ANGMA 2023 Conference

# **Conference**
## Program

University of NSW,  Kensington, | 9 – 11 November 2023

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**PBS Information:** Authority required for the treatment of EoE in adults. Refer to the PBS Schedule for full authority information.

# 01

## Message
### from the President

It is with the greatest pleasure that I welcome you to our 2023 ANGMA Conference, and for the first time in Sydney/NSW.

The Local Organising Committee, led ably by Peter Wu and Michal Szczesniak, have worked tirelessly to put together an exciting program, consisting of state-of-the-art talks by renowned invited speakers, presentations of the latest original research by our members in the form of posters and talks, a workshop including live demonstrations of motility studies, as well as a public lecture.

I wholeheartedly thank the LOC, on behalf of our membership, for providing us with this excellent opportunity to meet in person, discuss the latest research, deepen long standing relationships, establish new collaborations, and form new friendships.

I would also like to express, on behalf of the ANGMA Council and the LOC, my gratitude to our sponsors. We are a small society with limited resources, and organising such an important meeting would be challenging without the generous support of our sponsors. So, thank you so much for your support!

I wish you all a wonderful meeting and look forward to speaking with many of you!

Christine
**Feinle–Bisset**
President, ANGMA

# BiomeBank

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**References:**
1. Biomictra Product Information
2. TGA website - *https://www.tga.gov.au/resources/artg* accessed 13 July 2023
3. Tucker E.C., Haylock-Jacobs S., Rapaic M., et al. J Gastroenterology and Hepatology Open 2023: 1- 6.

**Please review the Product Information before prescribing.**
PI is available at trade display and
www.biomebank.com/biomictra-product-information.pdf

**PBS Information – Biomictra™ is not PBS listed**

# 02
# Location
## John Niland Scientia Building
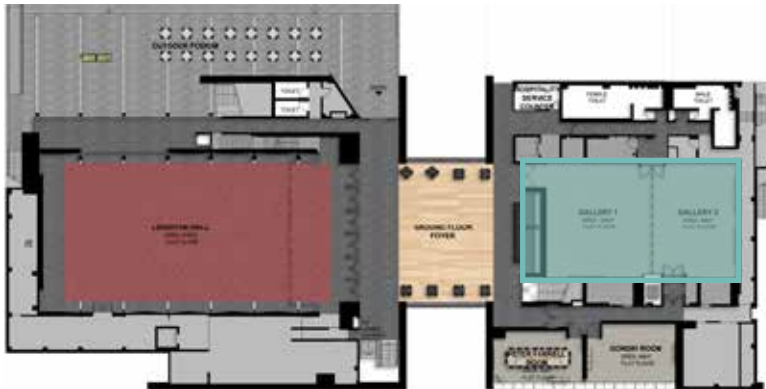


### Gallery 1 & 2 – Ground Floor

✓ Poster Presenations

✓ Morning tea, lunch, afternoon tea

✓ Sponsors & Exhibition Space

All food will be served in the Galleries Room Posters can be viewed during breaks on Friday 10th Nov. Poster numbers are indicated in the back of this booklet.
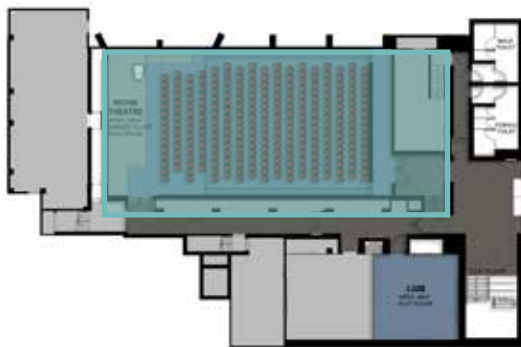


### Ritchie Theatre – Lower Ground Floor

✓ Scientific Sessions

All talks and lectures will be held in the Ritchie Theatre

*"Kindly be aware that there may be ongoing examinations in the adjacent Leighton Hall. We kindly request that you minimise noise in the foyer and while proceeding to the Galleries during your breaks."*

### ✓ Parking

The most convenient parking station for access to the Scientia Building is the Botany Street Carpark, entry via Gate 11, Botany Street.

### ✓ Wi-Fi

Visitors can access Wi-Fi at UNSW by connecting to the UNSW Guest Network.

### ✓ Security

UNSW has a 24-hour security on presence on campus. Contact UNSW Security on 9385 6000, or 9385 1515 in an emergency.

### ✓ Location

Located on Library Road, within the UNSW Kensington Campus. Map reference G19 on the UNSW Kensington Campus Map. Google Maps reference here.

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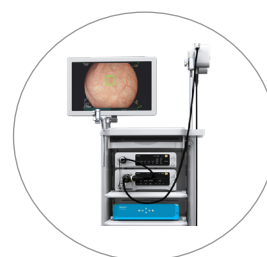
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# 03

# Program

**Thursday**
**November 9 | 2023**

**PUBLIC LECTURE**  – 05:30PM – 07:00PM

**Gut Feelings: Exploring the Role of the Microbiome in Digestive Disorders**

05:15 PM     **Doors Open**

05:30 PM     **Introduction**
Dr Peter Wu

05:35 PM     **Prof Emad El-Omar**
The Hidden World Within: Understanding the Human Microbiome

05:55 PM     **Prof Sunny Wong**
The Gut Microbiome Unveiled: How to Manipulate It for Better Health

06:15 PM     **Prof Nick Talley**
Microbes, indigestion, your gut and your brain: searching for the cause(s) and cure

06:35 PM     **Discussion and Q&A**

07:00 PM     **Close**

# 04

# Program

**Registration open** – 08:15 AM

09:00 AM **Welcome and Introduction**
Prof Christine Feinle-Bisset

President of ANGMA, University of Adelaide

**SYMPOSIUM 1** – 09:15AM – 10:30AM
**Microbes on the Mind: Microbiome Discoveries in Neurogastroenterology**
Chairs: Emad El-Omar, Howard Yim

09:15 AM **Prof Georgina Hold**
Bowel Health from Within: Harnessing the Power of the Microbiome

09:35 AM **Dr Rohan Williams**
Metagenomics and multi-omics for analysing microbiomes: a 20 min tour d'horizon

09:55 AM **Eunice Cheng**
Are bugs eating our memories? The microbiome in dementia

10:15 AM **General discussion**

10:30–11:00 AM **Morning tea & poster viewing**

**SYMPOSIUM 2** – 11:00AM – 12:30PM
**Neurogastroenterology in Parkinson's Disease: Unraveling the Gut–Brain Connection**
Chairs: Nick Talley, Sunny Wong

11:00 AM **Prof Carolyn Sue**
From Gut to Brain: Understanding How the Microbiome Impacts Parkinson's Disease

11:20 AM **Dr Shanti Diwakarla**
The gut barrier and Parkinson's disease

11:40 AM **Dr Michal Szczesniak**
A song of pressure and flow: Oro-pharyngeal dysphagia in Parkinson's Disease

12:00 PM **Myat Noe Han**
Early gastrointestinal changes in the A53T transgenic model of Parkinson's disease

12:15 PM **General discussion**

12:30–01:30 PM **Lunch & poster viewing**

# 05

# Program

**Friday**
**November 10 | 2023**

**SYMPOSIUM 3** - 01:30PM –04:30PM

### Live Motility Workshop: **Oesophagus and Pharynx**

Chairs: Taher Omari, Magnus Halland, May Wong

| | |
|---|---|
| 01:30 PM | **Dr Charles Cock**<br>Introduction to oesophageal manometry |
| 01:45 PM | **Dr Santosh Sanagapalli**<br>Solid testing - how I do it |
| 02:00 PM | **Dr Jenny Myers & Dr Santosh Sanagapalli**<br>Live demo - Oesophageal Manometry |
| 02:20 PM | **Prof Taher Omari**<br>Introduction to pharymgeal impedance-manometry |
| 02:35 PM | **Dr Michal Szczesniak & Dheeraj Pandey**<br>Live demo - Pharyngeal impedance-manometry |
| 02:45 PM | **Prof Taher Omari**<br>Live Demo - Swallow Gateway |
| 02:55 PM | **Panel Discussion** |
| 03:05–03:20 PM | **Short Break** |

