to HC. Morphological evidence of increased degranulation in the ileum of the IBS group was also observed (IBS: 78.2±9.07; HC: 60.7±6.98%, *P*=.0190). Moreover, Bcl-3 expression was induced by tryptase in Caco-2 cells; an effect that was inhibited by FSLLRY-NH2, a selective PAR2 peptide antagonist (*P*=.0245).

Conclusion: Our findings suggest that intestinal epithelial Bcl-3 expression is induced by mast cells, a mechanism that may modulate epithelial integrity and mucosal inflammation in IBS.

Policy of full disclosure: None.

224 | Hypnotherapy as complementary treatment for pain in chronic pancreatitis: A pilot study of four patients

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Background: Chronic pain is the hallmark symptom of chronic pancreatitis (CP). Its treatment is complicated, and often the patients have side-effects notwithstanding that pain is not ameliorated in many cases. Hypnotherapy has been shown to improve symptoms of irritable bowel syndrome including abdominal pain and, as such, may serve as an interesting remedy to relive pain. However, the effect has never been investigated in pancreatitis and the aim of this open-label pilot study was to test the effect of hypnotherapy for pain in patients with CP, the feasibility of the treatment protocol, and the safety of the intervention in terms of self-reported adverse effects.
Methods: Four patients with CP and chronic abdominal pain were included and followed for four consecutive weeks. The primary efficacy parameter was pain relief. After one week of baseline patients received a one-hour session of hypnotherapy. This was repeated at day 15 and day 23 and supplemented by self-administered hypnotherapy.
Results: Three of four participants completed the trial and experienced short lasting pain reduction during the trial. The reported pain relief was in the range of 20%-39% compared to baseline. Hypnotherapy improved self-reported sleep, vitality, and social life.
Conclusions: The results suggest that hypnotherapy may reduce pain related to CP. Furthermore, no adverse effects were reported and the majority of participants completed the trial. The findings warrant further prospective controlled trials to examine the potential of hypnotherapy.
Policy of full disclosure: None.

225 | Can mast cell stabilizers reduce pain in pancreatitis?

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Objective: Both acute and chronic pancreatitis lead to strong abdominal pain and often require the use of potent opioids. Former investigations revealed an association between this neuropathic pain and accumulation of active mast cells around intrapancreatic nerves. This study aims to investigate whether mast cell stabilizers might be a new analgesic therapy for pancreatitis-associated pain.
Methods: Pancreatitis was induced in C57BL/6-mice via intraperitoneal injections of caerulein (50 mg/kg bw) in 1-hours intervals. After a dose-finding study, the mice were randomized into 4 groups with the test groups receiving therapeutic doses of either cromoglycate (500 mg/kg bw), ketotifen (10 mg/kg bw) or metamizole (500 mg/kg bw) as positive control. The openfield test was used for quantifying spontaneous pain and the von Frey Filament stimulation to measure evoked pain. Blood samples for amylase and lipase levels, and pancreas, lung and brain tissues for histopathology were collected.
Results: While there was no effect of mast cell stabilizers on pain in acute pancreatitis during the dose-finding study, the mice in the chronic pancreatitis model showed a significant reduction of pain (cromoglycate: 9.9±0.3 vs saline: 11.1±0.3, *P*=.025). Also during the main experiments, treatment led to reduced pain reactions in mice with chronic pancreatitis compared to the control group. In the von Frey assay mice receiving cromoglicate (Cr) presented a significant reduction of the abdominal mechanical sensitivity, while for mice treated with ketotifen (Ke) the decrease was not significant (Cr: 9,3±2,4; Ke: 10.4±0.7; saline: 11±0.6; metamizole: 8.4±1.8). Since there was no difference of locomotor activity in the openfield test, treatment apparently did not affect spontaneous pain behavior. In acute pancreatitis, mast cell stabilizers showed no effect on evoked pain (von Frey test: Cr: 10.3±1.0; Ke: 10.2±1.1; Me: 6.8±3.1; saline: 9.6±2.6).
Conclusions: Mast cell stabilizers may be a new analgesic therapy or adjuvant with opoid treatment in chronic pancreatitis. Studies in humans should further evaluate the effect of mast cell stabilizing medication.
Policy of full disclosure: None.

CHALLENGES IN SEVERE DIGESTIVE DISORDERS

226 | The influences of visceral fat area on the sites of esophageal mucosal breaks and symptom severities in subjects with gastroesophageal reflux diseases

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Background: Some studies have suggested the central obesity as a risk factor for gastroesophageal reflux diseases (GERD). However, the associations between visceral adipose tissue (VAT) and the sites of esophageal erosions or the symptom severities of GERD have not been studied yet. The aim of this study was to evaluate the influences of visceral fat area on the locations of erosions and symptoms of GERD.
Methods: The subjects who underwent abdomen computerized tomography and esophagogastroduodenoscopy for routine checkup at the same day were collected from January 2007 to October 2016. 177 subjects who had erosive esophagitis (LA class A to D) were enrolled. Questionnaires including gastrointestinal symptoms were written before examinations. The abdominal obesity was evaluated by measuring visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), ratio of VAT to SAT, total adipose tissue (TAT), body mass index (BMI) and waist circumference (WC).
Results: Lesser curvature (LC) side of esophagogastric junction (EGJ) was the most frequent site of mucosal breaks (103 cases, 58.2%) followed by posterior wall side (71 cases, 40.1%), anterior wall side (25 cases, 14.1%) and fundus side (16 cases, 9.0%). Mucosal breaks in LC side were frequently observed in male subjects (61.3% vs 36.4%, *P*=.04). BMI (25.6±4.5 vs 24.2±3.1, *P*=.019) and WC (89.0±11.8 vs

85.0±9.1, *P*=.01) were significantly higher in LC group. Moreover, VAT, ratio of VAT to SAT, and TAT were significantly higher in LC group. In the multivariate analysis, a higher VAT area (odds ratio (OR) 3.47, 95% confidence interval 1.38 to 8.73, 1st quartile vs 4th quartile, *P*<.01) and ratio of VAT to SAT (OR 2.99, 95% CI 1.15 to 6.70, 1st quartile vs 4th quartile, *P*=.02) were strongly associated with the mucosal breaks in LC side. However, TAT was not significant in the multivariate analysis. Lower HDL-cholesterol levels (OR 0.28, 95% CI 0.11 to 0.67, *P*<.01) and much coffee consumption (OR 2.50, 95% CI 1.06 to 5.86, *P*=.035) were associated with the severities of GERD.

Conclusions: Mucosal breaks in LC side of EGJ were associated with visceral obesity measured by VAT, ratio of VAT to SAT, BMI and WC. Life style modification such as in left decubitus sleeping position might be emphasized in the subjects with visceral obesity.

Policy of full disclosure: None.

227 | Esophageal stasis on barium esophagogram in achalasia patients without symptoms after treatment does not predict symptom recurrence

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Objective: After achalasia treatment, some patients have poor esophageal emptying without having symptoms. There is no consensus on whether or not to pre-emptively treat these patients. We aimed to compare asymptomatic patients with and without stasis after treatment. We included 99 adult achalasia patients who were in clinical remission (Eckardt≤3) at 3 months after treatment. Patients were divided into two groups, based on presence or absence of esophageal stasis on a timed barium esophagogram performed after 3 months. Before initial treatment, groups were comparable regarding age, gender, achalasia subtypes, treatment allocation, Eckardt score, LES relaxation, barium column height and maximum esophageal diameter. After 3 months, the median barium column height at 5 minutes was 4.4 cm (IQR 2.6-6.2) in the stasis group. The distal esophagus was significantly wider in patients with stasis (2.5 cm; IQR 2-3.9) than in patients without stasis (2 cm; IQR 1.7-2.3), *P*<.001. The Eckardt score and number of patients with inadequate LES relaxation after treatment were comparable between the groups. Two years after initial treatment, in patients with stasis, the esophageal diameter had increased from 2.5 to 3.0 cm, which was significantly wider than in patients without stasis (1.8 cm; IQR 1.5-2.7), *P*<.001. Also, they still had significantly more stasis (3.5 cm; IQR 1.9-5.6) than the no-stasis group (still 0 cm; IQR 0-0) *P*<.001. The proportion of patients receiving additional treatment was identical in the stasis group (17%) and the no-stasis group (19%), *P*=1.00. Also, median time to retreatment was comparable between

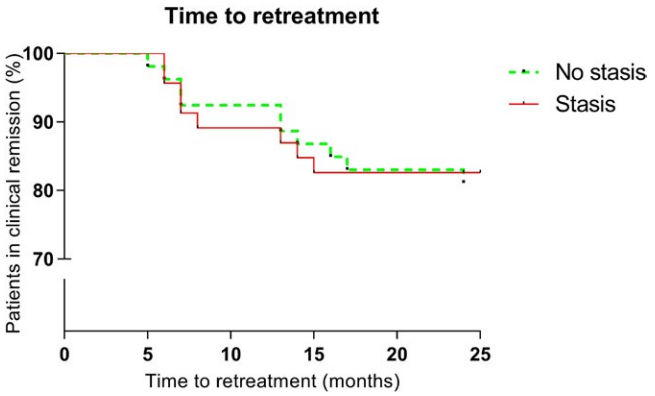

FIGURE 1 Cox "survival" function between patients with and without stasis

patients with stasis (8 months; 95% CI 5.1-10.9) and patients without stasis (13 months; 95% CI 4.7-21.3); *P*=.893. After 2 years, there was still no difference in Eckardt score, quality of life and reflux symptoms between the two groups.

Conclusion: Although patients with stasis initially had a wider esophagus and 2 years after treatment also more stasis and a more dilated esophagus, compared to patients without stasis, they did not have a higher chance of requiring retreatment. We conclude that stasis in symptom-free achalasia patients after treatment does not predict treatment failure within 2 years and can therefore not serve as a sole reason for retreatment.

Policy of full disclosure: AJB received research funding from Endostim, Medical Measurement Systems, Danone and Given and received speaker and/or consulting fees from MMS, Astellas, AstraZeneca and Almirall. FBH and AJS have no conflicts of interest.

228 | A pilot feasibility study of gastroparesisclinic.org: An online assessment and psychological treatment program for gastroparesis patients

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Objective: Gastroparesis is associated with significant psychological distress and impaired quality of life (QoL). This study explored the feasibility of an online psychological treatment program for individuals with gastroparesis. It was expected that individuals who completed the program would report reduced gastroparesis symptom severity, psychological distress, and maladaptive coping, in addition to improved illness perceptions, adaptive coping, and QoL at post-intervention assessment compared to baseline.

Methods: Adults with gastroparesis were recruited for a six-week online treatment program targeting gastroparesis-related psychological distress. Participants completed a self-report questionnaire assessing gastroparesis symptom severity, illness perceptions, coping styles,

psychological distress, and QoL at baseline and post-intervention. Participants also provided feedback on the program. Of 97 participants who completed the baseline assessment, six also completed the post-intervention assessment.

Results: Participants who completed the program demonstrated trends of reduced symptom severity, negative illness perceptions and psychological distress, increased adaptive and maladaptive coping, and improved QoL. Feedback indicated a generally positive response to the program, which participants thought was an acceptable way of dealing with gastroparesis symptoms and would willingly recommend to a friend. Participants provided suggestions regarding module and program duration, and quantity of homework. Completers and non-completers did not differ significantly on symptom severity, illness perceptions, coping styles, psychological distress, QoL, or demographic variables except for a geographical influence.

Conclusions: These preliminary results indicate that a structured online program is a feasible method of providing targeted psychological support for the gastroparesis cohort. The program was associated with a trend of improvement in symptom severity, illness perceptions, psychological distress, and QOL. Future research should address feedback from participants, which may also improve attrition, and conduct a randomized controlled trial as the next step in validation of the gastroparesisclinic.org program.

Policy of full disclosure: None.

229 | Therapeutic effects of mesenchymal stromal cells on anxiety and depression-like behavior in a model of radiation-induced persistent visceral hypersensitivity

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Objective of the study: Each year, millions of people worldwide are treated for primary or recurrent pelvic malignancies, involving radiotherapy in almost 50% of cases. Many cancer survivors exhibit overlapping symptoms resulting from multiple visceral organ dysfunctions which have been recently recognized as a new pathology called "pelvic radiation disease or PRD". Persistent abdominal pain can affect those patients's quality of life and may be a factor in the development of psychiatric co-morbidity. Using our model of radiation-induced visceral hypersensitivity, we have yet shown that mesenchymal stromal cell (MSC) treatment decreases mechanical allodynia. The aim of the present study was to assess, in this model, (i) anxiety and depression-like behavior and (ii) to test on those parameters MSC therapeutic strategy.

Methods: A 29 Gy colorectal irradiation (CI) were performed on Sprague-Dawley rats. Time-dependent effects of radiation (at 7, 14 and 28 days) on anxiety and depression-like behavior was first analyzed using different tests. The anxiety-like behavior assessment was performed using Marble Burying (MB), Elevated Plus Maze (EPM) and Sociabilization (S) tests. For depression symptoms, Forced Swimming Test (FST) was used. To test MSC efficiency, 5 millions of cells were administered intravenously 3 weeks after irradiation and 1 week later MB, EPM, S tests and FST were realized.

Results: Irradiated rats buried more balls, spent less time in open arm of EPM, had fewer interactions between us compared to control rats. Concerning FST, irradiated rats spent more time immobile in the water in comparison with control rats. CI, associated with persistent visceral hypersensitivity, seems to cause a predisposition to anxiety and depression-like behavior. The anxiety-like behavior appears very early after irradiation and get worse over time. For the first time, we showed that administration of MSC decreased most of anxiety and depression-like behavior.

Conclusion: This work provides new insights into the potential use of MSC in the treatment of visceral hypersensitivity and co-morbidities associated to PRD.

Policy of full disclosure: None.

230 | Amyloid in the intestines: Myopathy seems the most likely cause of dysmotility

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Objective: Amyloidosis is characterised by pathological deposition of proteins in many organs, including the gastrointestinal tract, the latter may result in intestinal dysmotility. Two underlying mechanisms have been suggested: neuropathy due to amyloid deposits in the neural plexus in primary (AL) amyloidosis and myopathy due to deposits within smooth muscle fibres in secondary (AA) amyloidosis, respectively.

Objective: We systematically studied histological characteristics of intestinal dysmotility in AL and AA patients in relation to the presence of amyloid depositions in the intestines.

Methods: Archival autopsy tissue sections from AL amyloidosis patients (small bowel n=6; colon n=7), AA amyloidosis (small bowel n=5; colon n=5) and controls (small bowel n=14; colon n=14) were systematically analysed. Histological evaluation of the enteric nervous system, the network of interstitial cells of Cajal (ICC) and the muscularis propria was performed using haematoxylin and eosin, Congo red, periodic acid Schiff (PAS) and Elastic von Gieson staining, and immunohistochemistry with HuC/D, S100, CD117, and α-smooth muscle actin (α-SMA) antibodies.

Results: All AL and AA patients showed amyloid depositions in the blood vessel walls. We could not find any histological features that are correlated with neuropathy. There were no depositions in the neural plexus, nor were there differences in nerve fiber density or the ICC network. In both patient groups were signs of myopathy present.

In the colon of the AA group, significantly lower α-SMA intensities were present in the circular layer (*P*=.002), in combination with amyloid depositions in mucosa and muscularis mucosae. One of the patients also presented with PAS-positive inclusion bodies. In the small bowel, PAS-positive inclusions were found in 2/5 of AL and 1/5 of AA patients.

Conclusions: We did not find signs of neuropathy in the intestines of patients with amyloidosis, however, a number of patients showed histological features of myopathy.

Policy of full disclosure: None.

231 | Demand for psychological support: Perspective of patients with inflammatory bowel disease (IBD)

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Objective: The relative contribution of psychological factors to onset and course of inflammatory bowel diseases (IBD) is a matter of constant debate among researchers and practitioners. We aimed to investigate the patients' perspective of their need in psychosocial interventions as a part of treatment.

Methods: Psychometric tests including the Short-Form IBD Questionnaire (SIBDQ), the ADAP test measuring demand for psychotherapy, and the Fear-of-Progression Questionnaire Short Form as well as disease related questions were placed on the internet between 12/2014-01/2016. The study was advertised through DCCV (German branch of the European Federation of Crohn´s and Ulcerative Colitis Associations). Multiple regression was performed in order to identify factors associated with high psychotherapy demand.

Results: 631 patients responded to the invitation and 578 completed the survey (356 Crohn Disease (CD), 219 Ulcerative Colitis (UC), 3 unclear). Two hundred and nighty six patients had previous experiences with psychotherapy, while 282 had not. When all available data were entered into a (stepwise-forward) regression model, psychotherapy demand was dependent on previous experience (*P*<.001), fear of progression (*P*<.001), quality of life (*P*=.001), smoking (*P*=.003) and previous surgery (*P*=.005) with the total model explaining 29.7% of the variance. The total explained variance of this model was higher in UC (37.6%) than in CD alone (25.4%).

Conclusions: Lower quality of life and higher fear for disease progression together with other social factors support patients' seek for a psychotherapy as additional treatment option in IBD.

Policy of full disclosure: None.

232 | Gastroesophageal reflux and esopahgeal dysmotility prevalence in candidates for lung transplantation and the influence of esophagogastric gradients

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Introduction: GERD is prevalent in patients with end-stage lung disease candidates for lung transplantation (LTx). The proximal extent of reflux is more dangerous because of the risk of microaspiration. Also, hypotensive lower esophageal sphincter (LES) and ineffective peristalsis had been reported in these patients.

Aim: To determine the frequency of GERD and esophageal motor disorder (EMD) in candidate patients for LTx. Also, to study if the esophagogastric pressure gradients (EGPGs) favor GERD.

Material and methods: Observational cross-sectional study carried out with 74 prospective patients for LTx. High resolution manometry (HRM) (Manoscan®) and double channel pH monitoring off PPI were performed. Chicago 3.0 classification was used to classify EMD. Positive GERD: % time pH<4: >4.5% distal esophagus; >0.9% proximal esophagus. GEPGs were calculated for both respiratory phases (intrathoracic pressure minus intra-abdominal pressure) and referred to the atmospheric pressure.

Results: Patient´s characteristics: male 42 (56.8%); mean age 53.6 (51.3-56); BMI 24.5 (23.6-25.6); Abdominal perimeter 94.3 (91.7-96.8). Pulmonary hypertension patient´s were younger (*P*=.014) and frequently female (*P*=.021) compared to other disease groups (restrictive, obstructive, pulmonary hypertension and others). The frequency of GERD and GEGPs according to the different lung diseases are shown in the figure. There were no differences in GEPGs among different lung diseases (*P*=.165). There was correlation between greater negative intrathoracic pressure in inspiration and higher acid exposition in supine in distal esophagus (*P*=.004) also in proximal esophagus; (*P*=.061). The frequency of hypotensive LES and EMD was 32.4% and 39.2% respectively. There were no significant differences according to the type of lung disease (*P*=.696 and *P*=.805 respectively).

Conclusions: GERD is very frequent in patients with end stage pulmonary disease, especially in restrictive diseases. Proximal GERD is very common in cystic fibrosis. Hypotensive LES and EMD are also frequent in prospective patients for LTx. GEPGs were similar in the different lung diseases. A more negative thoracic inspiratory pressure was associated with more acid exposition in supine in distal and proximal esophagus.

Policy of full disclosure: Advisory board for Allergan.

233 | Postprandial response of gut regulatory hormones by standardized meal stimulation among patients with gastrectomy

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Objective: Anatomic and gut hormonal changes induced by gastrectomy is clinically important, including decreased appetite, weight loss or dumping syndrome. The aim of this study is (i) to evaluate the independent hormone influenced on the weight reduction (ii) to compare the effect of gut hormonal change at preoperative and postoperative periods according to different gastrectomy modalities.

Methods: This prospective study was conducted included 14 controls and 21 patients with gastrectomy; 16 underwent subtotal gastrectomy (STG) because of gastric cancer (early, n=11; advancer, n=5), 5 laparoscopic sleeve gastrectomy (LSG) because of obesity. Serum ghrelin, peptide YY (PYY), insulin, HOMA-IR, glucagon like peptide-1(GLP-1) and gastric inhibitory peptide (GIP) in response to a standardized test meal (200 Kcal, protein 17.6%, fat 30.3%) was recorded at multiple time point before and after gastrectomy 4 and 12 months.

Results: Compared to those of STG and control groups, the LSG group has significantly greater baseline insulin, HOMA-IR, GLP-1, GIP, PYY serum levels, except for PYY and ghrelin levels. The greatest weight reduction occurred on the 4th postoperative month and subsequent weight reduction was not observed. Baseline insulin, HOMA-IR, GLP-1, and GIP were positively correlated with weight reduction(r=−.78, P=.001; r=−.78, P=.001; r=−.81, P<.001; r=−.77, P=.001) meanwhile, baseline ghrelin was negatively correlated with weight reduction at the 4th postoperative month. GLP-1 and GIP were significantly decreased after LSG, however, these hormones had increasing trend on the 4th postoperative month in STG group.

Conclusions: A different pattern of hormonal change was observed in the STG and LSG. Insulin, GLP-1, GIP and body weight decreased significantly 1 year after sleeve resection, it is probably related with metabolic improvement after weight reduction. However, a trend of activation of GLP-1, GIP and PYY after subtotal gastrectomy might attribute the aggravation of weight loss.

Policy of full disclosure: None.

234 | Inhibition of human gastric dysrhythmia by sildenafil, not by Ano1 inhibitors

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	Initial Regular Contractions		Irregular Contractions		N
	Max tension (% increase)	Frequency (% change at max)	Time of onset (min)[1]	Frequency (% change)[1]	
motilin 300nM	1186±597	-20±7	7±2	-56±4	6
+water	1959±917	-28±10	6±1	-48±14	4
+DMSO 0.1%	611±233	-22±4	8±4	-55±5	3
+E-4031 100μM	1043±600	-31±17	5±2	-31±17	3
+MONNA 100μM	477±417	-21±10	6±1	-65±6	3
+$CaCCh_{inhA01}$ 100μM	131±86	-10±5	7±2	-24±24	3
+sildenafil 100μM	1164±995	-29±10	-	-	4*

TABLE

Objective: Nausea is associated with gastric dysrhythmia. Motilin can induce nausea and in human isolated stomach, cause dysrhythmia of muscle contractions (1). The aim was to prevent motilin-induced dysrhythmia by modulating functions of interstitial cells of Cajal.

Method: Mucosa-free circular muscle strips of human stomach (sleeve gastrectomies; non-diabetic obese; informed consent) were suspended in tissue baths (Krebs; 37°C; 95/5% O^2/CO^2, 2 g tension) for isometric measurement of contractions (g/g wet weight of tissue, frequency as contractions/min; cpm). Data shows means±error; n=number of patients.

Results: In the presence of tetrodotoxin 1 μmol/L, atropine 1 μmol/L and L-NAME 300 μmol/L, the amplitude and frequency of spontaneous contractions (1.9±0.2 g/g, 2.9±0.1 cpm, n=45) were unaffected by ω-conotoxin GVIA 100 nmol/L, DMSO 0.1% or water (P>.1, n=3-4 each), abolished by nifedipine 100 μmol/L (n=2) and not consistently changed by E-4031 100 μmol/L (ERG K+channel blocker), MONNA, CaCChinhA01 (100 μmol/L; Ano1 inhibitors) or sildenafil 100 μmol/L (PDE-5 inhibitor) (P>.2, n=4 each). Their effects on the response to motilin was then determined; alone, motilin slightly increased muscle tension and greatly increased regular contraction amplitudes (reducing frequency) which then became irregular.

Conclusion: Sildenafil inhibited motilin-induced irregularity, without affecting the ability of motilin to initially facilitate coordinated contractions. We speculate that increased cGMP inhibits intracellular communication via gap junctions (2), preventing spread of irregular but not coordinated contractions.

Policy of full disclosure: None.

STRESS AND FUNCTIONAL GASTROINTESTINAL DISORDERS

235 | A prospective assessment of bowel habit in patients with non-constipated Irritable Bowel Syndrome (IBS)

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Background & Aims: The prevalence of irritable bowel syndrome (IBS) varies according to geographically based populations, sex, age and socioeconomic status. Many studies have reported the prevalence of IBS, but the effects of socioeconomic status and quality of life have not been well described. And there is no long-term follow up study for IBS and alternating IBS in Korea. Therefore, we conducted to determine different characteristics of IBS subtype and factors influencing the IBS subtype.

Methods: A prospective study, using a reliable and valid questionnaire based on the fulfillment of the Rome III criteria and EuroQol five dimensions (EQ-5D) questionnaire was performed. The patients with physician-diagnosed IBS symptoms fill out the questionnaire at baseline, 3 month, 6 month and 12 month follow up period. Algorithms to classify subjects into IBS-D, IBS-C, and IBS-U groups used questionnaire information and modified Rome III definitions.

Results: Among 39 non-constipated IBS patients, there were no differences between groups except for stool frequency. The proportion of patients in each subgroup remained the same over the year is 20.51% and most patient changed to either of the other 2 subtypes at least once. There were no differences in age, sex, BMI and EQ-5D score between groups which stayed the same subtypes and changed the subtypes.

Conclusions: In our study, non-constipated IBS patients commonly transition between subtypes and there were no influencing factor that changing subtypes. We questioned the meaning of existing classification of IBS subtypes using Rome III criteria and further studies will be needed to support our opinion.

Policy of full disclosure: None.

236 | The increased level of depression and anxiety in Irritable Bowel Syndrome (IBS) patients compared with healthy controls: Systematic review and meta-analysis

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Background/Aim: Irritable bowel syndrome (IBS) patients commonly experience psychiatric disorders, such as depression and anxiety. This meta-analysis sought to compare depression and anxiety levels between IBS patients and healthy controls.

Methods: We searched major electronic databases (MEDLINE, EMBASE, Scopus, and Cochrane library) to find comparative studies on IBS patients and healthy controls. The primary outcome was a standardized mean difference (SMD) of anxiety and depression levels; sub-group analyses were conducted according to IBS-subtypes.

Results: In total, 2293 IBS patients and 4951 healthy controls from 27 studies were included. In random effect analysis, depression and anxiety levels were significantly higher in IBS patients (pooled SMD=0.76, 95% CI 0.62-0.90; *P*<.001; I2=77.2% and pooled SMD=0.84, 95% CI 0.67-1.01; *P*<.001; I2=85.6%, respectively). Both analyses' funnel plots showed symmetry. In meta-regression analysis, heterogeneity was due to the studied region and questionnaire type for both depression and anxiety. In sub-group analyses of IBS-subtype, the pooled SMDs of depression and anxiety levels (IBS with predominant constipation: 0.83 and 0.81, IBS with predominant diarrhea: 0.73 and 0.65, and IBS with mixed bowel habits: 0.62 and 0.75; *P*<.001, respectively) were significantly higher in all IBS-subtypes, respectively.

Conclusions: The present meta-analysis showed depression and anxiety levels to be higher in IBS patients than in healthy controls, regardless of IBS-subtype. However, the gender effect on psychological factors among IBS patients could not be determined and should be evaluated in prospective studies.

Policy of full disclosure: None.

237 | Microbial regulation of hippocampal miRNA expression: Implications for transcription of kynurenine pathway enzymes

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Increasing evidence points to a functional role of the enteric microbiota in brain development, function and behaviour including the regulation of transcriptional activity in the hippocampus. No changes in CNS miRNA expression have yet been linked to the colonisation status of the gut. Given the pivotal impact of miRNA's on gene expression, our study was based on the hypothesis that this would also be altered in the germ-free state in the hippocampus. We measured miRNA's in the hippocampus of Germ free (GF), conventional (C) and Germ free colonised (GFC) Swiss Webster mice. miRNA's were selected for follow up based on significant differences in expression between groups based on gender and colonization status. The expression of miR-294-5p was increased in male germ free animals and was normalised following colonisation. Targets of the differentially expressed miRNA's were over-represented in the kynurenine pathway. We show that the microbiota modulates the expression of miRNAs associated with kynurenine pathway metabolism and, for the first time, demonstrate that the gut microbiota regulates the expression of kynurenine pathway genes in the hippocampus. We also show a sex-specific role for the microbiota in the regulation of miR-294-5p expression in the hippocampus. The gut microbiota plays an important role in modulating small RNAs

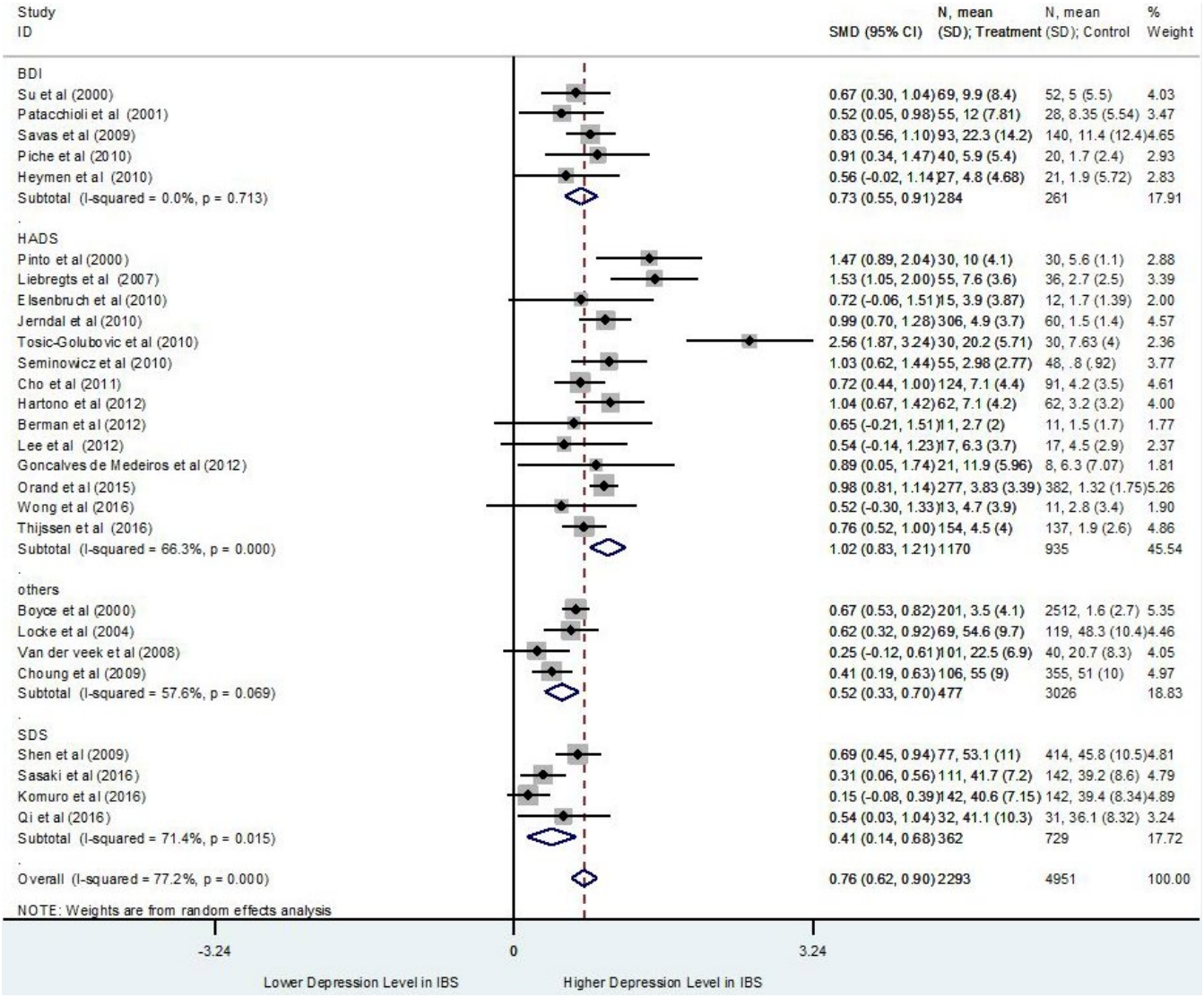

FIGURE 1 Forest plot of depression levels in IBS-subtype patients

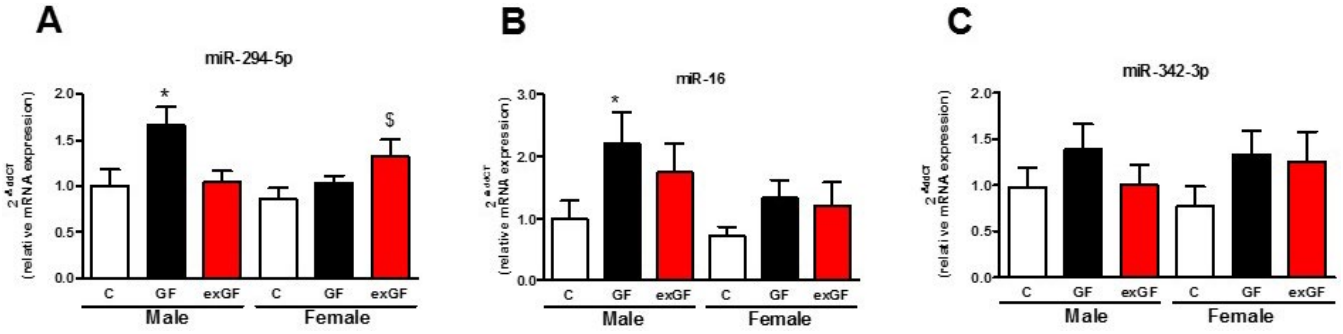

FIGURE 1 mir-294-5p expression in the hippocampus is regulated by the gut microbiota

that influence hippocampal gene expression, a process critical to hippocampal development.

Policy of full disclosure: None.

238 | Brain MRI reveals white matter tract abnormalities in patients with idiopathic fecal incontinence

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Objective: The pathophysiological mechanisms behind idiopathic fecal incontinence (IFI) is still partially unknown, however abnormalities within the central nervous system (CNS) have been proposed as part of the pathogenesis. The current study aimed to examine gray matter volume and cortical thickness characteristics as well as white matter (WM) tract characteristics in patients with IFI and healthy controls.

Methods: We enrolled 21 female patients with IFI (60.3±10.9 years) and 15 healthy controls (55.5±9.45 years). All subjects underwent a structural MRI and diffusion tensor imaging (DTI). We performed voxel-based morphometry analysis to investigate grey matter volume and surface-based morphometry analysis investigate cortical thickness. Additionally, we used tract-based-spatial statistics (TBSS) to characterize WM microstructure. Finally, associations between MRI based brain characteristics and previously collected latencies of rectal sensory evoked electroencephalography potentials were determined.

Results: Compared to healthy controls, IFI patients had significantly reduced fractional anisotropy (FA) values, implicating reduced integrity of fiber tracts, in the left hemisphere. Particularly in the superior longitudinal fasciculus (SLF) (P=.042), posterior thalamic radiation (PTR) (P=.047), and middle frontal gyrus (MFG) (P=.049), see Figure 1. No differences were observed in gray matter volume or in cortical thickness between the groups. The reduced FA values in the superior longitudinal fasciculus and middle frontal gyrus were negatively associated with prolonged latencies of cortical potentials evoked by rectal stimuli (all P<.05).