### Live Motility Workshop: **Stomach and Colon**

Chairs:  Vincent Ho, Jenny Myers

| | |
|---|---|
| 03:20 PM | **Prof Greg O'Grady**<br>High-Resolution Gastric Mapping 101 |
| 03:35 PM | **Dr Daphne Foong**<br>Live Demo - High-Resolution Gastric Mapping |
| 03:50 PM | **Dr Allison Malcolm**<br>Anorectal Manometry & Biofeedback |
| 04:10 PM | **Prof Phil Dinning**<br>Colonic Manometry - Why? |
| 04:30 PM | **Panel discussion** |
| 04:40–05:10 PM | **Afternoon tea & poster viewing** |

# 06

# Program

**Friday**
**November 10 | 2023**

05:10 PM **ANGMA AGM**

06:30 PM **Conference Dinner**
Black Bottle, Shop 2, 116-118 Darlinghurst Rd, Darlinghurst 2010
**Bus Pickup at 06:00 PM** near Gate 14, Barker St

**Saturday**
**November 11 | 2023**

**SYMPOSIUM 4** – 09:00AM – 10:15AM

**Paediatric Neurogastroenterology: Navigating the Developing Gut–Brain Axis**

Chairs: Usha Krishnan, Marlene Hao

09:00 AM **Dr Lincon Stamp**
Healing Tiny Tummies: Advancements in Cell Therapies for Paediatric GI Disorders

09:20 AM **Dr Hannah Evans–Barns**
Motility assessments in children with Hirschsprung disease

09:40 AM **Gayl Humphrey**
Clinical application of body surface gastric mapping to subgroup patient
phenotypes in paediatric gastroduodenal disorders

09:50 AM **Hui Yu**
Harnessing the neurogenic potential of enteric glia for the treatment of
Hirschsprung disease

10:00 AM **Panel Discussion**

10:15–10:45 AM **Morning Tea**

**State–of–the–Art Lecture** – 10:45AM – 11:45AM

Chair: Peter Wu, Charles Cock

10:45 AM **Prof Sunny Wong Recent Advances in Gut–Brain Axis
Research: Relevance for Clinicians and Scientists**

11:35 AM **Discussion and Q&A**

11:45AM – 12:15PM **Awards and conference close**

# 07

# State-of-the-Art Lecture

## Sunny Wong
Associate Professor at the Lee Kong Chian School of Medicine

**Recent Advances in Gut–Brain Axis Research: Relevance for Clinicians and Scientists**

This lecture explores the influence of psychiatric factors, metabolism, and the gut microbiome on various neurogastrointestinal disorders, including inflammatory bowel disease (IBD), functional dyspepsia, and others. Psychological factors such as anxiety, depression, and stress significantly impact the onset and severity of these conditions. Neurometabolic dysregulation, involving neurotransmitter imbalances and disrupted neuroendocrine signaling, contributes to altered gut function and pain perception. Additionally, the gut microbiome, comprising trillions of microorganisms residing in the gastrointestinal tract, plays a crucial role in neurogastrointestinal health. Imbalances in the gut microbial composition have been linked to the development and progression of these disorders. Integrating psychological interventions, pharmacological approaches, and interventions targeting the gut microbiome, such as probiotics and dietary modifications, shows promise in managing symptoms and improving patient outcomes.

This talk provides an overview of the interplay between psychiatric factors, metabolism, and the gut microbiome in neurogastrointestinal disorders, emphasizing the importance of a multidisciplinary approach that considers psychological, neurobiological, and microbial aspects. By understanding and addressing these complex interactions, healthcare professionals can enhance diagnostic accuracy, treatment efficacy, and patient well-being in these conditions.

# 08

# Organising Committees

## Local Organising Committee

Peter Wu, University of NSW
Michal Szczęśniak, University of NSW
Nick Talley, University of Newcastle
Santosh Sanagapalli, St Vincent's Hospital Sydney
May Wong, Royal North Shore Hospital
Alvin Cheah, St George Hospital Sydney

## Scientific Organising Committee

Michal Szczęśniak, University of NSW
Peter W, University of NSW
Charles Cock, Flinders Medical Centre
Santosh Sanagapalli, St Vincent's Hospital Sydney
May Wong, Royal North Shore Hospital
Nick Talley, University of Newcastle
Phil Dinning, Flinders Medical Centre
Taher Omari, Flinders Medical Centre
Simona Carbone, MIPS
Marlene Hao, University of Melbourne
Howard Yim, University of NSW
Daniel Poole, MIPS
Lincoln Stamp, University of Melbourne

09

# Abstracts
## Oral Presentations

## Clinical application of body surface gastric mapping to subgroup patient phenotypes in pediatric gastroduodenal disorders

Gayl Humphrey[1], Celia Keane[1], Gabriel Schamberg[1,2], Alain Benitez[3,4], Corey Bowerman[3], Stefan Calder[1,2], Christopher Andrews[6], Greg O'Grady[1,2], Armen Gharibans[1,2,4], Hayat Mousa[3,4]

[1] Department of Surgery, The University of Auckland, Aotearoa | New Zealand
[2] Alimetry Ltd., Auckland, Aotearoa | New Zealand
[3] Division of Gastroenterology, The Children's Hospital of Philadelphia, Philadelphia, USA
[4] Perelman School of Medicine, University of Pennsylvania, Pennsylvania, USA
[5] The Division of Gastroenterology, Cumming School of Medicine, University of Calgary, Canada

**Background**
Body surface gastric mapping (BSGM) is a new diagnostic technology capable of defining mechanisms of gastric symptoms using high-resolution electrophysiological recordings. This study evaluated BSGM in relation to gastric emptying scintigraphy (GES), symptom burden, quality of life (QoL) and mental well-being in pediatric patients with chronic gastroduodenal symptoms.

**Methods**
Patients were enrolled from Auckland, NZ and Children's Hospital of Philadelphia, USA. GES outcome was recorded and gastrointestinal symptoms, anxiety, depression, QoL, and functional disability were reported using validated questionnaires. High scores reflect better outcomes. A standardized BSGM protocol including continuous symptom monitoring was followed.

**Results**
Thirty-five participants were enrolled, median age 17 (IQR 14-18), 79% female, median BMI 20.3 (IQR 18.6-23.25). Of 29 undergoing GES, 14 had delayed emptying (5 mild,
4 moderate, 5 severe), 14 FD patients had normal GES and 1 had rapid emptying. 6 participants with a clinical FD diagnosis did not undergo GES. No differences were found between the symptom burden score, mental well-being, quality of life or gastrointestinal symptoms by clinical diagnosis (Table 1).

BSGM phenotypes demonstrated differences in physical disability index ($p = .041$, Table 2). The symptom burden score approached statistical significance ($p = .056$). The BSGM Normal patients, less symptoms but poor QoL and mental wellbeing, indicating potential gut-brain- axis associations.

**Conclusion**
Patients with a diagnosis of FD and GP have similar symptoms and wellbeing results making targeted management of these patients challenging. BSGM, by contrast, identified substantial differences between clinical subgroups. These findings suggest that BSGM can provide valuable data to classify disease phenotypes within these complex conditions, which could support differential treatment approaches.

**Table 1.** Patient demographics, Gastric Emptying Scintigraphy outcome, BSGM Total Symptom Burden, Mental Well-being, Quality of Life and Physical Symptom Domains

| | All Delayed Gastric Emptying Patients | Delayed Gastric Emptying+ | | | FD^ | Rapid | FD no GES# | p |
|---|---|---|---|---|---|---|---|---|
| | | Mild | Moderate | Severe | | | | |
| n | 14 | 5 | 4 | 5 | 14 | 1 | 6 | |
| Sex (m/f) | 4/10 | 2/3 | 1/3 | 1/4 | 1/13 | 0/1 | 2/4 | |
| Age (years) median IQR | 17 (12.75-18.0) | 18 (11.5-22.5) | 14.5 (12.0-17.5) | 17 (15.0-18.0) | 16.5 (15.5-18.0) | 17 | 12.5 (9.0-17.08) | |
| BMI | 19.6 (15.15-21.3) | 19.7 (16.15-22.48) | 19.9 (17.55-20.43) | 19.9 (17.55-22.5) | 21.5 (20.3-23.7) | 9 | 19.6 (17.08-24.5) | |
| **Questionnaires** | | | | | | | | |
| **GIS [1]** (median IQR) | 62.2 (39.05-72.97) | 62.2 (33.26-68.51) | 57.3 (57.26-81.33) | 47.94 (42.94-66.89) | 50.8 (28.29-68.92) | 76.0 | 76.7 (76.39-89.09) | |
| **PROMIS Profile 25 v2 Anxiety[2]** t-score ±SE | 54.8 ±5.0 | 49.9 ± 5.1 | 52.4 ± 5.0 | 57.2 ± 5.0 | 49.9 ± 5.1 | 59.5 ± 5.0 | 49.9 ± 5.1 | 0.82 |
| **PROMIS Profile 25 v2 Depression[2]** t-score ±SE | 52.3 ± 4.5 | 49.8 ± 4.6 | 49.8 ± 4.6 | 56.7 ± 4.4 | 52.3 ± 4.5 | 58.8 ± 4.3 | 43.5 ± 5.2 | 0.6 |
| **PedsQL- QoL [3]** (median IQR) | 51.6 (36.41-66.85) | 55.4 (41.3-68.48) | 47.8 (34.78-72.83) | 50.0 (26.09-76.09) | 58.7 (22.83-82.61) | 42.4 | 77.2 (57.61-96.74) | 0.65 |
| **Nausea Severity Scale [4]** (median IQR) | 25.0 (11.75-30.50) | 24.0 (7.0-28.0) | 21.0 (8.75-28.0) | 32 (13.0-30.5) | 27.0 (19.75-29.25) | 20.00 | 10.5 (6.0-18.0) | 0.09 |
| **Abdominal Pain Index [5]** (median IQR) | 28.0 (20.75-32.0) | 24.2 (13.5-34.0) | 30.5 (27.0–31.75) | 26.0 (13.5-34.0) | 20.0 (14.75-29.25) | 31.0 | 27 (16.5-32.25) | |
| **Functional Disability Inventory [6]** (median IQR) | 9.0 (5.5-32.0) | 9.0 (3.75-27.75) | 13.5 (3.00-24.75) | 31.0 (14.5-40.0) | 25.0 (11.75-35.0) | 18.0 | 3.0 (3.00-28.50) | |

+Delayed Gastric Emptying: Residual at 2 hours: Mild 61-65%, Moderate 65-75%, Severe >75%  Residual at 4 hours: Mild 10%, Moderate 20%, Severe 30%
^ FD: Functional Dyspepsia normal gastric emptying scintigraphy outcome
# FD no GES: L16Functional Dyspepsia with no gastric emptying scintigraph study

1 Varni JW, Bendo CB, Denham J, et al. PedsQL™ Quality of life research 2015; 24(2): 363-78.
2 Cella D, Weinfurt K, Revicki D, Pilkonis P, DeWalt D, DeVellis R, et al. Patient-Reported Outcomes Measurement Information System Pediatric Profile-25 v2.0. https://wwwassessmentcenternet/documents/PROMIS%20Pediatric%20Profile%20Scoring%20Manualpdf. 2017.
3 Varni JWP, Bendo CBP, Nurko SMD, Shulman RJMD, Self MMP, Franciosi JPMD, et al. The Journal of pediatrics. 2015;166(1):85-90.e2.
4 Russell AC, Stone AL, Wang A, Walker LS. Children (Basel). 2018;5(6):68.
5 Laird KT, Sherman AL, Smith CA, Walker LS. Journal of Pediatric Psychology. 2015;40(5):517-25.
6. Claar RL, Walker LS. Pain. 2006;121(1-2):77-84.

**Table 2.** Patients by BSGM Phenotype, BSGM Total Symptom Burden, and Mental Well- being, Quality of Life and Physical Symptom Domains

| | BSGM Phenotype Grouping | | | | |
|---|---|---|---|---|---|
| | Delayed | Low Stability/Low Amplitude | High Amplitude | Normal | p |
| **Total** | 9 | 10 | 1 | 15 | |
| **All Delayed Gastric Emptying** | 5 | 3 | | 6 | |
| Mild | 1 | 2 | | 2 | |
| Moderate | 2 | | | 2 | |
| Severe | 2 | 1 | | 2 | |
| **FD** | 1 | 6 | 1 | 6 | |
| **Rapid** | 1 | | | | |
| **FD no GES** | 2 | 1 | | 3 | |
| **Total Symptom Burden Score** (median IQR) | 13.6 (1.65-34.37) | 31.79 (16.65-36.04) | 13.52 | 5.0 (0.63-25.29) | .056 |
| **Questionnaires** | | | | | |
| **GIS [1]** (median IQR) | 60.06 (33.6-72.9) | 47.6 (44.6-68.1) | 27 | 70.6 (44.9-76.5) | 0.35 |
| **PROMIS Profile 25 v2_Anxiety[2]** t-score ±SE | 57.2 ± 5.0 | 54.8 ± 5.0 | 59.5 | 40.9 ± 4.4 | .084 |
| **PROMIS Profile 25 v2_Depression[2]** t-score ±SE | 49.8 ± 5.0 | 56.7 ± 5.0 | 58.8 | 43.5 ± 4.4 | 0.14 |
| **PedsQL- QoL [3]** (median IQR) | 57.2 (0.2-21.3) | 54.8 (47.84-64.6) | 59.5 (49.7-69.3) | 40.9 (29.9-51.8) | 0.1 |
| **Nausea Severity Scale [4]** (median IQR) | 49.8 (40.7-58.8) | 56.7 (48.0-65.3) | 58.8 (50.3-67.2) | 43.5 (33.3-53.6) | 0.7 |
| **Abdominal Pain Index [5]** (median IQR) | 61.9 (53.2-70.65) | 55.4 (38.8-58.9) | 22.8 | 66.3 (42.4-83.1) | .062 |
| **Functional Disability Inventory [6]** (median IQR) | 9 (5.25-16.0) | 24 (20.5-32.0) | 43 | 18 (1.0-33.0) | .041 |

1 Varni JW, Bendo CB, Denham J, et al. PedsQL™ Quality of life research 2015; 24(2): 363-78.
2 Cella D, Weinfurt K, Revicki D, Pilkonis P, DeWalt D, DeVellis R, et al. Patient-Reported Outcomes Measurement Information System Pediatric Profile-25 v2.0. https://wwwassessmentcenternet/documents/PROMIS%20Pediatric%20Profile%20Scoring%20Manualpdf. 2017.
3 Varni JWP, Bendo CBP, Nurko SMD, Shulman RJMD, Self MMP, Franciosi JPMD, et al. The Journal of pediatrics. 2015;166(1):85-90.e2.
4 Russell AC, Stone AL, Wang A, Walker LS. Children (Basel). 2018;5(6):68.
5 Laird KT, Sherman AL, Smith CA, Walker LS. Journal of Pediatric Psychology. 2015;40(5):517-25.
6. Claar RL, Walker LS. Pain. 2006;121(1-2):77-84.

# Harnessing the neurogenic potential of enteric glia for the treatment of Hirschsprung disease

Hui Yu[1,2], Matilde Oviedo Querejazu[1], Annette Bergner[1], Gunes Yildiz[1], Marlene Hao[1], Lincon Stamp[1]

[1]Dept. Anatomy and Physiology, University of Melbourne, Parkville, VIC, 3010, Australia
[2]Department of Pediatric Surgery, Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an 710004, China

## Introduction

Emerging evidence suggests that, under certain stresses, enteric glial cells function as the neural stem cells of the enteric nervous system and generate new neurons *in situ*. However, their utility as a cell source for transplantation to restore gut function of Hirschsprung disease (HSCR) has not yet been investigated. HSCR is a neurodevelopmental disorder arising from a failure of ENS progenitors to colonise the entire gastrointestinal tract, resulting in an aganglionic region of the bowel that lacks a functioning ENS. Here, we aimed to isolate enteric glia from adult *Sox10-creERT2;R26R-YFP* mice and to determine their potential for neurogenesis following transplantation into the aganglionic colon of the *Ednrb-knockout* (*Ednrb$^{s-l/s-l}$*) mouse model of HSCR.

## Methods

Adut enteric glia were isolated from *Sox10creERT2:R26R-YFP* mice 1week after tamoxifen induction. Following *in-vitro* culture expansion, enteric glia-derived "gliospheres" were transplanted into the muscular layers of the *Ednrb$^{s-l/s-l}$* aganglionic colon. All colons were collected for immunohistochemical analysis of cellular survival and neuronal differentiation.

## Results

After 10-14 days of *in-vitro* culture expansion, enteric glia proliferated and formed gliospheres. Sox10-YFP expressing cells were shown to survive in the aganglionic colon. A subpopulation of YFP-expressing cells had given rise to Hu C/D-expressing enteric neurons. These data indicate that enteric glia can survive and differentiate into neurons following transplantation into the aganglionic colon. We are also currently investigating their ability to function as enteric neurons using live calcium imaging.