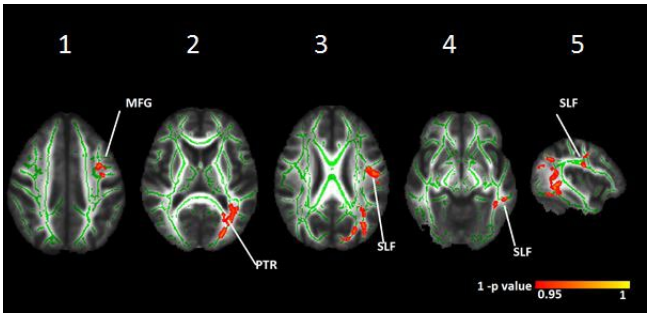

FIGURE 1 Brain regions showing a significant decrease in FA

Conclusions: IFI patients have no macrostructural brain abnormalities, but reduced integrity of left WM tracts that are relevant for sensory processing when compared to healthy controls. The clinical relevance of the microstructural findings is supported by their associations with functional changes expressed as prolonged latencies of cortical potentials evoked by rectal stimulation. Our findings support the theories of CNS changes as a central part of the pathogenesis in IFI.

Policy of full disclosure: None.

239 | Activity-based anorexia rats show an increased activation of nesfatin-1 immunoreactive neurons in distinct brain nuclei

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Disorders like anorexia nervosa are not completely understood so far. Animal models are useful to gain more insight in the pathophysiology of the disease and to find new treatment strategies. Here we investigated the potential role of the anorexigenic peptide nesfatin-1 in the pathogenesis of anorexia nervosa by using the activity-based anorexia (ABA) model in rats. Female Sprague-Dawley rats were divided into 4 groups (n=6/group): activity-based anorexia (ABA, food for 1.5 hours and 24-hours access to a running wheel); restricted feeding (RF, food for 1.5 hours); activity (AC, 24-hours access to a running wheel and food) and ad libitum (AL, 24-hours access to food) for 3 weeks. After developing ABA, animals were transcardially perfused and brains processed for Fos and nesfatin-1 immunohistochemistry. ABA increased the expression of nesfatin-1 immunopositive neurons in the paraventricular nucleus of hypothalamus (PVN, cells/section, mean±sem, ABA: 137.7±7.6, RF: 106.6±7.2, AC: 68.3±9.2, AL: 74.1±15.0), dorsomedial hypothalamic nucleus (DMH, ABA: 42.0±6.0, RF: 30.0±3.3, AC: 15.6±1.4, AL: 18.3±2.0), arcuate nucleus (Arc, ABA: 66.4±11.4, RF: 48.7±6.4, AC: 26.6±6.2, AL: 35.2±4.9), locus coeruleus (ABA: 117.2±5.0, RF: 102.2±7.7, AC: 79.1±4.8, AL: 80.8±7.9) and in the rostral part of the nucleus of the solitary tract (ABA: 31.2±3.0, RF: 24.7±1.6, AC: 19.2±3.6, AL: 22.3±2.1) compared to AC and AL (P<.05) but not RF rats (P>.05). Moreover, we observed significantly more Fos and nesfatin-1 double labeled cells in ABA rats compared to RF, AL and AC in the supraoptic nucleus (ABA: 11.7±4.7, RF: 1.2±0.6, AC: 0.08±0.08, AL: 0.04±0.04, P<.05) and compared to AC and AL in the PVN (ABA: 4.0±0.8, RF: 2.8±1.0, AC: 0.7±0.2, AL: 0.6±0.3), DMH (ABA: 6.2±1.9, RF: 2.4±0.6, AC: 0.5±0.3, AL: 0.6±0.4), Arc (ABA: 21.8±9.2, RF: 9.1±4.3, AC: 0.0±0.0, AL: 0.0±0.0), dorsal raphe nucleus (ABA: 13.5±2.1, RF: 10.7±4.1, AC: 2.4±1.1, AL: 2.6±1.1) and the rostral raphe pallidus (ABA: 5.8±1.9, RF: 3.3±1.5, AC: 0.7±0.2, AL: 1.0±0.2, P<.05). Since nesfatin-1 is involved in the inhibition of food intake and plays a role in the response to stress, we hypothesize that the observed changes of brain nesfatin-1 might

contribute to the reduction of food intake and body weight under conditions of ABA.

Policy of full disclosure: None.

240 | Preliminary evidence for increased parasympathetic activity during social inclusion and exclusion in adolescents with functional abdominal pain

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Objective: Peer victimization (eg, social exclusion) has been shown to be associated with physical health problems such as functional somatic complaints and especially symptoms of pain. To date, no study has investigated the mechanisms underlying this association in clinical pediatric samples. The aim of this study was to evaluate the parasympathetic reactivity during a social exclusion experience in adolescents with functional abdominal pain (FAP).

Methods: Twenty adolecents with FAP diagnosed by mean of Rome III pediatric criteria and twenty one matched healthy participants were compared regarding parameters of parasympathetic activation (based on heart rate variability analysis) before, during and after participating in the Cyberball-game, a well-established paradigm to induce social exclusion.

Results: Adolescents with FAP showed an increase in parasympathetic activation whereas the healthy control group remained stable. Repeated measure ANOVA showed a significant "group"×"phase" interaction for high frequency power (HF) ($F(1, 39)=2.88$, $P=.024$, η^2part=.069) and for root mean square successive difference (RMSSD) ($F(4, 156)=3.16$, $P=.034$, η^2part=.075) indicating different patterns in children with FAP during both—inclusion and exclusion—conditions of the Cyberball game. Baseline as well as post-stress measures were similar in both groups.

Conclusion: The parasympathetic activation pattern may indicate altered emotional processing of social stimuli and represent an enhanced psychophysiological vulnerability of adolescents with FAP regarding social evaluation.

Policy of full disclosure: None.

241 | Sex difference of eating behavior and gastrointestinal function in response to stress in rat

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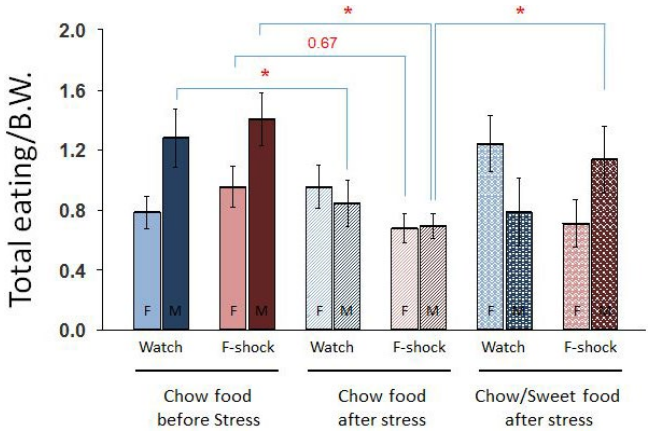

FIGURE 1 Food consumption after stress in male and female rats

Background/Aims: Stress could affect appetite and bowel function. The aim of this study is to investigate the difference of eating behavior and gastric motility according to the type of stressor, sex in rat and type of food.

Methods: Both sex of Spargue-Dawely rats were used. All rats underwent foot shock stress (FSS) for 5 minutes one day before experiment. We used two type of stressor physical stress "FSS" as physical stress and "watching other rat exposed to FSS" as psychological stress. In each type of stress, rats were randomly divided into three groups as (i) regular food supply before stress, (ii) regular food supply after stress, and (iii) concomitant regular and sweet food supply after stress in male and female, respectively. After stress session, food consumption, gastric emptying (GE), and sweet food preference, serum ACTH/corticostereone were measured in all rats.

Results: In male rats, both psychological and physical stress decreased the food consumption. In female rats, however, only physical stress decreased the food consumption. When food was supplied before stress, both psychological and physical stress delayed GE during stress in male rat. In female rats, however, only physical stress delayed GE during stress. When food was supplied after stress, physical stress increased GE in both male and female, however psychological stress showed no effect on GE. Restoration of stress-induced eating inhibition by concomitant sweet food was observed only in males rats exposed to physical stress.

Conclusions: These results indicate that sex as well as type of stressor is a crucial factor affecting on feeding behavior and gastric motility in stress response in rat.

Policy of full disclosure: None.

242 | Early-life stress promotes alterations in intestinal permeability in juvenile rats

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Early-life stress, such as maternal separation (MS) in rodents has been used to model stress-related psychiatric disorders and alterations in intestinal function, however most reports focus on the effects seen at adulthood. Here we evaluated the effect of MS (3h/day from post-natal day (PND) 2 to PND12) on colon barrier function in male Sprague-Dawley rats at PND21 and PND35, and compared them to non-separated (NS) controls. Permeability to macromolecules was evaluated through the everted gut sac technique, applying FITC-conjugated 4.4kDa dextran (FD4.4) on the mucosal side, and then measuring fluorescence in the serosal side for up to 3 hours, while transepithelial electrical resistance (TEER) was determined through Ussing chamber studies. There was no difference in FD4.4 permeability and TEER between MS and NS at PND21. However, at PND35 there was an increase in FD4.4 permeability in MS rats when compared to NS, while no differences in TEER where found between both groups. Additionally, when MS rats at PND35 were subjected to a 5 minutes swim stress, they showed a blunted corticosterone response in comparison to swim-stressed NS rats. Furthermore, FD4.4 permeability in swim-stressed MS rats was lower than in non-swim stressed MS rats. Together these data show that early-life stress affects permeability to macromolecules at PND35, and that this phenomenon is sensitive to corticosterone. Moreover, these results suggest that alterations in colon barrier function are observable in young individuals, which should be considered when discussing mechanistic aspects of early life stress-induced alterations in the gut brain axis. FUNDED BY: FONDECYT #1140776.

Policy of full disclosure: None.

243 | Dynamic gastrointestinal serotonergic responses to an acute stressor: Role of host genetics

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Objective: Host genetics influences the acute stress response, the impact of which is frequently manifested in altered gastrointestinal function and exacerbated in disorders such as irritable bowel syndrome. Gut-derived serotonin (5-HT) exerts physiologically and clinically important local and systemic effects. The role of host genetics on the dynamics of the gastrointestinal serotonergic system response to an acute stressor is poorly understood. We sought to define the characteristic gastrointestinal serotonergic system response to and recovery from an acute stressor in genetically-distinct mice strains.

Methods: Adult male NIH Swiss Webster, BALB/c, and C57/BL6 mice were randomly allocated to the unstressed control or stress group. Stressed animals were subjected to 15 minutes of restraint stress (n=4-8 mice/timepoint/strain) and sacrificed post-stressor +0, 5, 15, 30, 45, 60, or 240 minutes. Plasma corticosterone was assayed using an ELISA. 5-HT and its main metabolite, 5-HIAA, were measured in both the ileum and colon via HPLC. Results were analyzed by student's *t*-test or ANOVA, where applicable, and statistical significance was set at a $P<.05$.

Results: Plasma corticosterone was significantly ($P<.05$) elevated immediately after restraint stress compared to control group in each strain. C57/BL6 exhibited a greater ($P<.05$) plasma corticosterone concentration post-stressor compared to BALB/c or NIH Swiss Webster mice. Colonic 5-HT levels were higher than ileal 5-HT in all mouse strains. Distal ileal and proximal colonic 5-HT and 5-HIAA was elevated ($P<.05$) at several post-stressor timepoints in C57/BL6 compared to other strains.

Conclusions: Confirming that host genetics heavily influence the stress response. The C57/BL6 strain displayed the largest post-stress HPA axis activity. This strain also had higher levels of 5-HT in the colon and ileum at multiple timepoints post-stressor. Further studies are required to understand the implications of these findings for the control of stress-induced 5-HT-mediated gastrointestinal symptoms and to assess the role of the gastrointestinal microbiota and microbial metabolites in regulating the local gastrointestinal serotonergic system response to acute stressors.

Policy of full disclosure: The APC Microbiome Institute has conducted studies in collaboration with several companies including Suntory, Mead Johnson, Cremo, 4D Pharma, Wyeth, Pfizer, and GSK.

244 | The prevalence and psychopathology of functional gastrointestinal disorders in psychiatric outpatients

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Objective: In functional gastrointestinal disorders (FGIDs), psychological factors are known to be very important. This study aimed to investigate the prevalence and psychological characteristics of patients who experience FGIDs among psychiatric outpatients.

Methods: This survey was conducted on 170 patients who visited the outpatient unit in the Department of Psychiatry at Wonkwang University Hospital. Data from 144 patients was included in the analysis, excluding 26 patients with insincere responses. FGIDs were screened (Gastroesophageal Reflux Disease; GERD, Functional Dyspepsia; FD, Functional Constipation; FC, Irritable Bowel Syndrome; IBS) according to the Rome III questionnaire-Korean version. Demographic factors were investigated, and the Hospital Anxiety Depression Scale (HADS), the Patient Health Questionnaire-15 (PHQ-15), the Childhood Trauma Questionnaire-Korean (CTQ-K), and the State-Trait Anger Expression Inventory (STAXI) were used to evaluate psychosocial factors.

Results: The prevalence of FGIDs was 64 patients for GERD (44.4%), 29 patients for FD (20.1%), 26 patients for FC (18.1%), 24 patients for IBS (16.7%) and 44 patients for Overlap syndrome (30.6%). Among the differences in FGIDs according to mental disorder classification, only IBS showed a significant difference in prevalence (χ^2=11.408,

P=.022). Differences in psychological factors according to the classification of FGIDs were as follows: IBS patients showed significant differences in anxiety (t=−3.106, *P*=.002), depression (t=−2.105, *P*=.037), PHQ-15 (t=−3.565, *P*<.001), Trait-anxiety (t=−3.683, *P*<.001), Anger-in (t=−2.463, *P*=.015), and Anger-out (t=−2.355, *P*=.020), FD patients showed significant differences in anxiety (t=−4.893, *P*<.001), depression (t=−3.459, *P*<.001), PHQ-15 (t=−7.906, *P*<.001), Trait-anxiety (t=−4.148, *P*<.001), State-anxiety (t=−2.181, *P*=.031), Anger-in (t=−2.684, *P*=.008), Anger-out (t=−3.005, *P*=.003), and GERD patients showed significant differences in anxiety (t=−4.286, *P*<.001), depression (t=−3.402, *P*<.001), PHQ-15 (t=−7.162, *P*<.001), Trait-anxiety (t=−2.994, *P*=.003), State-anxiety (t=−2.259, *P*=.025), Anger-in (t=−2.772, *P*=.006), Anger-out (t=−2.958, *P*=.004).

Conclusion: The results of this study confirmed that the prevalence of FGIDs is high in patients with mental disorders, and that various psychosocial factors affect the prevalence of FGIDs. Therefore, mental disorder patients with FGIDs require attention and active intervention from healthcare workers. Keywords Mental disorders, functional gastrointestinal disorders, depression, anxiety, expression of anger.

Policy of full disclosure: None.

245 | Clinical features of Irritable Bowel Syndrome (IBS) in migraine patients

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Objective: The goal of the study was to research clinical features of irritable bowel syndrome (IBS) in migraine patients.

Methods: 114 migraine patients aged from 18 to 60 were studied. The M diagnosis were based on ICHD-3 (2013). Functional gastrointestinal disorders (FGIDs) were diagnosed using Rome IV criteria (2016). Quality of life of the patients was assessed using MOS SF-36 questionnaire. The anxiety levels were evaluated using The Beck Anxiety Inventory, and the depression levels—with Zung W. PSM-25 was used for structure evaluation of the stress experiences.

Results: There were 40 total IBS occurencies in patients (35,1%), with predominant constipation IBS prevailing (n=21, 18,4%). IBS with predominant diarrhea manifested itself in 10 patients (8,8%), IBS with mixed bowel habits—in 9 (7,9%). No significant difference in frequency of IBS co-occurring with different types of migraine was found. IBS co-occurred with GERD in 23 patients (57,5%) and with functional dyspepsia—in 36 (90%). Patients with migraine and IBS reported to have more severe social maladjustment and more frequent migraine attacks. Quality of life in different aspects has dropped in patients with migraine and IBS, with the Vitality scale (feeling stressed out, exhausted) levels dropping as well (PMW=0.024). Patients with migraine and IBS had reliably high depression levels (PMW=0.021), anxiety levels (PMW=0.017), more frequently percieved life events as stressful (PMW=0.046).

Conclusions: According to the modern views on the matter, FGIDs are a disruption of "brain-gut" interaction, which is a two-way communication carried out by visceral, afferent and efferent systems, in which vagus nerve plays an important part. Hyperexcitability of the brain that lies at the core of migraine pathogenesis, may create appropriate conditions to form FGIDs. The revealed connections between migraine, IBS, anxiety and depression unify functional interactions of the brain stem structures with anterior cingulate cortex, amygdala, hypothalamus and medial frontal cortex. This integrated system modulates motor, vegetative, neuroendocrine and psychosocial aspects of pain. These pathogenic mechanisms may help in explaining the close interconnection between migraine and FGIDs.

Policy of full disclosure: None.

246 | Add-on alginate to proton pump inhibitor therapy in patients with breakthrough symptoms: A post-hoc analysis using a clinically relevant responder rate

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Objective: In patients with gastroesophageal reflux disease (GERD), augmenting proton pump inhibitor (PPI) therapy with alginate can help relieve persistent symptoms. The objective of this post-hoc analysis was to examine clinically relevant responder rates.