## Conclusions

In this study, we harnessed the unique potential of enteric glia as a novel source of stem cells for generating neurons to populate the aganglionic colon in HSCR treatment.

# Early gastrointestinal changes in the A53T transgenic model of Parkinson's disease mice

Myat Noe Han[1], David I Finkelstein[2], Shanti Diwakarla[1], *Rachel M McQuade[1]

[1]Department of Anatomy and Physiology, Melbourne University, Melbourne, VIC, 3021, Australia
[2]The Florey Institute of Neuroscience and Mental Health, Parkville, 3010, Australia

### Introduction
Gastrointestinal (GI) symptoms in Parkinson's disease often occur years before motor deficits manifest, however, the underlying mechanisms remain elusive and effective therapeutics are yet to be developed. We used a transgenic mouse model that expresses the mutant human A53T alpha-synuclein (α-syn) protein under the direction of the mouse prion protein promoter to investigate them.

### Methods
Central deficits were assessed using the ledged beam assay and GI dysfunction was assessed using the bead expulsion test and whole gut transit test between 12-36 weeks of age. Changes at the tissue and cellular levels were assessed using immunohistochemistry and ex vivo calcium ($Ca^{2+}$) imaging via short high $K^+$ depolarisation and stimulation of the nicotinic cholinergic receptor.

### Results
Transgenic mice exhibited changes in motor coordination starting at 24 weeks old and GI function as early as 16 weeks characterised by slowed colonic motility ($p=.014$). However, whole gut motility remained comparable to that of wildtype (WT) mice. Number of glial cells in the myenteric plexus of the colon were lower in A53T mice at 12 weeks compared to WT (Sox10: $p=.34$, S100B: $p=.005$). Transient amplitudes of $Ca^{2+}$ signalling in the colonic myenteric plexus were significantly higher in 12-weeks-old A53T mice compared to WT ($p<.001$; $p=.001$).

### Conclusions
A53T transgenic mice exhibit early changes at the tissue and cellular level of the GI tract prior to the manifestation of motor deficits. Differential $Ca^{2+}$ signalling responses to physiological stimuli might be linked to changes in colonic motility. Understanding these pathophysiology mechanisms could unveil novel therapeutic options.

# Abstracts
## Poster Presentations

# Gastric Alimetry® expands patient phenotyping in gastroduodenal disorders compared to gastric emptying scintigraphy

William Jiaen Wang[1, 2], Daphne Foong[1], Stefan Calder[3,4,5], Gabriel Schamberg[4,5], Chris Varghese[3], William Xu[3], Charlotte Daker[6], Daniel Carson[3], Stephen Waite[5], Peng Du[4], Thomas Abell[7], Henry Parkman[8], Vivian Fernandes[9], Christopher Andrews[10], Armen Gharibans[3, 4, 5], Vincent Ho[1, 11], Greg O'Grady[3, 4, 5]

[1] School of Medicine, Western Sydney University, Sydney, Australia
[2] Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane, Australia
[3] Department of Surgery, Auckland City Hospital, Auckland, New Zealand
[4] Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand
[5] Alimetry Ltd, Auckland, New Zealand
[6] Department of Gastroenterology, North Shore Hospital, Auckland, New Zealand
[7] Division of Gastroenterology, University of Louisville, Louisville, United States
[8] Gastroenterology Section - Lewis Katz School of Medicine, Temple University, Philadelphia, United States
[9] Lumus Imaging Campbelltown and Camden, Sydney, Australia
[10] Division of Gastroenterology and Hepatology, University of Calgary, Calgary, Canada
[11] Department of Gastroenterology and Hepatology, Campbelltown Hospital, Sydney, Australia

## Introduction

Gastric emptying scintigraphy (GES), while useful for evaluating gastric motility, is not specific nor sensitive for neuromuscular disorders. Gastric Alimetry® (GA) is a novel test that uses high-resolution gastric electrical spectral mapping and validated symptom profiling. This study compares patient-specific phenotyping of GA with GES.

## Methods

Patients with chronic gastroduodenal symptoms completed GES and GA concurrently. Tests included a 30-minute baseline, 99mTC-labelled egg meal and 4-hour postprandial recording. The validated GA App profiled symptoms and subsequently phenotyping into: 1) Continuous (no correlation between symptoms and meal/gastric-activity); 2) Gastric Sensorimotor (correlation between symptoms and meal/gastric-activity); 3) or Other.

## Results

75 patients with chronic gastroduodenal symptoms were assessed; 77% female, median age 43, median BMI 24.0. Motility abnormality detection rates were 22.7% (GES); 33.3% (GA-spectral) with a combined yield of 42.7%. GA symptom phenotypes (Figure 1A) were: Gastric Sensorimotor 17%; Continuous 30%; Other 53%. Strong correlations between the Sensorimotor phenotype and gastric amplitude were observed (median r=0.61 vs r=0.08 and r=0.06 respectively; p=0.0002). Abnormal GA spectral and Continuous phenotypes correlated with gastrointestinal symptom burden and anxiety (p<0.05), while Rome IV Criteria and delayed gastric emptying did not (p>0.05). Delayed gastric emptying was not predictive of specific GA phenotypes (Figure 1B).

## Conclusions

GA generated a higher yield for motility abnormalities than GES in patients with chronic gastroduodenal symptoms. GA also identified patient-specific symptom phenotypes, showing correlations with health psychology variables not observed with Rome IV and delayed gastric emptying. GES and GA assess different aspects of gastric function, indicating these tests may have complementary roles.
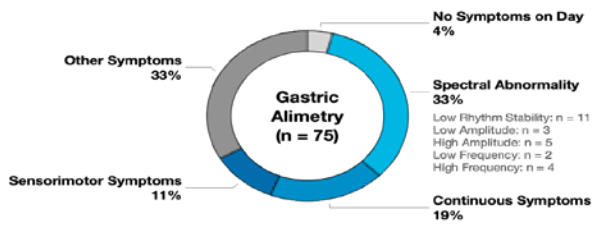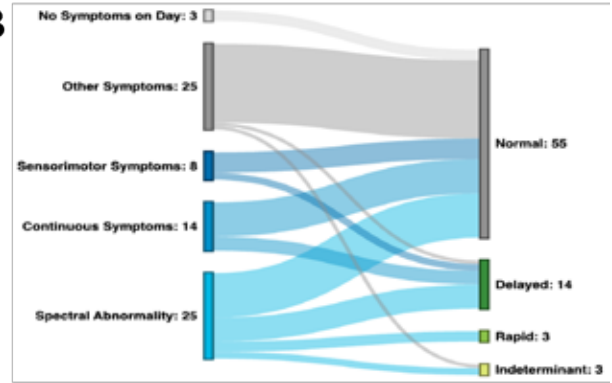
Figure 1: Diagnostic evaluation by GES and GA A) Overall diagnostic outcomes of GA spectral and symptom phenotyping. B) Sankey plot showing limited concordance between GA spectral or symptom phenotypes with GES abnormalities.

## How gut serotonin modulates gut motility.

Damien J Keating

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**Introduction**

The gut contains most of the body's serotonin (5-hydroxytryptamine, 5-HT). We have demonstrated that the microbiome modifies Enterochromaffin (EC) cell 5-HT to change host metabolism, but that intraluminal pressure triggers gut 5-HT release to modulate gastrointestinal motility by stimulating intrinsic primary afferent neurons. How EC cells do this has remained elusive.

**Methods**

Carbon fibre amperometry provides measures 5-HT release in mouse and human gut tissue. Transgenic mice and pharmacology were combined distinguish roles of neuronal and non-neuronal 5-HT in modulating gut motility. We utilised *in vitro* and *in vivo* measurement of gut motility and transit. 3-dimensional Confocal imaging measured the proximity of afferent nerves to EC cells and the anatomical nature of this neuroepithelial circuit.

**Results**

5-HT from EC cells, but not enteric neurons, controls gut motility [2]. Our findings show contraction-evoked EC cell secretion modifies gut contraction [3], including in a model of EC cell ablation [4], that the Piezo2 channel is required for this response [5] and this mechanism is reduced in aging human gut [5]. Microbial metabolites activate EC cells to modulate gut motility [6] and mucosal sensory nerves rarely contact EC cells, that EC cell "neuropods" are rarely sites of synaptic contact, and the nature of these circuits change with GI tract location.