Methods: The original 7-day, randomised controlled trial compared alginate with placebo (10 mL) four times daily (with once-daily PPI) (n=136). The post-hoc analysis used the following formula: Heartburn Reflux Dyspepsia Questionnaire (HRDQ) daily score=[HB severity*(HB frequency/(HB frequency+3))]+[RG severity*(RG frequency/(RG frequency+3))]. Responder rate was defined as ≥3 days reduction in the number of 'bad days' during treatment vs run-in, where 'bad day' was defined as a day with HRDQ daily score >0.70 (clinically relevant).

Results: From the original study, the change in HRDQ score was significantly greater for alginate/PPI compared with placebo/PPI, as well as for heartburn, but not regurgitation. Daily frequency but not severity of heartburn and regurgitation were significantly greater for alginate/PPI, as well as the number of nights without symptoms. For the post-hoc analysis, alginate offered significantly better overall control of reflux symptoms as demonstrated by the responder rate analysis (56% for alginate/PPI vs 29% for placebo/PPI; *P*<.005) (n=120), and more symptom-free nights (51% for alginate/PPI vs 27% for placebo/PPI; *P*<.05) (n=94), compared with placebo/PPI. Alginate/PPI also provided significant improvement in reflux symptoms (heartburn and regurgitation combined), heartburn symptoms (frequency and severity) and regurgitation severity (Figure 1). Alginate/PPI provided greater improvements in

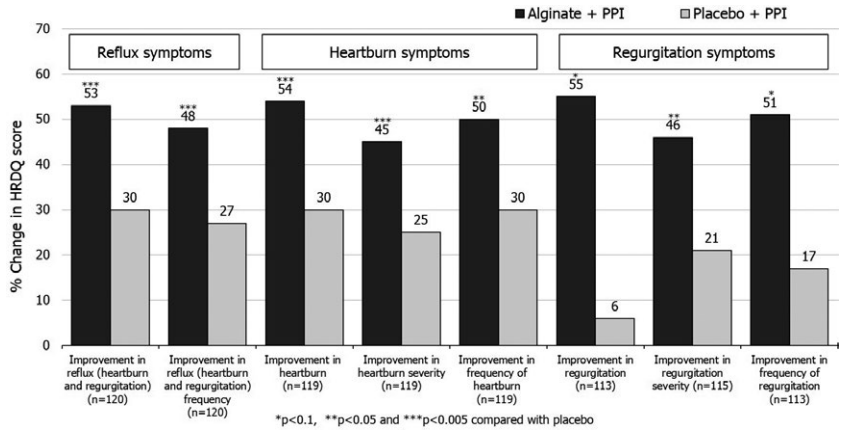

FIGURE 1 Change of symptoms and quality of life following alginate/placebo treatment in addition to PPI

regurgitation frequency but was not significant compared with placebo/PPI (Figure 1).

Conclusion: Using a clinically relevant responder rate analysis, alginate as add-on therapy to PPI significantly improves overall symptoms in patients with GERD.

Policy of full disclosure: C Coyle, T Pavion and F Lewis are RB employees. P Bytzer has served on an advisory board for RB and received honoraria and research funding from RB. Data presented previously at Gastro Update Europe 2017.

247 | The overlap of functional gastrointestinal disorders and quality of life of patients in the Department of Psychiatry

H.-S. Ryu[1]; S.-H. Jang[2]; S.-C. Choi[2]; Y.-S. Kim[2]; S.-H. Park[2]; M.-Y. Lee[3]; S.-Y. Lee[2]

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Objective: Functional gastrointestinal disorders (FGIDs) is known to cause very serious discomfort in daily life. Therefore, this study aimed to examine the quality of life for mental disorder patients showing FGIDs.

Methods: This survey was conducted on 144 patients who visited the outpatient unit in the Department of Psychiatry. FGIDs were screened using the Rome III questionnaire-Korean version and the severity was assessed. A health-related quality of life scale (The Short Form Health Survey-36; SF-36) was used to evaluate the quality of life, and the subdomains were analyzed [Vitality (VT), Physical functioning (PF), Bodily pain (BP), General health Perception (GHP), Physical Role Functioning (PR), Emotional Role Functioning (RE), Social Role Functioning (SR), Mental Health (MH), Physical Component Summary (PCS), Mental Component Summary (MCS)].

Results: Differences in quality of life according to the classification of mental disorders were observed for the subdomains of RE (F=2.607, *P*<.05), MH (F=5.623, *P*<.001), VT (F=3.913, *P*<.01), GHP (F=3.680, *P*<.01), and MCS (F=5.941, *P*<.001). The following differences in quality of life according to the classification of FGIDs were observed: for IBS, differences were observed in MH (t=2.397, *P*=.018), VT (t=3.817, *P*<.001), GHP (t=3.925, *P*<.001), PCS (t=2.149, *P*=.033), and MCS (t=3.020, *P*=.003); for FD, differences were observed in RP (t=1.996, *P*=.048), RE (t=2.780, *P*=.006), MH (t=4.666, *P*<.001), VT (t=4.606, *P*<.001), BP (t=2.699, *P*=.008), GHP (t=3.683, *P*<.001), PCS (t=2.895, *P*=.004), and MCS (t=5.043, *P*<.001)]; and for GERD, differences were observed in RP (t=2.154, *P*=.033), RE (t=2.592, *P*=.011), MH (t=3.941, *P*<.001), VT (t=4.477, *P*<.001), GHP (t=3.880, *P*<.001), PCS (t=2.329, *P*=.021), and MCS (t=3.937, *P*<.001). In terms od correlations between quality of life and severity of FGIDs, PCS showed very strong correlations with IBS (*r*=−.381, *P*<.01), GERD (*r*=−.395, *P*<.01), FD (*r*=−.298, *P*<.01), and FC (*r*=−.415, *P*<.01), and MCS showed very strong correlations with IBS (*r*=−.403, *P*<.01), GERD (*r*=−.441, *P*<.01), FD (*r*=−.402, *P*<.01), and FC (*r*=−.386, *P*<.01).

Conclusion: FGID severity in patients with mental disorders was found to be very closely related to quality of life. Therefore, various interventions by healthcare workers are required to improve the quality of life of mental disorder patients who complain of gastrointestinal diseases.

Policy of full disclosure: None.

248 | Ghrelin enhances GLP-1 induced neuronal activation in the distal colon

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Objectives: Irritable Bowel Syndrome (IBS) is a chronic condition characterised by bouts of cramping, abdominal pain, constipation and/or diarrhoea and afflicts 10%-20% of the population. IBS symptoms are exacerbated following a meal. The orexigenic hormone, ghrelin is secreted prior to a meal, whereas the incretin hormone, glucagon like peptide-1 (GLP-1) is secreted in response to the arrival of nutrients to the gut. Both hormones have been implicated in gut motility and thus, may be important in post-prandial exacerbation of IBS symptoms.

Methods: Colonic myenteric plexus tissue was prepared from adult male Sprague Dawley (SD) rat controls and Wistar Kyoto (WKY) rats, which are an animal model of IBS. Real-time calcium imaging experiments on myenteric neurons were conducted using a standard epifluorescence imager. Distal colon with intact vagal innervation from SD and WKY rats was placed in a tissue bath in carbogen-bubbled Krebs saline. Exposed colonic myenteric neurons were placed in the stimulus chamber and nerve activity from the vagus was recorded extracellularly in an adjacent recording chamber using a bipolar electrode.

Results: GLP-1 induced a small increase in intracellular calcium levels in myenteric neurons of both SD and WKY rats, whereas prior exposure to ghrelin enhanced the GLP-1 evoked response in both SD (n=24, $P<.01$) and WKY (n=40, $P<.001$) rats. Exposure of colonic myenteric neurons to GLP-1 and ghrelin in both SD and WKY also stimulated vagal nerve firing and the GLP-1-evoked neural response was potentiated by prior exposure to ghrelin in both rat strains (SD: $P<.001$, WKY: $P<.01$).

Conclusions: Prior exposure to ghrelin, as may occur during the ghrelin peak prior to food ingestion, appears to sensitise colonic myenteric neurons to the neurostimulatory effects of GLP-1. Moreover, sensitisation of myenteric neurons by exposure to ghrelin enhances the gut-to-brain signalling via the vagus nerve. However, this neuroendocrine modification was not different between the IBS rat model and the control comparator.

Policy of full disclosure: None.

249 | Influence of STW5, an herbal preparation, on the microbiome in an experimental model of functional dyspepsia

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Objective: Stress has been advocated a role in the etiology of functional dyspepsia (FD). Stress has also been claimed to affect the composition and function of intestinal microbiota in such patients. STW 5 is a fixed multi-component herbal preparation consisting of hydro-alcoholic extracts of bitter candytuft, lemon balm, chamomile, caraway fruit, peppermint leaf, Angelica root, milk thistle, celandine herb, and licorice root. It has been used successfully in treating FD1 but the potential involvement of gut microbiota has not been studied. The current study investigates the changes in intestinal microbiota induced by stress in an experimental model of FD and the influence of STW5 on such changes.

Methods: To induce FD, male Wistar rats were subjected to neonatal maternal separation (NMS) (pups removed from mother cage 30 min/day for 21 days). After reaching adulthood, they were subjected to restraint stress (RS) (90 min/day for 1 week). STW5 was given orally (2 and 5 mL/Kg) daily for 1 week while subjecting them to RS. Animals were sacrificed 24 hours after last drug administration and fecal samples were taken from the cecum to determine genomic DNA and changes in selected bacterial flora were assessed using quantitative Real Time-PCR. The main phyla studied were the Bacteroidetes, Firmicutes, Fusibacterium and Actinobacteria.

Results: The phyla studied were affected to variable extents by the FD model. In normal animals, STW 5 induced a decrease in the relative abundance of Actinobacteria associated with an increase in the relative abundance of Bacteroidetes and Firmicutes. STW 5 tended to improve the changes induced by the stress model.

Conclusions: The results provide evidence that the successful use of STW5 in FD could at least be partly due to influencing beneficially the intestinal microbiota deranged by stress.

References: (1) Schmulson MJ (2008) Nature clinical practice gastroenterology & hepatology, 5, 136-137.

Policy of full disclosure: The study has been funded in part by Steigerwald Arzneimittelwerk, GmbH, Bayer Consumer Health, Darmstadt, Germany.

250 | Acute stress diminishes unspecific treatment effects on gastric motility in Nausea

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Background: Nausea affects a broad spectrum of patients in nearly every medical specialty, be it as symptom or as side-effect of treatment (Holmes et al., 2009). A variety of antiemetic agents is available, but satisfactory alleviation of nausea remains challenging (Jordan et al., 2007). Nausea and the situations it occurs in impose stress and negative emotions, which can further compound symptoms (Roscoe et al. 2004). Contextual factors are a crucial component of every treatment, esp. in subjectively evaluated conditions like pain and nausea (Hrobjartsson et al., 2010). Their effect can be quantified using placebo interventions. We aimed to examine (i) the influence of contextual treatment factors on both behavioural and physiological parameters of nausea and (ii) the effect of acute stress on them.

Methods: Eighty motion-sickness-susceptible women were randomised in a two-factorial design to stress (Maastricht Acute Stress Test, MAST (Smeets et al., 2012))/no stress (control-MAST) and placebo intervention (sham acupuncture-point-stimulation)/natural history (Figure 1) and exposed to a visual vection stimulus. Assessed

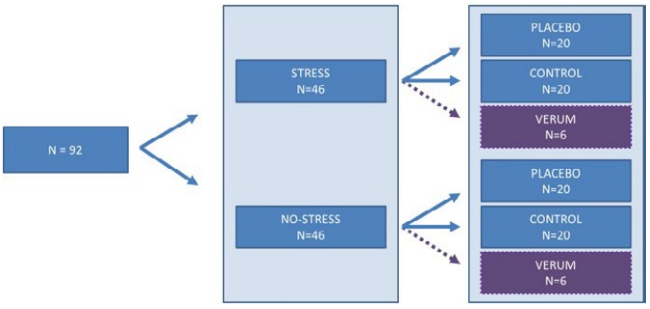

FIGURE 1 Study Design. Verum groups served for blinding purposes only and hence were not included in the analyses

parameters were, amongst others, symptom ratings, electrogastrogram (EGG), and electrocardiogram.

Results: A two by two analysis of covariance revealed that the placebo group experienced significantly less nausea than the natural history group ($F=30.43$, $P<.001$). Stress did not impact the behavioral placebo response ($F=0.30$, $P=.59$). However, there was a significant interaction between `stress´ and `intervention´ for the normo-to-tachy ratio in the EGG ($F=4.73$, $P<.05$), which was due to placebo-induced improvement of gastric activity only in the no-stress condition.

Conclusion: Results suggest powerful expectation effects on nausea, even affecting gastric motility. Acute stress abolished the peripheral placebo response. Thus, optimizing contextual factors, such as verbal suggestions and reduction of negative emotions, could facilitate nausea management.

Policy of full disclosure: None.

251 | Patients symptoms are not predictive for GERD when compared with esophageal functional test and endoscopy

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Introduction: GERD typical symptoms are not highly sensitive or specific for predicting the presence of GERD and present in other non-GERD conditions such as eosinophilic esophagitis or achalasia. ENT symptoms are not predictors for GERD and patients with these types of symptoms are a diagnostic challenge. AIM. To determine the utility of symptoms with respect to the esophageal functional test and endoscopy and evaluate the frequency of esophageal motor disorder (EMD) in both groups.

	ENT symptoms (N=89)		Typical GERD symptoms (N=113)	
General data				
Age	53.5 (50.8-56.2)		49.4 (46.7-52.1)ǂ	
Sex (♀)	56 (62.9%)		72 (63.7%)	
BMI	26.4 (25.6-27.3)		26.7 (25.7-27.7)	
Abdominal perimeter	94.6 (92-97.2)		96.1 (93.2-98.9)	
Previous treatment PPI	76 (85.4%)		102 (91.1%)	
Complete response to PPI	11 (14.5%)		29 (28.2%)+	
Endoscopy results				
Normal	22 (24.7%)		18 (15.9%) ǂ	
GERD lesions (esophagitis, Barrett)	9 (10.1%)		41 (36.3%)	
Others (gastritis, ulcers...)	41 (46%)		43 (38.1%)	
No data	17 (19.1%)		11 (9.7%)	
24 pH-monitoring				
Normal	63 (70.8%)		56 (49.6%) +	
Abnormal	24 (27%)		56 (49.6%)	
Hypersensitivity	2 (2,2)		1 (0.9%)	
HRM and 24-pH monitoring				
	ENT symptoms(N=89)		Typical symptoms (N=113)	
HRM results	pH normal (N=63)	pH abnormal (N=26)	pH normal (N=56)	pH abnormal (N=57)
LES diagnosis				
Normal	35 (55.6%)	16 (61.5%)	37 (66.1%)	28 (49.1%)
Hypotensive	21 (33.3%)	5 (19.2%)	11 (19.6%)	22 (38.6%)
EGJOO (IRP-4s >15 mmHg)	7 (11.1%)	5 (19.2%)	8 (14.3%)	7 (12.3%)
Esophageal body diagnosis				
Normal	51 (81%)	13 (50%)+	45 (80.4%)	39 (68.4%)*
Ineffective peristalsis	7 (11.1%)	12 (46.2%)	4 (7.1%)	13 (22.8%)
Aperistalsis	0	0	1 (1.8%)	2 (3.5%)
Hypercontractile	2 (3.2%)	1 (3.8%)	3 (5.4%)	1 (1.8%)
Distal spasm	3 (4.8%)	0	3 (5.4%)	2 (3.5%)

*$p < 0.001$; +$p<0.01$; ǂ > 0.05

TABLE Symptoms, patients characteristics andendoscopy, pH-monitoring and HRM results

Material and methods: Patients undergoing both double channel pH-monitoring off PPI testing and esophageal HRM (Manoscan®) for typical (heartburn and regurgitation) and ENT symptoms (laryngitis, pharyngeal foreign body (PFB) and cough) (113 and 89 respectively) over a 4-year period were prospectively evaluated. Gastrointestinal (GI) symptoms were evaluated using the modified DeMeester Questionnaire. Patients with a frequency of symptoms of at least once a week were included.

Results: Patients characteristics, results from the endoscopy, pH-monitoring and HRM are expressed in the figure. Patients with typical GERD symptoms had a better response to PPI, more peptic lesions and abnormal pH-monitoring compared to those with ENT symptoms. The diagnostic yield of symptoms considering the pH-monitoring as the gold standard was: Sensitivity: 50.4 (40.8-60.1); Specificity: 70.8 (60.8-80.8); PPV: 68.7 (50.1-79.3); NPV: 55.9 (48.9-63). When the endoscopy results and/or pH-monitoring were considered the results were: Sensitivity: 45.6 (38.7-52.5); Specificity: 78.3 (71.6-84.9); PPV: 73.7 (65.8-81.5); NPV: 51.9 (45.4-58.3). 12 (19%) of 63 patients with ENT symptoms and normal pH-monitoring, had an EMD that can impair esophageal clearance and 7 (11.1%) had EGJ outflow obstruction (EGJOO). Five out of 7 patients with EGJOO had PFB as their main symptom.

Conclusion: The sensitivity of symptoms to predict GERD is low. Typical GERD symptoms were associated with better response to PPI, higher frequency of peptic lesions and abnormal pH-monitoring compared to ENT symptoms. EMD and EGJOO that can impair bolus transit, should be considered in patients with ENT and typical GERD symptoms and normal pH-monitoring.

Policy of full disclosure: None.

252 | The impact of gut microbial short-chain fatty acids on psychosocial stress-induced deficits in brain physiology and behaviour

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There is a growing recognition of the involvement of the gastrointestinal microbiota in the homeostasis of brain physiology and behaviour. Bacterial-derived metabolites play a central role within this communication, of which short-chain fatty acids (SCFAs) are perhaps the most investigated molecules. SCFAs are the product of bacterial fermentation of host-indigestible dietary fibres and have already been demonstrated to play a pivotal role in gut function, host metabolism and immune system functionality. These factors have already been demonstrated to be adversely impacted by psychological stress, which is why we aimed to investigate whether SCFAs could alleviated stress-induced deficits, with particular emphasis on its consequences for brain physiology and behaviour. Mice were supplemented with a mix of the three principal SCFAs (ie, acetate, propionate and butyrate) in drinking water, starting 1 week before commencing psychosocial stress. After 3 weeks of psychosocial stress, animals were subjected to behavioural analysis assessing intestinal permeability, stress-responsiveness and anxiety- and depressive-related behaviour. We have found that SCFAs alleviated a stress-induced increase in in vivo intestinal permeability, stress-induced deficits in anhedonia in the female urine sniffing test, an increased stress-responsiveness in the stress-induced hyperthermia test, and heightened levels of corticosterone in response to a stressor as measure of increased HPA-axis reactivity. Subsequent analysis of colonic and brain tissue for gene expression by qrtPCR and neurotransmitter levels by HPLC showed corresponding changes in markers for colonic barrier permeability and brain signalling in the mesolimbic pathway. In conclusion, we have found that SCFA supplementation to mice undergoing psychosocial stress alleviates deficits in stress-responsiveness, anhedonia and intestinal permeability.

Policy of full disclosure: None.

253 | The influence of the multicomponent herbal preparation STW5 on intestinal inflammation and motility

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The multi-herbal preparation STW5 is a clinically proven drug to ameliorate symptoms of functional gastro-intestinal diseases such as irritable bowel syndrome. Its mechanisms of action include anti-inflammatory and motility-modulating effects. Both effects might be mediated by the enteric nervous system. We therefore investigated both isolated myenteric plexus and complete gut segments after induction of both spastic and inflammatory stimuli. In the present study, an in-depth investigation of the impact of STW5 on intestinal inflammation and motility was examined in the small intestine of adult mice. For that purpose, a proinflammatory cytokine mixture (IL1ß, TNFα, IFNγ) was injected into the supporting blood vessel arcades of small intestinal segments, to measure the intestinal motility under systemically induced inflammation. In order to evaluate the anti-inflammatory effect of STW5 in these intestinal segments, the cytokines in the gut wall homogenates were analyzed by Multiplex-ELISA. Cultures of myenterix plexus were likewise treated with cytokines and STW5. A reduced contractile activity of perfused intestinal segments could be demonstrated as soon as an inflammation was induced. Analysis with Multiplex-ELISA showed a higher amount of the proinflammatory cytokines G-CSF, IL-6 and LIF under inflammatory conditions in both gut wall homogenates and myenteric plexus cultures. Treatment with STW5 leads to lower cytokine concentrations in both gut wall homogenates and myenteric plexus culture supernatants. Myenteric plexus cultures that were treated with cytokines showed less varicosities.

Interestingly, the amount of varicosities increased when cultures were also treated with STW5. Moreover, we could observe morphological changes in enteric glia, whenever STW5 was applied. These results confirm the efficacy of STW5 as an anti-inflammatory drug. In conclusion, the multicomponent herbal drug STW5 shows in addition to its spasmolytic effects also prokinetic effects in perfused gut segments. There is evidence that this effect is, at least partly, mediated by the ENS, based on the decrease of proinflammatory cytokines released from the ENS.

Policy of full disclosure: None.

254 | A GLP-1 mimetic alleviates irritable bowel syndrome-like symptoms in the Wistar Kyoto rat

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Objectives: Irritable Bowel Syndrome (IBS) is a common functional bowel disorder affecting approximately 10%-20% of the population and is characterised by abdominal pain, altered bowel habit and bloating. Although IBS pathophysiology remains elusive, dysfunctional endocrine signalling and immune activation are implicated. Glucagon like peptide-1 (GLP-1) is an incretin hormone with reported antispasmodic and pain relieving effects in IBS patients. We aimed to assess the effects of the GLP-1 mimetic, Exendin-4 (Ex4), on IBS-like symptoms in the Wistar Kyoto (WKY) rat model of IBS.

Methods: Male WKY rats (n=12 per group) received an intraperitoneal (IP) injection of Ex-4 (1.0 mg/kg, 1 hour prior to behavioural assessment) or Ex-4 with anti-IL-6 receptor monoclonal antibodies (0.5 mg/kg, twice weekly IP injections) over 3 weeks and were compared to saline-treated WKY controls. Stress-induced defecation was assessed using an anxiogenic open field arena and visceral pain sensitivity was assessed by monitoring pain responses to colorectal distension (CRD)(0 mmHg-80 mmHg over 8 minutes). Inflammatory markers in plasma samples from each animal were examined using an ELISA bio-assay.

Results: WKY rats treated with Ex-4 exhibited decreased stress-induced defecation ($P<.001$) and decreased numbers of pain responses ($P<.05$) during CRD as compared to saline-treated WKY rats. WKY rats receiving Ex-4 and xIL-6R entered the inner exposed circle of the open field arena fewer times than untreated WKY rats ($P<.05$). Plasma analysis demonstrated that Ex-4 treated WKY rats had decreased circulating IL-2 ($P<.05$) and increased IL-10 ($P<.05$) in comparison to control WKY rats.

Conclusions: Peripheral administration of a GLP-1 mimetic has beneficial effects on bowel symptoms in the WKY rat model of IBS. Both the number of pain responses observed and stress-induced defecation were improved. However, treatment with the GLP-1 mimetic and xIL-6R increased anxiety-like behaviour in the stress-sensitive WKY model. The findings support a potential therapeutic role for GLP-1 in the treatment of visceral pain and altered bowel habit in IBS.

Policy of full disclosure: None.

255 | Acid ion channel 4 and 5-hydroxytryptamine receptors may modulate pain sensations: Results from a rat model of gastro-esophageal reflux disease

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Objective: Pain is a prominent symptom of gastro-esophageal reflux disease (GERD) and commonly related to acidic reflux. The mechanisms of acid induced activation in the esophageal afferent nerves are, however, not well understood. Serotonin, the ligand for 5-hydroxytryptamine receptors (5-HTR), was recently discovered to activate acid sensing ion channels. We analysed in our rat model the transcript expression of acid sensing ion channels (ASICs), of 5-hydroxytryptamin receptor (HTR) subtypes, of ASIC accompanying mechanotransducers (matrilin-2, stomatin, tubulins) and of the serotonin binding protein (SERT) in the esophageal tissue.

Methods: We established a subchronic model of GERD. Rats were pretreated with either the herbal multicomponent mixture STW5 (0.5 or 2 mL/kg), a multicomponent herbal preparation, or the proton pump-inhibitor (PPI) Omeprazole (O) (30 mg/kg). Esophagitis was induced surgically followed by a further 10d treatment. On day (d) 10 animals were sacrificed. RNA was isolated from defined tissue areas of the esophagi for Agilent whole genome microarray (rat). Data were analysed by Ingenuity®.

Results: Tissues of animals suffering from GERD showed a small, but significant increase in the expression of the ASIC-subtype 4 (3.8fold (f)), of HTR2A (3f), HTR2B (6.6f), HTR7 (9.3f) ($P<.001$), of stomatin (3.1f), of tubulin 6 (2.6f) ($P<.0001$) and a downregulation of SERT (−2.4f, $P<.001$) compared to "normal" tissue. In tissues of animals treated with either STW5 (2 mL/kg) or with O (30 mg/kg), the by inflammation increased ASIC 4 was down regulated (−4,8f, −4f, $P<.0001$) like HTR2A (−5.4f, −3.9f) HTR2B (−7.9f, 3.7f) and HTR7 (−15.4f, −6.8f), stomatin (−4.8f; −2.6f) as well as tubulin 6 (−2.8f; −2.1f) ($P<.0001$). The downregulated SERT was upregulated again by STW5 and O.

Conclusion: Data further support our hypothesis that ASIC4 and 5-HTR-subtypes in the esophageal mucosa play a role for the fast pain relief in responders to PPIs and to STW5. Both receptor types may form a communication network involved in pain signaling.

Policy of full disclosure: Disclosure of Interest: A. Shcherbakova: Financial support for research: DAAD-Scholar, H. Abdel-Aziz: Financial support for research: Received financial support for research from Steigerwald Arzneimittelwerke GmbH, Conflict with: joined Steigerwald Arzneimittelwerke GmbH as employee, O. Kelber Conflict with: employee of Steigerwald Arzneimittelwerke GmbH, G. Ulrich-Merzenich Financial support for research: Received financial support for research from Steigerwald Arzneimittelwerke GmbH (today Bayer Consumer Health).

256 | Isolation of human gastric interstitial cells of Cajal

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Objective: Interstitial cells of Cajal (ICCs) facilitate mammalian digestion through generation of electrical slow waves (O'Grady et al, Gastroenterol 2012:143:589-98). Dysfunctional ICCs are associated with symptoms of gastroparesis such as nausea (Angeli et al., Gastroenterol 2015;149.1:56-66). To help understand their pathophysiology we now describe a method for isolating ICC-enriched populations from human stomach.

Methods: Human stomach was obtained at surgery for obesity, with informed consent. Fundus or antrum (mucosa removed) was digested in a dissociation medium containing collagenase-D to generate single cell suspensions. Antibodies against cell surface epitopes for the ICC markers tyrosine protein kinase Kit (c-Kit) and anoctamin-1 (Ano-1) were utilised in fluorescent-activated cell sorting with a viability dye. RNA quality and concentration was assessed using a bio analyser. To determine expression of c-Kit, Ano-1 and Myosin Heavy Chain 11 (MYH11), a marker of smooth muscle, qPCR was undertaken using GAPDH as a housekeeping gene.

Results: Preliminary experiments indicated degradation of the c-Kit epitope during digestion (absence of signal in Q4, Figure), so further analysis used only the Ano-1 positive cells (Q1, Figure). In the gastric antrum, for example, between 50 000-200 000 Ano-1 positive cells were collected from ~10 to 20 g of tissue (n=3 patients). RNA from the Ano-1 positive population yielded a positive GAPDH signal in PCR reactions with an RNA integrity number >7.5 (n=2 patients). Both Ano-1 and c-Kit transcripts were detected, along with MYH11 (n=3 patients). Therefore the current aim is to further purify, selecting for ICC-specific gene expression.

Conclusion: Once optimised, changes in human ICC gene expression can be interrogated for the first time, in different patient populations and stomach regions.

Policy of full disclosure: This work is funded by Takeda and GlaxoSmithKline.

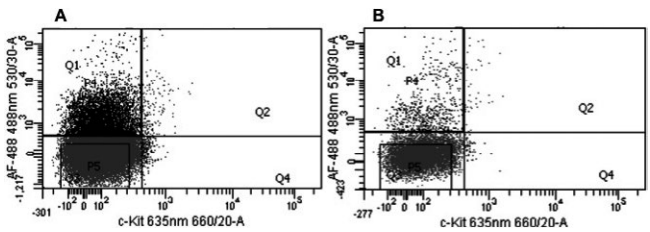

FIGURE 1 Collagenase-digested antrum stained with cKit-APC and Ano1-AlexaFluor488 (A) and isotope control (B) antibodies

LUMINAL SIGNALLING/FOOD ALLERGIES AND INTOLERANCES

257 | Self-reported food intolerance in Korean patients with Irritable Bowel Syndrome (IBS)

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Background/Aims: The majority of irritable bowel syndrome (IBS) patients attribute their symptoms to food, thus dietary intervention is considered to be an important option in the treatment of IBS. However, the food-related gastrointestinal (GI) symptoms in Korean patients with IBS have not been well evaluated. The aims of this study were to investigate the prevalence of food intolerance in Korean IBS patients and to determine the Korean food items and food groups perceived by patients to worsen GI symptoms.

Methods: A total of 101 IBS patients, 167 symptomatic non-IBS subjects, and 125 control participants were enrolled in the study. All subjects completed a questionnaire that assessed the occurrence of GI symptoms after the intake of 119 Korean food items and also the validated Rome III questionnaire for IBS. Symptom severity was assessed using the IBS symptom severity score.

Results: More patients in the IBS group and the symptomatic non-IBS group reported food-related GI symptoms compared with the control group (79.2% vs 74.3% vs 44.8%, $P<.001$). The number of food items causing GI symptoms was also highest in patients with IBS (mean 9.3, $P<.001$). IBS patients reported that the most problematic food groups causing GI symptoms were fatty foods (25.0%), flour-based foods (23.8%), spicy foods (15.0%), and dairy products (15.0%). The most common Korean food items causing GI symptoms in IBS patients was milk (34.7%), followed by Korean ramen (27.7%), black bean-sauce noodles/Chinese-style noodles with vegetables and seafood (24.8%), and pork belly (24.8%). In IBS patients, 49.5% reported GI symptoms related to at least one of the food items with fermentable oligo-, di-, monosaccharides, and polyols (FODMAPs). With increasing IBS symptom severity, patients reported more food items causing GI symptoms ($P=.020$). No significant association was found between IBS subtypes and the food related GI symptoms.

Conclusions: A large proportion of Korean IBS patients complained of food intolerance to certain food items, with fatty foods, flour-based foods, spicy foods, and dairy products being the main triggers. These data provide a basis for dietary recommendations for Korean IBS patients.

Policy of full disclosure: None.

258 | Effect of soluble mediators from Staphylococcus aureus on intestinal epithelial function and sensory signaling

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Enteritis is a known risk factor for the development of long-term visceral hypersensitivity. The resulting inflammatory response is thought to sensitise afferent nerves. Pathogens like Staphylococcus aureus however also produce mediators that directly activate sensory neurons. This and the observation that gut infection is frequently associated with pain and discomfort raised the question whether soluble mediators also affect intestinal nerves. Supernatants containing soluble mediators were produced from various strains of Staphylococcus aureus (SSA) and applied to sensory nerves in an ex-vivo preparation of mouse distal intestine. Bath-application of SSA from strain JE2 (20% v/v) transiently increased afferent nerve activity before it decreased below baseline. This inhibitory action was alleviated in strains producing different arrays of soluble mediators. It was not observed when SSA were boiled or perfused through the lumen. The effect of SSA on epithelial function was studied in Ussing Chambers. Basolateral application of SSA induced an early increase in short circuit currents and attenuation of electrical field stimulation-induced secretion. The early response was not sensitive to inhibitors of neuronal activity (tetrodotoxin, ω-conotoxin, capsaicin) or antagonists of serotonin (cilansetron) and ATP receptors (PPADS) and this suggests a direct effect of SSA on epithelial secretion. In conclusion, these results indicate that soluble mediators produced by Staphylococcus aureus affect the activity of intestinal sensory nerves and also have pro-secretory actions on the intestinal epithelium. Future investigations aim at understanding the mechanisms of nerve inhibition and its relevance for physiology.

Policy of full disclosure: None.

259 | Nutrition characteristics and impact on life quality in Irritable Bowel Syndrome (IBS): An example of Turkey

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Introduction and Aim: Irritable Bowel Syndrome (IBS) is a functional disorder characterized by pain/abdominal discomfort, bloating and changes in defecation. Symptoms can adversely affect quality of life. This a descriptive study which aimed to investigate nutrition characteristics and the impact of nutrition on life quality in IBS patients.

Materials and Methods: The research was conducted with 40 volunteer IBS patients at a tertiary center gastroenterology outpatients clinic. Demographic data, body mass index (BMI), smoking, alcohol consumption, use of laxatives and herbal medicine were recorded. Irritable Bowel Syndrome Life Quality Questionnaire (IBS-QOL) with subscales for dysphoria, activity, body image, health concern, food avoidance, social reaction and sexuality and IBS Individual Identification Form were used for data collection.

Results: The definitive characteristics of the patients were as follows: 22 female, 18 male patients; 51.2% of them were primary school graduates, 69.8% of them were married and 53.5% of them had a moderate financial status. 23.3% of them were smoking,

Table: Distribution and Relation of IBS QOL and Its Subscales by Descriptive Characteristics (n: 40)

	Dysphoria		Activity		Body Image		Health Concern		Food Avoidance		Social Reaction		Sexuality		Social Relationship		TOTAL SCORE	
$\bar{X}$± S.S.	29.3 ± 8.02		24.52 ± 6.89		14.87 ± 4.39		10.00±3.45		10.02±3.32		14.3±4.15		8.15±2.44		11.6±3.21		122.52±31.44	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Sex	-1.608	0.108	-0.654	0.513	-1.833	0.067	-1.683	0.092	-1.797	0.072	-0.971	0.331	-0.663	0.507	-1.663	0.096	-1.373	0.170
Socio-economic Situation	4.648	0.199	3.170	0.366	2.482	0.479	4.277	0.233	1.583	0.663	4.392	0.222	4.633	0.201	2.634	0.452	3.988	0.263
Smoking	1.900	0.387	0.772	0.680	0.686	0.710	0.489	0.783	0.897	0.639	1.539	0.463	2.310	0.315	0.049	0.976	**0.911**	**0.634**
Alcohol Use	3.936	0.140	2.491	0.288	5.018	0.081	3.289	0.193	2.891	0.236	1.665	0.435	2.407	0.300	4.510	0.105	**3.801**	**0.150**
Using Herbal Medicine	**-2.029**	**0.042**	-0.922	0.356	-0.782	0.434	-1.440	0.150	-1.279	0.201	-1.831	0.067	-1.020	0.308	0.868	0.385	**-1.636**	**0.102**
Coffee Consumption	-0.357	0.721	-0.754	0.451	-0.303	0.762	-0.661	0.509	-0.566	0.571	-0.798	0.425	-1.393	0.164	-0.650	0.516	**-0.588**	**0.556**
Using Laxatives	-1.853	0.064	-1.130	0.259	-1.770	0.077	-1.338	0.181	-1.729	0.084	-1.860	0.063	-0..933	0.351	-1.846	0.065	**-1.804**	**0.071**
BMI	-0.027	0.868	0.012	0.939	-0.146	0.370	-0.193	0.234	-0.092	0.572	-0.156	0.338	0.308	0.053	-0.055	0.735	-0.075	0.647

16.3% used to smoke, 12.5% of them were using herbal medicines, 57.5% of them consumed coffee and 10.0% of them used laxatives. 28.7% of them drank alcohol, 2.3% stopped drinking and mean BMI was 27.42±4.97. IBS-QOL scores in general were good ((X) :122.52±31.44). One of the subscale scores namely "food avoidance" was 10.00±3.32 (min: 3-max: 15) were found as moderate. No significant relation was found with IBS-QOL general and subscale averages with smoking, coffee and alcohol consumption, BMI and use of laxatives ($P>.05$). A negative correlation was found between use of herbal medicines and IBS-QOL subscale dysphoria (ZMU: −2.029, $P<.05$); but no relation was found with other subscales (Table).