**Conclusions**

Our data addresses several ongoing questions with relation to the role of EC cells in gut motility, the nature by which this occurs, and the likelihood that EC cell signalling to the nervous system occurs via a direct synaptic connection.

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## Convergence of aminergic and peptide signalling on neurons in the rodent lumbosacral defection centre

Mitchell T Ringuet[1], Ada Koo[1], John B Furness 2[1,2], Sebastian GB Furness [3] Stuart J McDougall [1,2]

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### Introduction
Dopamine, serotonin, and ghrelin receptor (GHSR) agonists lead to increases in propulsive colorectal motility. Experimental evidence indicates that effects are exerted in the lumbosacral spinal cord (L6-S1), possibly through actions at parasympathetic preganglionic neurons (PGNs). We sought to investigate which neurons express the receptors and whether dopamine, serotonin, and GHSR agonists each excite PGNs.

### Methods
We used whole cell electrophysiology to record ionic currents from neurons in the lumbosacral defecation centre, following drug application, and investigated their expression of relevant receptor, using Fluorescence in situ hybridisation (FISH), and the chemistry of their neural inputs, using immunohistochemistry (IHC).

### Results
In D2R-tdTomato adult mice, whole-cell electrophysiology conducted in L6-S1 revealed that dopamine, serotonin, α-methylserotonin, and capromorelin (GHSR agonist) each induced inward currents in overlapping populations of D2R neurons. Furthermore, dopamine increased the frequency of inhibitory and excitatory postsynaptic currents (IPSCs and EPSCs) in a subset of D2R neurons. The administration of tetrodotoxin blocked both IPSCs and EPSCs, revealing a post-synaptic excitatory action of dopamine. In lumbosacral PGNs of neonatal rats, only the postsynaptic effects of dopamine were observed. Additionally, the inward currents triggered by dopamine were reversed when slices were preincubated with YIL781 (GHSR antagonist). FISH showed expression of all three receptors in the same neurons. IHC revealed neurons receive both catecholamine and serotonin inputs.

### Conclusions
We conclude that the convergence of effects of endogenous ligands for D2R and 5-HT2R occurs at PGNs in the rodent lumbosacral defecation centre, and that GHSR serves as a cis-modulator for D2R within these neurons.
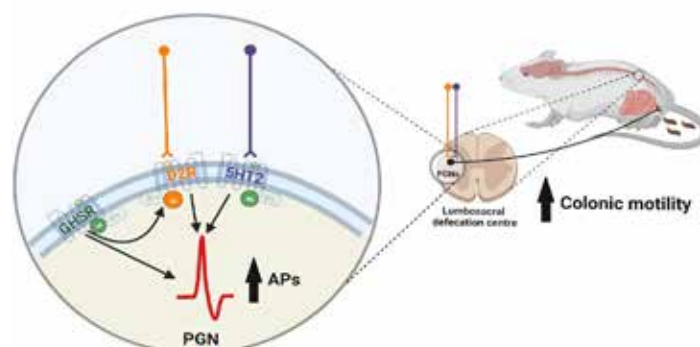
## Role of weakly acidic reflux and proximal impedance in gastro- oesophageal reflux disease and lung conditions

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University of Sydney
Royal North Shore Hospital

**Introduction**

Gastro-oesophageal reflux disease (GORD) is the backward flow of stomach acid into the oesophagus. Whilst GORD is extensively studied, role of weakly acidic reflux in common respiratory diseases is less explored. Furthermore, the contribution of proximal reflux events on respiratory disease remains to be elucidated. This study aimed to investigate the associations between weakly acidic reflux, GORD, and respiratory diseases.

**Method**

This was a retrospective cohort study that encompassed patients referred to the gastroenterology unit by a respiratory physician at RNSH, Sydney. Patient demographics and 24-hour pH impedance studies results were analysed for potential associations with respiratory complications.

**Results**

In the cohort of 1,005 patients, 382 had a diagnosis of GORD, 451 had no GORD and 123 had weakly acidic reflux. The presence of weakly acidic reflux appeared to be linked to a higher likelihood of bronchiectasis, asthma, and COPD ($p= 0.0477$) compared to those without GORD. Moreover, the results showed asthma ($p=0.0151$) was significantly more prevalent in those with GORD compared those without GORD.

There was a statistically significant association between bronchiectasis ($p=0.0375$), asthma ($p=0.0206$), combination of asthma and COPD ($p=0.00960$) and reflux severity, implying that patients with these respiratory diagnoses may experience more severe reflux compared to those without the condition. The presence of respiratory conditions did not show significant differences ($p>0.05$) in proximal impedance events.

**Conclusion**

The findings suggest of a link between weakly acidic reflux events and chronic respiratory conditions. Results align with current literature advocating a positive association between asthma and GORD.

## Interrogating gastric enteric neurons to align genotype and phenotype

John B Furness[1,2], Anna C Laddach[3], Chanya Gajanaike[1], Billie Hunne[1], Madeleine R Di Natale[1,2], Ana Oliveira[3], Franze Progatzky[3], Tiffany A Heanue[3], Cameron D Adams[2] and Vassilis Pachnis[3]

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**Introduction**
Recent single cell RNAseq studies of enteric neuron populations in the mouse intestines reveal neuron clusters that cannot be unambiguously aligned with functional classes. We reasoned that investigation of gastric enteric neurons would provide greater insights and be likely to successfully identify genotype/ phenotype alignments because functional types differ between the stomach and intestines.

**Methods**
We conducted single cell RNAseq analysis of 744 neurons from mouse stomach; regional localisation of neurochemical markers in enteric neuron somas with and without colchicine enhancement of peptide levels; localisation of markers in nerve terminals related to targets; and distinguished between sources of terminals using nerve lesion surgery.

**Results**
We tentatively identified the relations between genotype and phenotype for motor neurons to the muscle and we identified for the first time two possible classes of interneuron (immunoreactive for and expressing VIP (*Vip*) and Tachykinins (*Tac1*)). Intrinsic primary afferent neurons, identified by NMU (*Nmu*) in the intestines were rare in the stomach.
Classes of neurons corresponding to secretomotor neurons, including acid secretion stimulatory neurons, were tentatively identified as VIP/ GRP expressing. Unlike the intestine, the stomach does not appear to harbour somatostatin (*Sst*), CGRP (*Calca* or *Calcb*) or serotonin (*Tph1* or *2*) intrinsic neurons.

**Conclusions**.
Our hypothesis that investigation of genotype / phenotype relationships in the stomach will clarify the identification of functional classes of enteric neurons was supported by our investigations. The stomach appears to harbour two classes of vagally innervated interneurons, multiple classes of motor neuron, but few or no intrinsic sensory neurons.

# The Lymphocytic Choriomeningitis Virus (LCMV) clone Armstrong induces enteric inflammation

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**Introduction**
It is only recently that the importance of interactions between the enteric nervous system (ENS) and the immune system has been recognised. Enteric neurons closely communicate with anti-inflammatory muscularis macrophages (MM) and the ENS is target of several systemic viral infections. In this study we used infection with the Lymphocytic Choriomeningitis Virus (LCMV) as a model of systemic viral infection.

**Methods**
Female C57BL/6 mice (8-10 weeks) were intraperitoneally infected with LCMV Armstrong strain. One week before infection, mice received an intravenous injection of LCMV-specific CD8$^+$ T cells (P14 cells). Mice were sacrificed eight or thirty days after infection. Whole mounts of the *muscularis externa* were prepared for immunohistochemistry.

**Results**
No lymphocytes were observed in the *muscularis externa* of non-infected controls. However, after LCMV infection, we identified numerous CD8$^+$ T cells, including P14 cells, within the *muscularis externa*, some of them entering the myenteric plexus. Moreover, MM became significantly smaller. These changes were most prominent 8 days after infection, even though the virus was systemically cleared by day 3-4 post infection. Thirty days after infection, the macrophage morphology returned to normal and there were fewer CD8$^+$ T cells in the *muscularis externa*.

**Conclusions**
The results reveal enteric inflammation is accompanied by infiltration of CD8$^+$ T cells and a change in muscularis macrophage morphology indicating a potential shift towards a pro-inflammatory phenotype. Although this suggests that there could be effects on enteric neurons, neuronal changes and neuronal inter-play with MM remain to be investigated.