Conclusion: It is known that quality of life in IBS patients is decreased due to the disorder per se. No impact of BMI, smoking, alcohol consumption, use of laxatives and herbal medicine on quality of life could be found. Studies with wider population and other nutritional elements should be conducted.

Policy of full disclosure: None.

260 | Role of oxidative stress and TRPA1 channels in oxaliplatin-induced gastrointestinal disturbances

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Nausea and vomiting are among the most common and severe side effects in oxaliplatin-treated patients. It has been proposed that disruption of enterochromaffin (EC) cells in the gastrointestinal (GI) tract results in massive 5-hydroxytryptamine (5-HT) release that targeting 5HT3 and 5HT4 receptors in terminals GI sensory neurons triggers nausea/vomiting. However, the pathway through which oxaliplatin exposure leads to EC disruption and the massive 5-HT release is still poorly defined. Transient receptor potential ankyrin 1 (TRPA1) channel is a cation channel highly expressed in nociceptors and in other non-neuronal cells, including EC cells. A functional TRPA1 channel has been documented in EC cells, whose activation causes 5-HT release. TRPA1 in nociceptors is known to be activated by oxidative stress byproducts. As oxaliplatin is known to produce a remarkable generation of oxidative stress byproducts, we hypothesized that oxaliplatin-induced oxidative stress could activate/sensitize TRPA1 in EC cells. By pharmacological tools, we investigated whether oxidative stress activates TRPA1 in EC cells. Additionally, we examined the effect of oxaliplatin on in vivo GI motility changes and PICA behavior (indicative of nausea linked-behavior) to set up an in vivo mice model to investigate our hypothesis. We demonstrated that similarly to a selective TRPA1 agonist, allyl isothiocyanate (AITC), oxaliplatin contracts isolated strips of guinea pig ileum. These responses were attenuated by the selective TRPA1 antagonist, HC-030031. Moreover, we showed that also H2O2 produces a concentration-dependent contractile response in guinea pig ileum, and this effect is abated by HC-030031. Oxaliplatin (3 mg/kg, i.p.) treatment in mice increased upper and lower GI motility as well as induced PICA behavior. Thus, results support our hypothesis and we propose that oxaliplatin, by generating oxidative stress byproducts, activates/sensitizes TRPA1 in EC cells, thereby releasing 5-HT to contracts the ileum. Oxaliplatin increased GI motility and pica behavior in mice indicates phenomena reminiscent of the GI disturbances experienced by patients treated with oxaliplatin.

Policy of full disclosure: None.

261 | Gluten intolerance in Korean patients with Irritable Bowel Syndrome (IBS)

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Objective: The most of Irritable bowel syndrome (IBS) patients attribute their gastrointestinal (GI) symptoms to food. The gluten is one of the most common ignite and constant the IBS symptoms. However, gluten related symptoms has not been evaluated, the aims of this surveillance were to investigate the prevalence of gluten related Gluten symptoms in Korean IBS patients and to determine the characteristics of them.

Methods: A total 80 IBS group and the symptomatic non-IBS group (124 persons), and 56 control participants were enrolled in the study, All subjects completed a questionnaire that assessed the IBS symptoms with validated Rome III questionnaire for IBS and food. Symptoms severity was assessed using the IBS symptom severity score.

Results: Gluten contained food is the most problematic food (control 28.6%, symptomatic non-IBS 20.4%, IBS 23.8% of patients). However the prevalence is similar with fatty foods(control 28.6% vs 16.1%, symptomatic non-IBS 20.4% vs 21.0%), IBS 23.8% vs 25.0%). Proportion of subjects with GI symptoms related to intake of gluten contained food is significantly higher than control (control 15.2% vs symptomatic 41.7%, esp. symptomatic non-IBS 40.7%, IBS 43.6%). Gluten-induced GI symptoms were reported various and gas related symptoms was most common symptom (gas distension/bloating 39.1%, Epigastric fullness 30%, loose stool 10.3%, abdominal pain 8.3%). 6.3% of IBS and 3.2% of symptomatic non-IBS persons were reported very similar symptoms with non-celiac gluten sensitivity.

Conclusion: Gluten contained food is most common food induced GI symptom. It is suggests that there is a possibility of non-celiac gluten sensitivity in S. Korea.

Policy of full disclosure: None.

262 | Better response to low FODMAP diet in JH negative patients with disorders of gut-brain interaction

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Objective: Introduction Previous studies have shown a reduction of gastrointestinal (GI) symptoms in patients with disorders of gut-brain interaction (FGID) when following a diet low in FODMAPs. In addition, there is evidence for an association between GI symptoms and joint hypermobility (JH). However, there is no clear data regarding response rates to a diet low in FODMAPs in patients suffering from JH. In this study we aimed to analyze the response to a diet low in FODMAPs in JH+ and JH− patients with FGIDs. Methods Data of patients presenting with FGID at our functional bowel clinic between 01/2015 and 07/2016 were analyzed. FGIDs were diagnosed according to Rome III criteria. JH was assessed by physicians using Beighton score and rated positive for scores ≥4/9 points. Patients received professional nutritional counseling on a diet low in FODMAPs. A global symptom response was assessed by a professional nutritionist after 4-6 weeks following a low FODMAP diet. Results Of all 84 patients screened for JH, 62 (73.8%) were female and 22 (26.2%) were male. Median age was 35 [range 18-79] years. Females were more likely to exhibit JH compared to males (38/62 [61.3%] vs 6/22 [27.3%]; *P*=.006). Global symptom response rate to a diet low in FODMAPs was 64/84 (76.2%). Our data showed significantly better response to a low FODMAP diet in JH negative patients than in JH positive patients (36/40 [90.0%] vs 28/44 [63.6%], *P*=.005, ITT). Response of 7 patients was unknown because of early therapy discontinuation before nutritional re-counseling. When excluding 7 patients with therapy discontinuation from our calculations, the difference in diet response between JH negative and JH positive patients remained significant (36/39 [92.3%] vs 28/38 [73.7%]; *P*=.036) Table 1. Discussion Our data indicate an association between global symptom response to a diet low in FODMAPs and JH status in FGID patients. An underlying structural pathology or different pathophysiological factors (motility, intestinal permeability) causing GI symptoms in JH+ patients and limiting response to low FODMAP diet should be considered. Our findings represent a further step towards pathophysiological features in FGIDs and might help to select patients for individually appropriate therapies.

Policy of full disclosure: None.

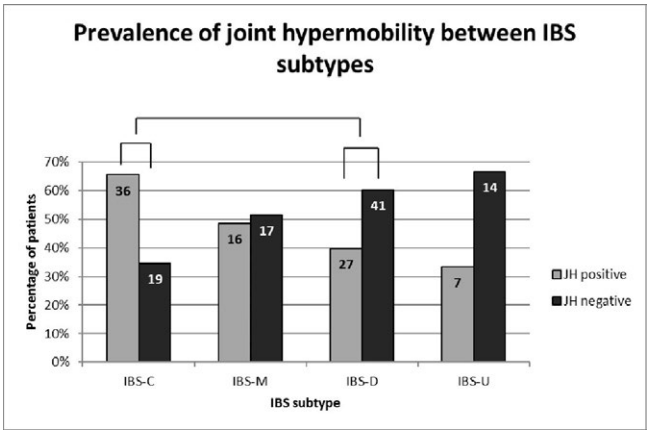

FIGURE 1

263 | Associations between microbiota, colonic volume and breath gasses during a low FODMAP diet

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Objective: Ingestion of poorly digested carbohydrates can induce functional gastrointestinal symptoms, which may be relieved by a low FODMAP diet. How the dietary changes alter intestinal microbiota and gut function remains unclear. The aim of the study was to test whether a low FODMAP diet would affect colonic volume (CV), and whether adding a FODMAP supplement would abolish this effect. The microbiota was assessed to explore its relationship with CV, breath gasses and diet.

Methods: 37 healthy subjects followed a low FODMAP diet for 7-days also taking a daily supplement of either oligofructose (OF,n=19) or maltodextrin (MD,n=18). CV and transit were assessed by MRI pre- and post-intervention, as were fasting breath hydrogen (H2) and methane (CH4). Stool was collected for microbiota analysis by 16S MiSeq sequencing.

Results: There was an increase in CV in both groups and the breath H2 levels were significantly increased in OF group and degreased in MD group after intervention. There was a significant drop in the in the total microbial load after the intervention in the MD compared to the baseline. Microbial taxa from the phyla Clostridia was decreased where as Actinobacteria were increased in the OF group compared to the baseline. The decline of Lachnospiraceae was associated with increase in CV. The FODMAP diet had a pronounced effect to the breath gases, 72% of MD group lost ability to emit H2, whereas in OF group 71% of the subjects retained their ability to emit H2. This change in the breath gasses could be associated with other health parameters such as microbial diversity, SCFA levels and CV.

Conclusion: Increase in Actinobacteria and H2 confirm previous reports after OF or the low FODMAP diet. The increase in certain taxa after MD may reflect the effect of polysaccharides included in the low FODMAP diet. Larger CV was associated with specific bacteria as well as parameters suggesting bacterial fermentation. This study is the first able to compare changes in microbiota, CV and breath gasses in response to FODMAP intervention. Concurrent assessment provides

insights into the relationship between microbiology and physiology, and the interacting mechanistic effects of therapy.

Policy of full disclosure: None.

MIXED TOPICS II

264 | Clinical characteristics of functional dyspepsia depending on chemosensitivity to capsaicin

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Introduction: Chemical hypersensitivity has been demonstrated in patients with functional dyspepsia (FD). The capsaicin capsule test has been established as a non-invasive test of chemosensitivity and has good reproducibility and negligible placebo response (Führer et al., NGM 2011). We studied whether basal clinical characteristics as well as daily gastrointestinal symptoms differ in patients with and without chemical hypersensitivity.

Methods: N=49 outpatients with confirmed FD received the capsaicin sensitivity test with 0.75 mg capsaicin at two occasions 1 week apart, Symptomatic response to the capsaicin sensitivity test allowed stratification of patients to capsaicin positive (chemosensitive) and capsaicin negative (not chemosensitive) patients, an aggregate perception score above 9 after capsaicin ingestion was considered a positive test. Graded symptom diaries were filled out at bedtime, median scores (25%/75% CI) were given, a *P*-value <.05 was considered significant, NS=not significant, HAD=Hospital Anxietyand Depression Scale, SF12=ShortForm 12 Quality of Life Scale.

Results: Aggregate perception scores at the first (11.0 (3.0-17.0)) and second capsaicin test (10.0; 3.0-14.0) were similar (*P*=.15). In both tests 26 patients (53%) had a positive test and 23 (47%) had a negative test. NcNemar Test for marginal homogeneity was *P*=1.0, Crohnbach alpha was 0.85. Basal clinical characteristics such as age, gender, FD subgroup and concomitant IBS, quality of life (SF12), and anxiety and depression (HAD) did not differ between hypersensitive and non-hypersensitive patients (NS). Daily symptom scores of epigastric pain, nausea and distension were comparable between groups (NS), but satiety (*P*<.001) and epigastric bloating (*P*<.01) was significantly more intense in chemosensitive than in capsaicin negative patients (overall upper abdominal symptom scores *P*<.05). Lower abdominal pain, distension, meteorism and satisfaction with bowel movement were similar in both groups (NS) during the observation week.

Conclusion: Clinical characteristics are comparable in chemosensitive and non-chemosensitive FD patients, but some upper abdominal symptoms were more intense in chemosensitive patients, while lower abdominal symptoms did not significantly differ between chemosensitive and non-chemosensitive patients. Good reproducibility of the capsaicin sensitivity test was confirmed.

Policy of full disclosure: None.

265 | Clinical symptoms in children and adolescents with functional abdominal pain disorders in relation to the Laktose Breath-H2-Test

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Objective: Lactose malabsorption is generally considered as a possible cause of chronic abdominal pain in children. However, lactose malabsorption and abdominal symptoms correlate poorly. The AIM was to evaluate whether lactose malabsorption or rather lactose intolerance correlates with clinical symptoms in children with chronic abdominal pain.

Methods: A lactose BHT with 20% lactose solution, 2 g/kg body weight (up to 50 g) was performed in 59 patients (age: 6-17 years; 26 male, 33 female) with functional abdominal pain disorders. Clinical symptoms were quantified by a faces graded scale (0=no symptom, 5 extreme symptom) evaluating severity of pain, nausea, meteorism, flatulence and diarrhea in the 4 weeks before the BHT. An overall symptom score was calculated by adding each single score. Definition of malabsorption: increase of breath-H2 concentration ≥20 ppm over baseline. Symptoms during BHT were ascertained for up to 9 hours using a validated faces graded scale. Definition of lactose intolerance: increase of one or more symptoms by 2 points or more over baseline. Median (25th/75th percentile) are given, *P*<.05 was considered significant.

Results: 16 out of 59 were lactose malabsorbers, 20 patients were lactose intolerant. Among the malabsorbers, 10 were intolerant and 6 were not. Clinical symptom scores were 5.0 (3.5/6.5) in malabsorbers and 6.75 (4.0/10.5) in lactose-absorbers (NS). Patients with lactose-malabsorption had comparable clinical symptoms, independent whether they were lactose-intolerant or not. In contrast, lactose-absorbers had significantly higher overall clinical symptom scores if they were lactose-intolerant as compared to lactose-tolerant absorbers (*P*<.01); nausea (*P*=.01), meteorism (*P*<.05) and flatulence (*P*<.05), but not pain and diarrhoea (NS) were scored significantly higher in lactose-intolerant absorbers than in lactose-tolerant absorbers. Lactose-intolerant malabsorbers had significantly lower clinical symptom scores than lactose-intolerant absorbers (overall score: *P*<.01; nausea *P*<.05, meteorism *P*<.01 and flatulence *P*<.05, pain NS, diarrhoea NS), while lactose-tolerant patients had comparable clinical symptoms, no matter whether they absorbed or malabsorbed lactose (NS).

Conlusion: Paediatric patients with functional abdominal pain who can absorb lactose but are lactose-intolerant during the BHT have more severe clinical symptoms as compared to lactose-malabsorbers—no matter whether the latter react sensitive to lactose or not.
Policy of full disclosure: None.

266 | Submucous plexus neurons are mechanosensitive

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Objective: We recently described mechanosensitive enteric neurons (MEN) in the myenteric plexus which responded to normal stress stimuli. Since distension evoked, nerve mediated chloride secretion occurs in submucosa/mucosa preparations we postulate the existence of MEN also in thesubmucous plexus (SMP).
Methods: Neuroimaging experiments were performed in freshly dissected guinea pig colonic SMP preparations to record neuronal activity with the voltage sensitive dye DI-8-ANEPPS. As mechanical stimuli we used intraganglionic volume injection (compressive stress) of physiological solution and ganglionic stretch (tensile stress).
Results: 14% of SMP neurons responded to intraganglionic volume injection (n=88 neurons) with spike discharge of 2.3±1.1 Hz in a reproducible manner. The spike frequency significantly increased with higher injection pressure or duration by 45% or 330%, respectively. Neurons typically responded during the dynamic phase of mechanical deformation and adapted thereafter. In contrast, neurons responding to ganglionic stretch (1.6%, n=181 neurons) fired throughout the distension stimulus. None of the stimuli produced inhibition as the frequency of spontaneous spike discharge remained unchanged.
Conclusion: We demonstrated the existence of SMP neurons which responded to mechanical stress stimuli. They primarily respond to the dynamic phase of a compressive stimulus but encode a distension stimulus without adaptation. These features are relevant for reflex control of epithelial functions.
Policy of full disclosure: None.

267 | Sensitivity of enteric neurons to osmotic stimuli

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Background and Objective: Neurons of the enteric nervous system (ENS) are located inside the gut wall, which allows them to sense changes in their microenvironment. Amongst those are fluctuations in osmotically active molecules during digestive and interdigestive periods. Our hypothesis is that enteric neurons of the submucosal plexus, which are strategically located close to epithelial cells and blood vessels may sense and respond to shifts in osmolality.
Methods: We used neuroimaging techniques with a voltage sensitive dye to record spike discharge in freshly dissected guinea pig colonic preparations. As a stimulus, we briefly exposed individual ganglia to hypo- or hyper-osmolar stimuli (94 mOsm/kg-494 mOsm/kg) using a micro perfusion system. In addition, we analysed the volume change of enteric neurons during osmotic shifts. The involvement of TRPV4 was investigated using qRT-PCR and pharmacology with the agonist GSK1016790A and the antagonist HC-067047.
Result: 13% of submucosal neurons responded to the application of a hypoosmolar stimulus (94 mOsm/kg) with a peak spike frequency of 6.5 [2.8/9.3] Hz which was associated with a 8.5±4.2% decrease in cell surface. About 8% of submucosal neurons responded to a hyperosmolar stimulus (494 mOsm/kg) with a lower peak spike frequency of 0.5 [0.0/1.0] Hz. Among the likely osmosensors is TRPV4 which is supported by the finding that TRPV4 activation caused spike discharge in 15.0 [7.5/24.5]% of submucosal neurons. In the presence of the TRPV4 antagonist the proportion of hypoosmosenstive neurons significantly decreased to 5%. Furthermore the presence of TRPV4 in the submucosal plexus could be verified at mRNA level.
Conclusion: Submucosal neurons respond to hypoosmolar as well as hyperosmolar stimuli. This suggests that the ENS is capable of sensing changes in osmolality and providing adequate responses to them. Results strongly indicate involvement of TRPV4 in sensing hypoosmotic stimuli.
Policy of full disclosure: None.

268 | Association between gastroesophageal reflux disease and paroxysmal non-valvular atrial fibrillation

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Objective: The aim of this study was to evaluate the association between gastroesophageal reflux disease (GERD) and paroxysmal non-valvular atrial fibrillation (AF). Inflammation induced by esophagitis or higher atrial vulnerability due to heart rate variability decreasing seems to explain this association.
Material and method: A prospective case-control study was conducted at a tertiary care center from North-East of Romania over a period of 13 months (July 1, 2014-July 31 2015). We included patients older than 18 years diagnosed with paroxysmal non-valvular AF and GERD as study patients and we compared with patients with paroxysmal non-valvular AF and non-GERD as controls. All patients underwent upper endoscopy and heart rate variability in time (SDNN) and frequency (low-frequency (LF)/high-frequency (HF) ratio) domains by 24-hour electrocardiographic Holter monitoring.

Results: The study included 75 patients with AF: 36 GERD patients (55.6% females, mean age 62.44±9.37 years) and 39 non-GERD patients (56.4% females, mean age 60.18±7.98 years); *P*>.05. Esophagitis increased the relative risk of the association of AF and GERD by 2.5 times (*P*<.005). Heart rate variability in terms of time-domain parameter (SDNN) was lower in patients with AF and GERD (*P*<.005). Hypertriglyceridemia was statistically significantly higher in AF and GERD patients than in AF and non-GERD patients (*P*=.050).
Conclusion: These results suggest both the theory of inflammation and of the autonomic nervous system imbalance as a pathophysiological mechanism involved in the association between GERD and AF.
Policy of full disclosure: None.

269 | Combinations of high resolution manometry parameters are useful for diagnosis of gastroesophageal reflux disease

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Background: Esophageal dysmotility and defects of an antireflux barrier are important pathophysiologic mechanisms of gastroesophageal reflux disease (GERD). Aims: We aimed to determine the features of high resolution manometry (HRM) that can be used to diagnose GERD.
Methods: We retrospectively reviewed and compared HRM parameters in consecutive symptomatic patients who underwent endoscopy and impedance esophageal pH testing for diagnosis of GERD, and 23 controls.
Results: Among 167 patients (77 males, mean age, 51 years), 62 were diagnosed with GERD (34 erosive esophagitis and 28 non-erosive reflux disease) and 105 non-GERD. GERD patients had a lower distal contractile integral (DCI) and esophagogastric junction contractile integral (EGJ-CI), shorter abdominal portion of the lower esophageal sphincter (LES), longer LES-crural diaphragm separation, and less frequent type I EGC morphology. We used DCI, EGJ-CI, and EGJ morphology as candidate diagnostic HRM parameters. When DCI was low (≤630 mmHg s cm), and EGC-CI was also low (≤30 mmHg s cm), the diagnostic sensitivity was 94.4% (95% CI, 90.3%-99.7%) and specificity was 59.4% (40.6%-76.3%). When EGJ morphology was not type I, and DCI or EGC-CI were low, the diagnostic specificity was high, 94.9% (87.5%-98.6%)-97.2% (90.3%-99.7%), but sensitivity was moderate, 32.4%-58.8%.
Conclusions: Combination of two of the following HRM parameters: low DCI, low EGJ-CI and absence of EGJ morphology type I, were diagnostic for GERD. EGJ morphology was a specific parameter, while low DCI and EGJ-CI were sensitive parameters. HRM reduces the need for ambulatory pH monitoring in patients whose HRMs show pathognomonic features.
Policy of full disclosure: None.

270 | Prevalence of reflux esophagitis with or without nontuberculous mycobacterial lung disease

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Abstract Background/Aims: GERD (Gastroesophageal reflux disease) is defined as a condition which develops when the reflux of stomach contents causes troublesome symptoms and/or complications. GERD is reported to be associated with several respiratory diseases, including asthma, COPD (chronic obstructive pulmonary disease), and idiopathic pulmonary fibrosis. It may also affect the NTM (nontuberculous mycobacteria) lung infection. Repetitive aspiration of gastric acid in patients with reflux esophagitis (RE) causes chronic inflammation of the bronchial tree and may result in destruction of the normal mucosal barrier of the bronchial epithelium. This study investigated the prevalence of reflux esophagitis in patients with NTM lung disease as compared to a control group (healthy individuals).
Methods: Ninety-three patients with NTM lung disease were included in the study, all of which underwent esophago-gastro-duodenoscopy between January 2011 to December 2012 in our clinic. For the control group, we referred to the multi-center prospective study of Korea in 2008 by Lee and colleagues.
Results: Minimal change disease was included in reflux gastritis in this study. The prevalence of reflux gastritis was 33.3% (31/93) among the 93 NTM patients, which was significantly higher than that of the control group (19.8%, 5062/25 536).
Conclusion: Patients with NTM lung disease showed higher prevalence of reflux gastritis than normal control group.
Key Words: NTM (nontuberculous mycobacteria); Reflux esophagitis; GERD.
Policy of full disclosure: None.

271 | Food related to functional digestive disorders in working age adults

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Background & Aims: Few population-base studies evaluated the role of specific food in symptoms occurrence of functional digestive disorders. The main objective of this transversal study was to explore the association between eating habits, nutritional status and functional digestive pathology.
Methods: The study included a representative sample of working age adults, randomized from the lists of family doctors and the subjects were invited for interview in the doctor's office. Selected subjects were

evaluated for dietary habits using a food frequency questionnaire and the diagnosis was established according to Montreal criteria for gastro esophageal reflux disease (GERD) and Rome criteria for dyspepsia and irritable bowel syndrome (IBS). Socio-demographic factors and objective evaluation of overweight were also included in interview. Results from logistic regression were presented as odd ratios and 95% confidence intervals.

Results and discussions: 158 adults (45.8±13.67 years) finally attended the study. Prevalence was 28.5% for GERD, 7.6% for dyspepsia and 15.19% for IBS, increasing with age ($P<.05$). Obesity (21.5%) was associated with GERD ($P=.033$). The frequency intake of certain foods was significantly correlated with both digestive disorders and nutritional status. Predictors for functional digestive disorders, derived from multivariate analysis adjusted for socio-demographic factors, were the following foods: animal fats for GERD (OR=3.470; IC95%=1.071-11.241; $P=.038$) and IBS (3.058; 1.038-9.011; $P=.043$), processed meat for IBS (11.754; 2.405-57.455; $P=.002$), canned food for GERD (17.260; 3.570-83.441; $P<.001$), dyspepsia (5.735, 1.21-27.09, $P=.028$) and IBS (4.83, 1.70-13.70, $P=.003$), pulses (21.985; 2.514-192.272, $P=.005$) and grain cereals (14.342; 3.680-55.891; $P<.001$) for IBS, cafeteria products for GERD (4.886; 1.493-15.983, $P=.009$) and compotes for IBS (7.140; 2.143-23.789; $P=.001$). Patients with digestive pathology consumed these products more frequently than non-symptomatic subjects. The obese people consumed less frequently vegetable (0.218; 0.060-0.788; $P=0.020$) and grain cereals (0.225; 0.090-0.564; $P=0.001$) than normal weighted subjects.

Conclusion: This study updated prevalence data and reveals a strong association between certain foods and functional digestive disorders. This is a challenge for future research regarding the role of foods in etio-pathogenesis and treatment of these disorders.

Policy of full disclosure: None.

272 | Gastroesophagel reflux disease: A complication of post-corrosive esophageal stricture

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Introduction: Esophageal stenosis caused by ingestion of caustics in childhood, followed by esophageal plasty using stomach or colon is encumbered by major complications at adulthood like gastroesophageal reflux disease.

Material and method: A prospective study was conducted at the Institute of Gastroenterology and Hepatology, Iasi, Romania, between January 2015 and March 2017 and. It included 10 adult patients (50% women, 50% male, mean age: 21±5.62 years old) with a history of postcaustic esophageal stenosis and esophageal plasty with colonic or gastric tube under 18 years old. The patientes were clincally and endoscopic evaluated and the diagnostic of gastroesophageal reflux diseas was based on a GERD-Q score higher than 8.

Results: Of the 10 patients, 5 had plasty with gastric tube (group A) and 5 with colonic tube (group B). Most of the patients in group A were women but male were more pevalent in group B. Except one of the patients with gastric tube, all the other patients came from rural areas. The mean age was 25 years in group A and 20.2 years in group B. The diagnostic of gastroesophageal reflux disease was established at 4 patients (40%), all having esophageal plasty with gastric tube. The 4 patients had beside the typical symptoms of gastroesophageal reflux disease also nocturnal chronic cough. None of the patients declared the chronic consumption of alcohol or tobacco.

Conclusions: Gastroesophageal reflux disease is a common complication of the adult patient who had an esophageal plasty with gastric tube for postcaustic stenosis after accidentally ingestion of caustic soda in childhood.

Key-words: Esophageal stricture, ingestion of caustic, gastroesophageal reflux diasease.

Policy of full disclosure: None.

273 | Can we predict the presence of colonic bubbles with bowel symptoms during colonoscopy?

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Backgroud/Aims: In colonoscopic examination, luminal visibility is frequently limited due to intraluminal bubbles. There is a lack of data about bowel symptoms related to colonic bubbles after bowel preparation. The aim of this study was to assess the association between bowel symptoms and colonic bubbles in patients with colonoscopic examination.

Methods: We performed a prospective study in outpatients undergoing colonoscopies. Bowel preparation was performed using 2L PEG with ascorbic acid. Bubbles were scores as follows: 0, minimal or none; 1, covering at least half the luminal diameter; 2, covering the circumference of the lumen; 3, filling the entire lumen. We asked to participants questionnaire about bowel symptoms such as pain, loose stool, abdominal fullness, bloating, or gas.

Results: A total 45 patients were enrolled (M:F=25:20). Bubble score was as follows: 0 in 16 patients (35.6%), 1 in 14 (31.1%), 2 in 10 (22.2%), and 3 in 5 (11.1%). Mean age was 56.8 years old (32-80). The presence of bubble was not associated with loose stool, bloating, and abdominal pain. The symptoms with abdominal fullness and gas were associated with the presence of colonic bubbles (26.7% vs 73.3%; $P=.009$, 33.3% vs 66.7%; $P=.002$, respectively).

Conclusions: The symptoms with abdominal fullness and gas were possible predictors of colonic bubbles in patients with colonoscopic examination.

Policy of full disclosure: None.

274 | Fecal transplantation in Irritable Bowel Syndrome (IBS): An RCT

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Objective: Irritable bowel syndrome (IBS) is a common condition characterized by abdominal pain, bloating, and poor quality of life. Gut microbial dysbiosis has been suggested as a possible cause of IBS. This double blind randomized trial compared fecal microbiota transplant (FMT) to placebo in patients with IBS (ClinicalTrials.gov Identifier: NCT02154867).

Methods: General practitioners in Northern Norway enrolled subjects with IBS (defined by the ROME III criteria, without dominating obstipation), and randomized to FMT from healthy donors or placebo. Donor transplant was either processed freshly or previously stored frozen; transplantation of own feces served as placebo. The primary endpoint was symptom relief measured with the IBS symptom severity score (IBS-SSS) after 3 months.

Results: Ninety patients were included: 60 (30 fresh and 30 frozen) in the FMT and 30 in the placebo group. At 3 months, 43% in placebo, 50% in fresh FMT, and 79% in frozen FMT groups were responders (>75 point decrease in IBS-SSS); *P*=.013. At 12 months, 36% in placebo, 46% in fresh FMT, and 66% in frozen FMT groups were responders; *P*=.073. Adjusting for other functional comorbidity at baseline demonstrates a clear significant effect of FMT (global *P*=.004; repeated measures ANOVA).

Conclusion: FMT induces a significant symptom relief in patients with IBS. Self reported comorbid functional diseases is a negative predictor to effect of FMT treatment.

Policy of full disclosure: None.

275 | Neurogenic effect of Wnt signalling on murine and human postnatal enteric progenitor cells

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Objective: Neural progenitor cells from the enteric nervous system have been postulated as a putative cell source for the treatment of enteric neuropathies. To use this cell pool for future cell-based therapies it is necessary to understand the different signaling mechanisms that regulate the proliferation and cell specific differentiation. We systematically investigated the influence of canonical Wnt signaling on proliferation and differentiation of cultured enteric progenitor cells from neonate mice and human infants.

Methods: Activation of Wnt-Signaling pathway was performed in cultured murine and human ENS progenitor cells that were characterized by proliferation and cell death assays, immunocytochemistry, Western blot, and qRT-PCR experiments.

Results: In proliferating enterospheres derived from ENS progenitor cells we verified the expression of Wnt-receptors frizzled 1-10 and the co-receptors LRP5 and LRP6. Pharmacological stimulation with Wnt agonists induced an intracellular accumulation of Wnt-dependent β-catenin and upregulated the expression of known Wnt-target genes axin2, lef1, and lgr5. Furthermore, activation of the canonical Wnt pathway enhanced the growth of spheres during cell expansion and strongly increased the number of new-born neurons derived from both, murine and human progenitor cells.

Conclusion: Our results give new insights into the molecular mechanisms of Wnt signaling pathway regulating progenitor cell population of the enteric nervous system. The neurogenic effect of Wnt agonists on ENS progenitors highly encourages the use of such pharmacological agents in the generation of a sufficient cell pool for future autologous cell replacement therapies. Moreover, Wnt-receptors might ease the identification and isolation of neural progenitors from patient biopsies.

Policy of full disclosure: None.

276 | Effects of mosapride in patients with minor disorders of esophageal peristalsis using high-resolution manometry

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Background/Aims: Minor disorders of peristalsis are frequently observed esophageal motility, which may be shown dysphagia or reflux symptoms. Unfortunately, therapeutic options for minor peristaltic disorders are limited and the role of prokinetics has been controversial so far. This study investigated the efficacy of mosapride in patients with minor peristaltic disorders.

Methods: A total of 10 patients with esophageal symptoms, who were diagnosed as minor disorders of peristalsis by gastroscopy and high-resolution manometry (HRM) using the Chicago Classification v3.0, were prospectively enrolled. Patients received mosapride 30 mg daily for 2 weeks. Symptoms assessment including abbreviated version of the World Health Organization quality of life scale (WHOQOL-BREF) and the HRM study were performed before treatment and after 2 weeks.

Results: Complete response (≥80%), satisfactory response (≥50%), partial response (<50%), and refractory response rates were 20.0%, 60.0%, 10.0%, and 10.0%, respectively. Overall response rate (complete+satisfactory) was 80.0%, and overall score of WHOQOL-BREF was significantly increased after treatment (*P*=.025). In the HRM metrics, lower esophageal sphincter (LES) respiratory mean pressure was significantly increased after treatment (*P*=.014). The values of LES length, LES residual pressure, effective swallows, and distal contractile

integral were increased after treatment, however there was no significant difference before and after treatment.

Conclusions: Mosapride improved overall symptoms and increased LES respiratory mean pressure with presenting a tendency to increase LES contraction and esophageal motility in patients with minor peristaltic disorders. However, further large-scale studies are needed to identify the effect of mosapride.

Policy of full disclosure: None.

277 | Influence of comorbidities on self-rated health and health seeking behavior in primary care patients with IBS

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Objective: We investigated to what extent comorbidities influence self-rated health and health seeking behavior among IBS patients in primary health care (PHC) with also gastrointestinal symptom burden, self-rated stress and sense of coherence considered. We hypothesized that certain particular comorbidities and psychological factors are of more importance for perceived health and health seeking behavior than either number of comorbidities or gastrointestinal symptom burden.

Methods: 169 IBS patients in a Swedish PHC setting meeting ROME III criteria completed questionnaires including self-rated health, psychological factors and a gastrointestinal diary. Co-morbidity data and number of PHC contacts during a 5 years period were derived from a population-based Health Care Register. The patients were then divided into those with many PHC contacts and those with less. Extra-intestinal pain diagnoses, depression, anxiety and sleeping disturbances were also identified. The question about self-rated health was dichotomized.

Results: Abdominal pain (OR 1.77, C.I 1.25-2.5), sense of coherence (OR 0.932, C.I 0.88-0.97) and age (OR 1.06, C.I 1.02-1.09) made unique statistically significant contributions to the logistic regression model regarding self-rated health with all factors mentioned above included. Regarding PHC contacts, only number of comorbidities (OR 1,19, C.I. 1,09-1,3) and sleeping disturbances (OR 3,85, C.I 1,08-13,75) made unique statistically significant contributions.