## Designing, developing and validating a set of standardized pediatric pictograms to support pediatric-reported gastroduodenal symptoms

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### Introduction
Persistent upper gastroduodenal symptoms such as nausea, abdominal pain, and bloating are common reasons for specialist consultations and hospital admissions in children. Children find identifying, describing and differentiating their symptoms difficult, making diagnosis and treatment decisions challenging. This study aimed to develop and validate a set of static and animated gastroduodenal symptom pictograms for children.

### Methods
A three-phase co-creation process was implemented. Phase 1 used experience design methods to create ten static and animated symptom pictograms (Fig.1). Phase 2 assessed acceptability and face and content validity. Phase 3 assessed preference between existing pictograms used with adult patients and the novel pediatric pictograms.

### Results
Eight children aged 6-15 years (5 female) participated in Phase 1, 69 children in Phase 2 (median age 13 years: IQR 9-15), and 118 participants in Phase 3 (median age 15: IQR 12-17). Face and content validity were higher for both pediatric sets than for adult pictograms (78% vs. 78% vs. 61%). Participants with worse gastric symptoms had superior comprehension of the pediatric pictograms ($\chi^2_8 < .001$). The pediatric static pictograms were preferred by all participants over the animation and adult sets ($\chi^2_2 < .001$).

### Conclusion
Co-creating ten novel pediatric gastroduodenal pictograms resulted in high face and content validity when evaluated with children aged 6 to 18. Validity was superior when children reported more problematic symptoms. These pictograms could be used in clinical and research practice to enable standardized symptom reporting for children with gastroduodenal disorders.
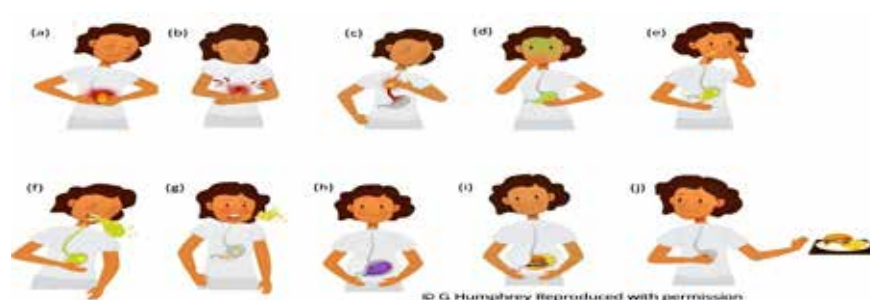


**Fig 1.** Pediatric gastroduodenal symptom pictograms. (a) stomach burn, (b) upper gut pain, (c) heartburn, (d) nausea, (e) reflux, (f) vomiting, (g) belching, (h) bloating, (i) excessively full, and (j) early Satiety. (The same images were used to create the Animation set).

# Internet-of-Medical-Things Device for Anorectal Biofeedback Therapy in Faecal Incontinence: A Pilot Study

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## Introduction

Biofeedback therapy is useful for treatment of faecal incontinence (FI) but is not widely available and labour intensive. We developed a novel Internet-of-Medical-Things (IoMT) device (Fig. 1), enabling self-guided, home-based biofeedback therapy. This pilot study assesses the safety and efficacy of self-guided biofeedback therapy using IoMT device in compared to standard in-clinic therapy.

## Methods

Patients experiencing urge or seepage FI (≥1 episode/week) were randomly assigned to either our IoMT system or the conventional anorectal manometry-based therapy. Both interventions comprised six weekly sessions, focusing on enhancing anal strength, endurance, and coordination. The novel device facilitated self-guided therapy via visual instructions on a companion app. Primary outcomes included safety/tolerability, changes in Vaizey FI severity scores, and alterations in anorectal pressure profiles.

## Results

A total of twenty-five FI patients (22 females, 3 males) participated, with 13 in the novel device group and 12 in the standard therapy group. Both groups showed significant reductions in FI severity scores: IoMT device group -4.2 (95% CI: -4.06, -4.34, p=0.018), and the standard therapy group -4.8 (95% CI: -4.31, -5.29, p=0.028). Anal sphincter resting pressure and sustained squeeze time improved significantly in both groups, and the novel device group demonstrated an increase in maximum sphincter squeeze pressure. There were no significant differences between the therapy groups. Importantly, the novel device was well-tolerated with no serious adverse events.

## Conclusions

This pilot study highlights the comparable efficacy of IoMT device delivered biofeedback therapy and traditional therapy using anorectal manometry. It demonstrates the potential of the IoMT device as a safe, self-guided method for FI therapy, offering convenience and effectiveness in FI management.

# Characterization of neuromuscular transmission and projections of muscle motor neurons in the rat stomach

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## Introduction

The stomach is the primary reservoir of the gastrointestinal tract, where ingested content is mixed and broken down into small particles which are propelled into the small intestine. Coordinated relaxation and contraction is essential for rhythmic motility and digestion, but how the muscle motor innervation is organized to provide appropriate graded regional control has not been established.

## Methods

Neuromuscular transmission to the gastric circular muscle of Sprague- Dawley rats was recorded using intracellular microelectrodes to investigate the spread of influence of intrinsic motor neurons. Microanatomical investigations and pharmacological analysis were conducted to investigate neuromuscular relationships.

## Results

Inhibitory neurotransmission was graded with both stimulus strength and circumferential distance from the stimulation site. The influence of inhibitory neurons declined with circumferential distance; in the antrum the decline was about 20%, in the corpus 30%, and in the fundus 50%. Stimulation of inhibitory neurons elicited biphasic hyperpolarizing potentials often followed by prolonged depolarizing events in the distal stomach, but only hyperpolarizing events in the proximal stomach. Excitatory neurotransmission influence varied greatly between the proximal stomach, where depolarizing events occurred in the muscle, and the distal stomach, where no direct electrical effects on the muscle were observed. Structural studies confirmed a dominant circumferential projection.

## Conclusions

We conclude that inhibitory and excitatory neural influences extend around the gastric circumference. The effectiveness of enteric innervation can be graded by the recruitment of different numbers of nerve terminals to finely control gastric motility and the ways that neurons influence the muscle differ between anatomical regions.

# Live calcium imaging as a tool to explore cellular activity in pediatric biopsies and resection specimens in Hirschsprung's disease

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## Introduction

Hirschsprung's disease (HSCR) is characterized by the absence of the enteric nervous system (ENS) in the most distal part of the colon, yet the ENS that is still present in the more proximal colon might be affected too. Owing to its intricate positioning within the gut wall, access to enteric ganglia is inherently challenging, thereby impeding the effective recording of their activity. To better understand ENS dysfunction in HSCR, we optimized our in-house developed imaging strategy and tailored it to enable live activity measurements in the ENS of pediatric intestinal samples.

## Methods & Result

Intestinal pediatric tissues were collected from children with HSCR suspicion or diagnosis; colorectal biopsies were taken during colonoscopy and resection specimens were removed during surgery (Figure 1). The nerve plexus was isolated by microdissection and pinned flat in a customized recording chamber. After loading the tissue with the fluorescent $Ca^{2+}$ indicator Fluo-4 (1 µM with 0.01% Cremophor), the tissue was visualized on an upright widefield microscope and ENS-related structures were identified. These structures were systematically stimulated, either electrically via a focal electrode or chemically with a high $K^+$ (75 mM) Krebs solution via a local perfusion tip. Changes in fluorescence were recorded as a readout for activity within the submucous (biopsies) and myenteric plexus (resection specimens) in HSCR. Post-hoc immunohistochemistry was performed to attribute observed (in)activity to neurons (HuCD$^+$) and glia (s100β$^+$).

## Conclusions

Live calcium imaging of freshly taken pediatric intestinal biopsies and resection specimens allows assessing cellular activity and will help advance our understanding of physiological dysfunction in HSCR.
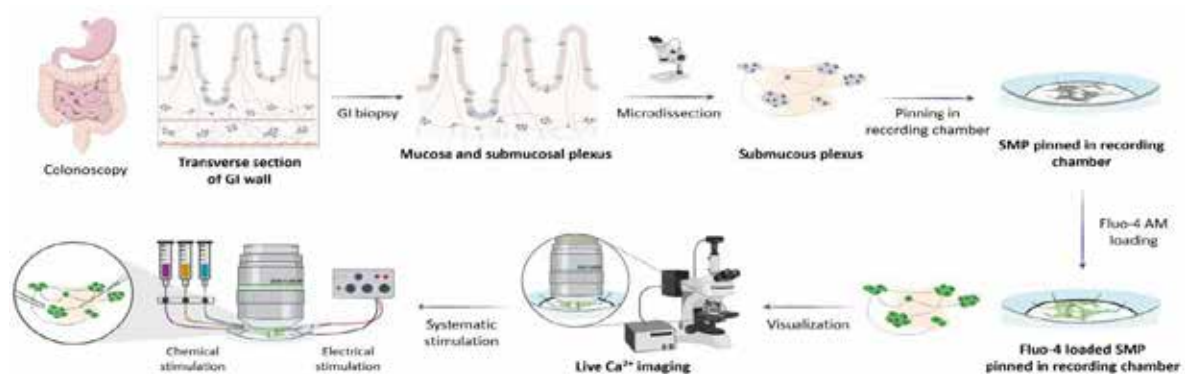


**Figure 1.** Schematic overview of the developed workflow to study cellular activity in Hirschsprung's disease in pediatric intestinal nerve tissue using live $Ca^{2+}$ imaging.