Conclusion: IBS patients with low sense of coherence, intense abdominal pain and higher age had poorer self-reported health. Neither self-rated stress or number or types of comorbidities mattered. However IBS patients with many comorbidities and sleeping disorders in particular seek more health care, regardless of gastrointestinal symptoms and psychological factors.

Policy of full disclosure: None.

278 | Effects of bisacodyl on the human colon muscle: An in vitro study

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Background/Aims: Although bisacodyl has being widely used for constipation, its underlying mechanism of action is still largely unknown. This study aimed to investigate effects of bisacodyl on response of human colon muscle to electrical field stimulation (EFS).

Methods: Sigmoid colon muscle strips (13 longitudinal and 12 circular muscles) were obtained from 13 subjects who underwent colectomy for colon cancer. Isometric force measurements were performed in response to EFS (0.3 ms in trains of 10 Hz for 20 s, 150 V). Peak and nadir (tone) during and after stimulation were measured in control state and after sequential addition of bisacodyl (1 μmol/L), atropine (1 μmol/L), N-nitro-L-arginine (L-NNA, 100 μmol/L), MRS2500 (1 μmol/L) and tetrodotoxin (TTX, 1 μmol/L) to the organ bath.

Results: Transient phasic contractions were observed during stimulation and after cessation of stimulation. In longitudinal muscles, nadir during stimulation and tone after stimulation increased and peak during and after stimulation decreased after addition of bisacodyl. Increased nadir and tone persisted after sequential addition of atropine, L-NNA, MRS2500, and TTX. However, peak decreased further after sequential addition of atropine and L-NNA. On the other hand, effects of biscodyl were not profound in the circular muscles. To confirm a direct action of bisacodyl on the colon smooth muscle, we added TTX before perfusion of bisacodyl in the longitudinal muscle strips (n=4). Pretreatment of TTX abolished EFS-induced phasic contraction but perfusion of bisacodyl increased tone without a contraction.

Conclusions: Our data suggest that a direct action of bisacodyl on the smooth muscle increases the tone of longitudinal muscle in the human sigmoid colon, which is probably involved in colonic propulsion.

Policy of full disclosure: None.

279 | Can symptoms predict findings of high resolution ano-rectal manometry

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Introduction: Constipation is a common problem thought to affect up to 27% of the general population. The health care costs of constipation are significant as evidenced by the hundreds of million euros spent yearly on laxatives alone. We aim to characterise the care pathway and diagnostic & treatment modalities in the care of these patients. HRAM not only allows for measurement of anorectal sphincter function but also allows for a better understanding of the dynamic processes of defecation. Poor toileting behaviour and inappropriate

recruitment of pelvic floor muscle is a significant factor on the pathophysiology of constipation.

Methods: We retrospectively reviewed HRAM tracings of 130 consecutive patients attending our GI Function Laboratory between October 2015 and March 2017. 39 of these patients (5 M, 34 F) presented primarily with features of constipation. We analysed the pattern of simulated defecation as per Rao classification of dyssynergic defecation. In addition we reviewed the medical records of these 23 patients and recorded their primary symptoms.

Results: 39 of 130 patients who attended our laboratory for HRAM were classified as presenting with symptoms of Constipation. Of these 39 patients 87% were female, (mean age 48±17 years). The predominant symptom in these patients was incomplete evacuation (66.6%). Of these 34.6% were categorised with HRAM patterns revealing Rao Type IV Defecatory Dyssynergia, 26.9% RaoType III, 19.2% Rao Type I & 11.5% Rao Type II. 3.8% of patients with symptoms of incomplete evacuation had a normal defecatory pattern on HRAM. In those patients with constipation who did not complain primarily of incomplete evacuation (33.3% disparate symptoms) 38.4% of them were classified as Rao Type IV, 15.4% Rao Type II, 15.4% Rao Type I & 15.4% Rao Type III. One of these patients had a normal defecatory pattern (7.7%) and one had a large rectal prolapse and Rao was not classified.

Conclusion: In this small patient group we find that defecatory dyssynergia is a common finding among those presenting with constipation. Symptoms of incomplete evacuation alone do not solely predict obstructed defecation. In those with disparate symptoms of constipation such as abdominal pain, infrequent bowel movements & pain on defecation defecatory dyssynergia is also a common finding.

Policy of full disclosure: None.

280 | Supra gastric Belching symptoms: Prevalence and associated disease

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Introduction: Supragastric belching (SGB) is considered a behavioral disorder where air is ingested in to esophagus and immediately expelled again; it can result in symptoms of excessive belching and has a significant impact on quality of life. With the advent of the intraluminal impedance recording technique, it has become possible to monitor the passage of air through the oesophagus, either in ab-oral or ad-oral directions.

Aims & methods: Our aim was to determine the prevalence of excessive SGB within our tertiary referral gastrointestinal physiology unit and evaluate the predominant presenting symptoms and examine its association with gastro-esophageal reflux disease and motility disorders. Data was collected retrospectively from our data base system; we reviewed 442 reports of 24-hours PH-Impedance and high resolution oesophageal manometry from January 2015 to December 2016. SGB was defined as a rapid raise in impedance >1000 Ω, moving in an aboral direction, followed by return to the baseline. (i), excessive SGB was defined as more than 13 episodes per 24 hours. (ii) Symptoms were reviewed from patients' charts. These included the predominant presenting symptom, symptoms of acid reflux and heartburn, regurgitation and dysphagia.

Results: Out of 442 report reviewed, a total of 98 (22%) patients were found to have excessive SGB. 71% of these patients were female. The mean age was 45 years. The predominant symptom among these patients was heartburn (55%), but only 18% had pathological acid exposure on the 24 hour pH/Z studies. Dysphagia was reported in 23% and regurgitation in 7%. However, only 7% presented with isolated symptom of belching. Esophageal hypomotility was seen in 48% of patients.

Policy of full disclosure: None.

281 | Severe intestinal dysmotility: Correlation between small bowel manometric patterns and histopathological findings in full-thickness small bowel biopsies

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Objective: The diagnosis of intestinal dysmotility relies on the identification of intestinal manometric pattern abnormalities defined by predetermined criteria and/or neuromuscular findings at histopathology. However the correlation between these two diagnostic approaches remains unknown. Objective of this study was to prospectively compare intestinal manometric abnormalities with histopathological findings in small bowel biopsies.

Materials and Methods: 38 patients (32F; 16-65 years) with severe and chronic symptoms of gut dysmotility were investigated with standard intestinal manometry and full-thickness intestinal biopsy. Mechanical obstruction and macroscopic gut lesions were excluded by a thorough diagnostic workup. Manometric diagnosis was established using previously published criteria (Gut 1987; 28:5-12). Intestinal specimens were processed for evaluation by traditional staining and a panel of immunohistochemical markers for muscle, interstitial cells of Cajal, glia and neurons and classified according to the London classification (Gut 2010; 59:882-7). Manometric concordance was obtained in 35 and their histopathology was compared with manometric findings.

Results: Patients with neuropathic, myopathic or obstructive manometric patterns had abnormal histopathology in 73% of cases. Manometric patterns did not match with the specific neuromuscular findings. Histopathology was abnormal in 62% patients with neuropathic pattern and of these only 23% had an enteric neuropathy, whereas 62% had neuromuscular inflammation and 15% enteric myopathy. All

patients with a myopathic or obstructive pattern had abnormal histopathology, however none of them with signs of enteric myopathy.

Conclusion: Intestinal dysmotility detected by manometry is often associated with abnormal neuromuscular findings in full-thickness biopsies. Although there is no correlation between the manometric patterns and the histopathological findings, an abnormal manometric study predicts a higher probability of abnormal histopathology.

Policy of full disclosure: None.

282 | Antibiotic driven changes in gut motility suggest direct modulation of enteric nervous system

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Background: Ever since oral antibiotics were shown to manage hepatic encephalopathy through modulation of the gastrointestinal flora, there has been interest in establishing a mechanistic basis for the effects of antibiotics on gut-brain signalling. Many animal studies have attributed the neurophysiological and behavioral effects of antibiotics almost entirely to hi-dose antibiotic-induced perturbation of the microbiota. The possibility that these effects occur through direct action of antibiotics on the host nervous system has been relatively neglected. We hypothesize that acute exposure of the gastrointestinal tract to antibiotics directly modulates neuronally-dependent reflexes such as peristalsis.

Objective: To determine whether transient luminal exposure to antibiotics can directly induce changes to enteric nervous system (ENS) dependent propagating contractile clusters (PCCs).

Methods: 4 cm colon segments, excised from adult male Swiss Webster mice, were submerged in an organ bath chamber and cannulated at both oral and anal ends. Gut lumens were then perfused with Krebs saline control solution followed by Bacitracin (3×10^{-4}, 10^{-3}, 3×10^{-3} or 10^{-2} M), Penicillin V (3×10^{-3}, 10^{-2} or 3×10^{-2} M) or Neomycin (3×10^{-4}, 10^{-3}, 3×10^{-3} or 10^{-2} M). Alterations in gut motility parameters were captured by video and converted to spatiotemporal diameter maps for quantitative analysis. Paired *t*-tests were performed for before and after measurements.

Results: Bacitracin, Penicillin V and Neomycin, all elicited dose-dependent short latency (<10 minutes) changes to PCC parameters between control and antibiotic treatments, suggesting that they act directly on the ENS. Specifically, $>3\times10^{-4}$ M Bacitracin, 3×10^{-4} -3×10^{-3} M Neomycin and 3×10^{-3} -10^{-2} M Penicillin V all increased PCC velocity and frequency in the colon.

Conclusion: Our results suggest that transient exposure of the gastrointestinal lumen to antibiotics may directly modify enteric nervous system function. Therefore, studies attributing the effects of antibiotics on gut-brain signaling solely to disruption of the gut microbiota should be reinterpreted.

Policy of full disclosure: None.

283 | Intestinal microbiota modulates Reg3-dependent epithelial host responses

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The intestinal microbiota plays a major role in modulating the interaction between the gastrointestinal (GI) epithelium and the immune system. Many GI tract diseases are assumed to be influenced by microbial dysbiosis. Even though it is known that antibiotics change the microbial composition in the gut, the impact of antibiotic-related microbiota disturbances on epithelial immune parameters is not yet fully uncovered. In this project we investigated the effects of antibiotic-induced microflora modulation on immunological responses in adult C57BL6 inbred mice. 16S RNA sequencing of feces revealed distinct patterns of microbiota changes after antibiotic treatments, the most prominent being dysbiotic-like changes in rifaximin (RFX)-treated animals. On the epithelial level, mice treated with RFX also showed a downregulation of Reg3b, a lectin with antimicrobial and anti-inflammatory properties. Further, we investigated the relevance of this finding by inducing a DSS colitis and observing that RFX pretreatment and Reg3b deficiency in KO animals show a more severe colitis phenotype. In order to investigate the pro-homeostatic role of Reg3b, we hypothesized that Reg3b influences the intestinal stem cell compartment. By using small intestinal (SI) organoids as an experimental model, we showed that application of cecal content of RFX-treated animals downregulates stem cell markers, while treatment of organoids with IL-22, PAMPs and even Reg3b upregulate these. Another aim of this project is explaining how dysbiosis influences Reg3b downregulation. By looking at bacteria reduced after RFX treatment, we spotted Clostridia cluster XIVa, the main producer of the anti-inflammatory metabolite butyrate, and found that fecal butyrate levels are significantly lower in RFX-treated mice compared to non-treated animals. Analysing Reg3b expression in butyrate receptors KOs, we observed a significant Reg3b downregulation. This finally provides a link between microbiome changes and epithelial Reg3b regulation. In summary, we found that experimental manipulations of gut microbiota induce distinct host responses with Reg3b as an important factor for intestinal homeostasis.

Policy of full disclosure: None.

284 | Effectiveness of botulinum toxin A injection for the patients with dysphagia due to decreased relaxation time of upper esophageal sphincter

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Background: We attempted to examine the role of botulinum toxin A for the patients with dysphagia due to shortened relaxation time of upper esophageal sphincter (UES).
Methods: From 2016.1.8 to 2016.10.30, videofluoroscopic swallowing study(VFSS) and high resolution manometry (HRM) were performed in 34 patients with dysphagia. Among these patients, 5 patients were selected for botulinum toxin A injection due to shorted relaxation time of UES. After injection of botulinum toxin A 100 U into UES, subsequent HRM and VFSS were performed 1 month later. Changes in HRM metrics (Pressure (basal, median intra bolus, nadir), relaxation time interval of the UES, and mesopharyngeal and hypopharyngeal contractility (as a contractile integral; CI)) were examined using HRM. The parameters of VFSS were vallecular residue, pyriform sinus residue, vallecular overflow, penetration, and aspiration.
Results: A single injection of botulinum toxin A could increase relaxation time of UES significantly in 5 patients ($P<.05$). However, for 1 patient, 2nd injection of botulinum toxin was needed for increased relaxation time of UES. Also, the amount of residue in the pyriform sinus was significantly decreased after injection of botulinum toxin A.
Conclusion: Injection of Botulinum toxin A significantly increased relaxation time of UES in patients with dysphagia and decreased residue in the pyriform sinus. (study is still under way).
Policy of full disclosure: None.

285 | Comparison of magnetic resonance enterography and video capsule endoscopy in cases with suspected small bowel Crohn's disease

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Background: Magnetic Resonance Enterography (MRE) and Video Capsule Endoscopy (VCE) are the most sensitive small bowel imaging modalities available to detect small bowel Crohn's Disease (CD). Despite differences in the information obtained by CE and MRE, one of these is usually helped in detecting small bowel involvement in CD.
Aim: Compare MRE and VCE in detecting small bowel involvement in suspected cases of CD Methodology: Retrospectively, 104 records of patients referred for VCE to Mercy Hospital in 2016, with a clinical suspicion of CD small bowel involvement. CD was suspected in the presence of suggestive clinical symptoms (diarrhoea, abdominal pain and weight loss) and biochemical signs of inflammation (raised CRP/fecal calprotectine). All patients who performed both MRE and VCE, 3 month apart were included. Upper endoscopy and ileocolonoscopy were performed in all patients. Exclusion criteria were patient who had no MRE in 2016.
Results: Small bowel pathologies were found in 11 out of 104 patients: Crohn's disease (n=3), IBS-diarrhea (n=4), NSAID-induced enteropathy (n=3), worm infection (n=1). VCE and MRE separately showed sensitivities of 100% and 33% and specificities of 12.5% and 75% respectively. In 6 patients, VCE depicted mucosal pathologies missed by MRE. In Conclusion Both VC and MER are complementary methods for diagnosing small CD. VCE is highly sensitive in diagnosing small bowel CD. VCE is more capable of detecting limited mucosal lesions that may be missed by MRE. In contrast, MER is helpful in identifying transmural CD with transmural lesions and exclude strictures.
Policy of full disclosure: None.

286 | Prevalence and clinical characteristics of non cardiac chest pain with reflux esophagitis

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Background/Aims: Noncardiac chest pain (NCCP) is substernal, squeezing chest pain, unrelated to the cardiac problem. Our study aimed to define the prevalence and clinical characteristics of reflux esophagitis in NCCP patients in Korea.
Materials and Methods: We reviewed medical records of patients who visited the cardiology department of St. Paul's hospital due to chest pain and had normal coronary arteriography and had been taken endoscopy within 6 months. Patients who had peptic ulcer or gastric cancer history were excluded and they were classified into two groups according to their endoscopic result; reflux esophagitis group and the control group.
Results: Two hundred seventeen NCCP patients were enrolled and 96 (44.2%) patients were diagnosed as reflux esophagitis: 68 patients (31.3%) in LA-M; 26 patients (12.0%) in LA-A; 2 patients (0.9%) in LA-B. There were no patients with severe erosive reflux disease. There were no significant different characteristics in reflux esophagitis group and the control group.
Conclusion: The prevalence of reflux esophagitis in NCCP patients in Korea was 44.2%. Most patients had mild reflux esophagitis.
Policy of full disclosure: None.

287 | An Oxytocin Feast: Novel signalling of the oxytocin receptor and the growth hormone secretagogue 1a receptor heterodimer

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The oxytocin receptor (OXTR) is suggested to play a central role in social behaviours, anxiety, depression and more recently in food preference and other eating behaviours. Similarly, the centrally expressed growth hormone secretagogue receptor 1a (GHSR1a) has been shown to be involved in appetite regulation and food intake, and more recently implicated in stress, anxiety and depression. It was long believed that such GPCRs were monomeric proteins, but pharmacological and biochemical studies have demonstrated the presence of both homo- and

heterodimerisation of receptors within the GPCR family. This has led us to investigate the possible formation of a GHSR1a/OXTR heterodimer and its potential role in social eating. Lentiviral plasmids encoding the OXTR-tagRFP sequence (tagged Red Fluorescent Protein) were cloned into lentiviral vectors and transfected into stable HEK293A cell lines expressing GHSR1a-eGFP receptor (Green Fluorescent Protein). Similarly, Lv plasmids with GHSR1a-tagRFP sequence were transfected into HEK293A cells expressing OXTR-tagGFP. Using confocal microscopy, co-localisation and co-internalisation of both receptors were then analysed. Functional assays including calcium mobilisation assays and IP-ONE assays were used to evaluate any alterations in receptors signalling. We have demonstrated that the OXTR and GHSR1a expressed in HEK293A cells can co-localize. Moreover, functionality of each receptor is decreased when another is co-expressed. Future work includes characterising the presence of these heterodimers and functionality in hypothalamic primary cell cultures and brain slices. The relative amounts of heterodimers to their homodimer GPCRs may shed some light on the susceptibility of certain individuals to eating as a consequence of anxiety and stress.

Policy of full disclosure: None.