# Gut Dysfunction in Ageing Precedes Systemic Inflammation

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### Introduction

Systemic inflammation is a pillar of age-related disease and contributes to reduced life expectancy. Interestingly, increased intestinal permeability, a prominent feature of inflammatory GI diseases like IBD, is known to contribute to systemic inflammation. Collectively, these findings suggest a link between leaky gut and longevity, yet exact relationship between leaky gut, age-related systemic inflammation, and longevity remains unknown.

### Methods

Male c57bl/6 mice were assessed for gut pathology and systemic inflammation at 4-, 7-, 10-, 13- and 16-months of age. Leaky gut was assessed via oral delivery of 4kDa FITC dextran, the concentration of FITC in blood plasma determined by fluorometry. Faecal pellets were collected and assessed for water content, intestinal absorptive capacity and faecal energy loss via bomb calorimetry. Systemic inflammation was assessed in plasma via BioPlex and ELISA. Small and large intestines were collected and processed for histological and immunohistochemical analysis.

### Results

Histological changes in villus height to width ratio and muscle thickness were found throughout the gut as early as 10-months of age ($P \leq 0.05$). Meanwhile, feacal water content was significantly decreased, and intestinal permeability significantly increased from 13- months of age onwards ($P \leq 0.001$). Interestingly, plasma concentrations of systemic immune markers did not show any significant change until 16 months of age, with TNF-$\alpha$, IL-10, IL-17, RANTES and Eotaxin concentrations significantly increased compared to 4-months of age ($P \leq 0.05$).

### Conclusions

Collectively, these data indicate that gut dysfunction precedes systemic inflammation in ageing mice. Alterations in the gut associated with ageing may play a role in the development of systemic inflammation.

# Intraduodenal calcium enhances the suppression of energy intake and stimulation of gastrointestinal hormones, including cholecystokinin (CCK), peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1), by L-tryptophan in healthy males

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**Introduction**

In humans, intraduodenal infusion of L-tryptophan stimulates plasma concentrations of gastrointestinal hormones, including CCK, PYY and GLP-1, as well as pyloric pressures, both of which are key determinants of slowing of gastric emptying and suppression of energy intake. Preclinical studies indicate that these effects may be mediated by the calcium-sensing receptor, and enhanced in the presence of extracellular calcium. This has not been evaluated in humans.

**Methods**

We studied in 15 healthy, lean men (age 26±7 y; BMI 22±2 kg/m$^2$), on three separate occasions, in double-blind, randomised order, the effects of 150-min intraduodenal infusions of solutions of i) 500 mg $CaCl_2$ ('L-Ca'), ii) 1000 mg $CaCl_2$ ('H-Ca') or iii) isotonic 0.9% saline ('control'), all combined with L-tryptophan (0.1 kcal/min) from t=75–150 min, on plasma CCK, PYY and GLP-1, pyloric pressures and *ad libitum* energy intake at a buffet meal post-infusion (t=150-180 min). The L-tryptophan load used is known to be associated with a submaximal appetite-suppressant effect.

**Results**

In response to calcium alone, H-Ca and L-Ca stimulated PYY, while only H-Ca stimulated GLP-1 and pyloric pressures (all P<0.05). Moreover, H-Ca, but not L-Ca, enhanced the effects of L-tryptophan to stimulate CCK, PYY and GLP-1, compared with control (all P<0.05). Both H-Ca and L-Ca reduced energy intake compared with control (both P<0.05) (Figure), and this reduction was directly related to the calcium dose (R=0.39, P<0.05).

**Conclusions**

Intraduodenal calcium enhances the effect of intraduodenal L-tryptophan to suppress energy intake, associated with greater stimulation of plasma CCK, PYY and GLP-1, in health.
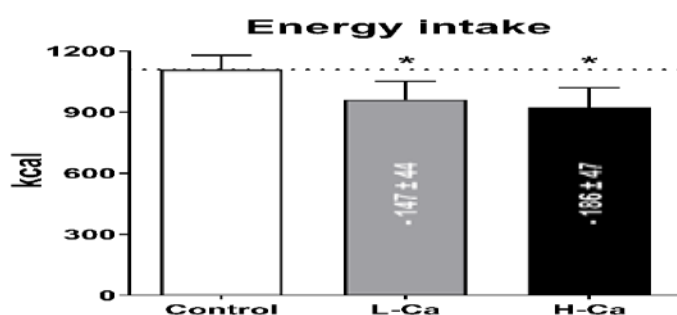


**Figure:** Energy intake at the buffet meal post-infusion; Data are expressed as means ± SEMs; *n* = 15.

# The impact of the plasma L-tryptophan to large neutral amino acids (Trp/LNAA) ratio on the ability of intraduodenal L-tryptophan to stimulate cholecystokinin (CCK) secretion, serum serotonin (5-HT), and to suppress energy intake in healthy males

Anjom-Shoae J[1], Fitzgerald PCE[1], Hajishafiee M[1], Coleman R[2], Martin AM[2], Poppitt SD[3], Lee MD[4], Higgs S[5], Rehfeld JF[6], Horowitz M[1], Feinle-Bisset C[1]

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**Introduction**

Intraduodenal administration of both L-tryptophan (0.15 kcal/min) and L-leucine (0.45 kcal/min) stimulates plasma CCK, associated with suppression of energy intake. That the circulating L-tryptophan to large neutral amino acids (Trp/LNAA) ratio, an indirect measure of brain serotonin (5HT), is also inversely related to energy intake suggests that postabsorptive mechanisms may contribute to appetite suppression. However, the extent to which these responses are related to plasma CCK and/or serum 5HT is uncertain.

**Methods**

12 healthy lean men (mean±SD: age 24±1 years; BMI 23±0.5 kg/m$^2$) received, on four separate occasions, in double-blind, randomised order, 90-min iso-osmotic intraduodenal infusions of i) L-tryptophan (0.15 kcal/min), ('Trp'), ii) L-tryptophan (0.15 kcal/min) + L-leucine (0.22 kcal/min), ('Trp+Leu-0.22'), iii) L-tryptophan (0.15 kcal/min) + L-leucine (0.45 kcal/min), ('Trp+Leu-0.45') or iv) isotonic 0.9% saline ('control'). Plasma CCK, plasma amino acids and serum 5-HT concentrations were measured during the infusions, and immediately post-infusion *ad libitum* energy intake at a buffet meal was quantified.

**Results**

Trp and Trp+Leu-0.45, but not Trp+Leu-0.22, decreased energy intake and increased plasma CCK, compared with control (all $P<0.05$). This decrease in energy intake was correlated directly with increases in plasma CCK ($R=0.17$, $P<0.05$) and the Trp/LNAA ratio ($R=0.13$, $P<0.05$). Plasma CCK was also directly correlated with the Trp/LNAA ratio ($R=0.11$, $P<0.05$). There was no effect on serum 5-HT.

**Conclusions**

The suppression of energy intake in response to L-tryptophan is related to both gastrointestinal and postabsorptive, including potentially central, mechanisms. The mechanistic interrelationships between these factors require further investigation in future studies.

# Gastrointestinal symptoms and glucagon-like peptide-1 receptor agonists (GLP-1 RAs) in overweight/obesity without type 2 diabetes (T2D): a systematic review and meta-analysis of randomised controlled trials (RCTs)

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Introduction
GLP-1 RAs, developed for the treatment of T2D, are now widely used in the management of obesity without concomitant T2D. RCTs, which have demonstrated clinically meaningful weight reductions with GLP-1 RAs in adults with T2D, have reported a high prevalence of gastrointestinal symptoms, which, not infrequently, compromise long-term adherence. Weight loss induced by GLP-1 RAs is greater in individuals with obesity without T2D, and higher doses of GLP-1 RAs are used, but there is little information about gastrointestinal symptoms in this group.

Methods
A systematic literature search, following PRISMA guidelines, was performed in relevant medical databases until May 2023. 26 RCTs, investigating the effects of GLP-1 RAs, compared with placebo, in adults with overweight/obesity (BMI ≥ 27 kg/m$^2$), and reporting data on gastrointestinal symptoms, were included. The trials included a total of 14,586 GLP-1 RA-naïve participants (age 18-70 years), and treatment durations of 12–68 weeks. In all cases, symptoms were assessed by participant self-report. Random-effects models were used to calculate pooled odds ratios (ORs) and 95% confidence intervals (CIs) for relevant gastrointestinal symptoms.

Results
All GLP-1 RAs were associated with an increased risk of nausea (pooled OR: 2.6, 95%CI: 2.4-3.0), vomiting (pooled OR: 3.5, 95%CI: 3.2-4.2), diarrhoea (pooled OR: 1.5, 95%CI: 1.3-2.0), and constipation (pooled OR: 1.8, 95%CI: 1.7-2.1) (Table).

Conclusions
Nausea and vomiting are common gastrointestinal symptoms in individuals with obesity treated with GLP-1 RAs, as is the case in T2D. Therefore, weight loss, induced by GLP-1 RAs, is likely to be, in part, an aversive effect.

**Table:** Summary risk estimates for the associations between different GLP-1 RAs and gastrointestinal symptoms in adults with overweight/obesity

| GLP-1 RAs (dosage) | No. of trials | Nausea Relative risk (95% CI) | Vomiting Relative risk (95% CI) | Diarrhoea Relative risk (95% CI) | Constipation Relative risk (95% CI) |
|---|---|---|---|---|---|
| Exenatide QW (2 mg/week) | 1 | 2.2 (0.6-7.7) | 2.8 (0.3-25.8) | 0.9 (0.2-4.3) | 1.9 (0.1-19) |
| Efpeglenatide (≤6 mg/week) | 1 | 3.1 (1.7-5.4) | 8.1 (3.1-21.2) | 1.1 (0.6-2.0) | 2.2 (0.8-5.6) |
| Efpeglenatide (>6 mg/two weeks) | 1 | 2.9 (1.7-5.2) | 3.7 (1.3-10.9) | 1.3 (0.7-2.3) | 2.1 (0.8-5.4) |
| Liraglutide (≤1.8 mg/day) | 6 | 3.2 (2.4-4.4) | 2.6 (1.5-4.4) | 1.2 (0.8-1.8) | 1.3 (0.9-1.9) |
| Liraglutide (>1.8 mg/day) | 14 | 2.8 (2.6-3.1) | 4.1 (3.4-4.9) | 1.8 (1.6-2.0) | 2.0 (1.8-2.3) |
| Semaglutide (≤0.4 mg/day) | 1 | 3.8 (2.6-5.5) | 3.4 (1.5-7.8) | 4.4 (2.7-7.1) | 4.5 (2.0-10) |
| Semaglutide (2.4 mg/week) | 5 | 2.6 (2.2-2.9) | 3.4 (2.7-4.3) | 1.8 (1.6-2.1) | 1.9 (1.6-2.3) |
| *Overall* | *26* | *2.6 (2.4-3.0)* | *3.5 (3.2-4.2)* | *1.5 (1.3-2.0)* | *1.8 (1.7-2.1)* |

## Pharyngeal weakness exacerbates aspiration risk in dysphagic patients with normal upper oesophageal sphincter (UOS) function.

Schar, MS[1,2]., Omari, T[1]., Wu, P [3]., Szczesniak, MM[3]., Tack, J [4][5]., Rommel, N[4,5]. & Cock, CC[1,2]

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### Introduction
In patients with oro-pharyngeal dysphagia, pharyngeal high-resolution manometry with impedance (P-HRM-I) can identify upper oesophageal sphincter (UOS) dysfunction and/or pharyngeal weakness. Diagnostic criteria for UOS dysfunction are established [1], however, defining pharyngeal weakness is unclear. This study investigated the relationship between weak pharyngeal contractility and aspiration.

### Methods
P-HRM-I recordings from 509 patients (18– 91 years; 295 male) were analysed. Penetration-aspiration score (PAS; scale 1-8) from videofluoroscopy was available for 320 (63%) patients. Normal values from 120 controls [1] were used to define abnormal pharyngeal contractility (<5$^{th}$ percentile). 10ml liquid bolus challenges were included.

### Results
Patients with UOS dysfunction (n=138) had increased velopharyngeal contractility (contractile integral for ABN UOS 110 [90, 135] v ABN UOS 90 mmHg.cm.s [77, 99], p <0.05). Patients with weak mesopharyngeal (n=90) and hypopharyngeal (n=124) contractility had increased aspiration scores (mean [CI] PAS for ABN MCI 5 [4, 6] vs. NOR 3 [3, 3], p<0.001; PAS for ABN PeakP 5 [4, 6] vs. NOR 3 [3, 3], p<0.001). Furthermore, patients with weak contractility had reduced UOS relaxation pressures (IRP) and hypopharyngeal intra-bolus pressures (IBP) (Figure 1). In patients with normal UOS function, pharyngeal weakness was associated with increased aspiration (Table 1).

### Conclusions
Dysphagic patients with UOS dysfunction have increased velopharyngeal pressures, most likely as a compensatory response to downstream flow restriction. Dysphagic patients with pharyngeal contractile weakness, bolus propulsive forces are also reduced. In patients with normal UOS function, pharyngeal weakness exacerbates aspiration risk.
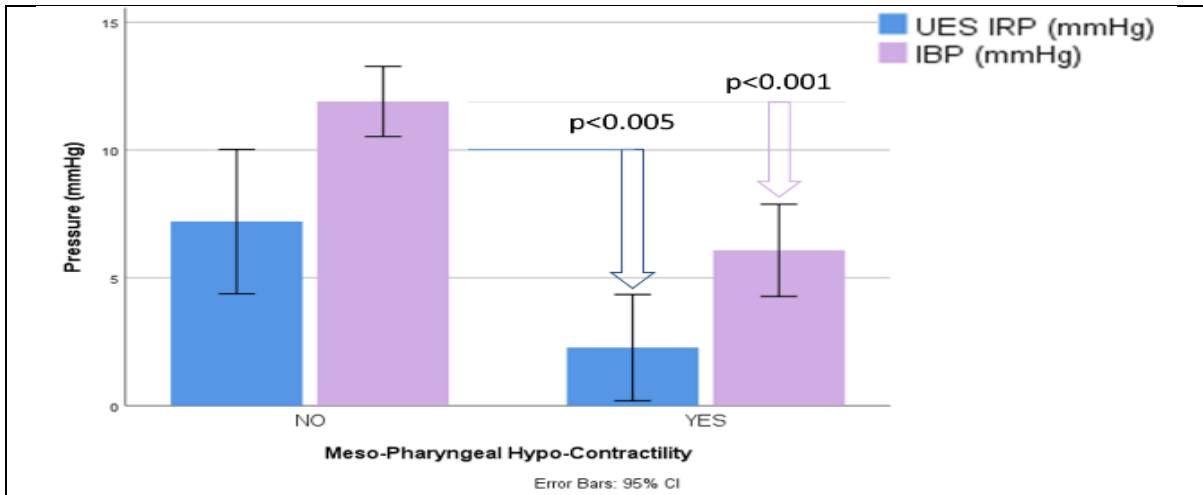
**Figure 1:** Graph shows patients with weak pharyngeal contractile pressures demonstrate reduced upper oesophageal sphincter integrated relaxation pressure (UOS IRP) and hypopharyngeal Intrabolus Pressure (IBP) when compared with patients with normal pharyngeal contractility.

**Table 1:** Penetration-Aspiration Scale comparing normal and abnormal upper oesophageal sphincter (UOS) function groups; and normal and weak hypopharyngeal peak pressures.

| Penetration-Aspiration Score (PAS) Levels 1-8 | | | |
|---|---|---|---|
| | **Normal hypopharyngeal peak pressure Mean (CI)** | **Reduced hypopharyngeal peak pressure Mean (CI)** | **Significance (t-test)** |
| **Normal UOS function** | 2.4 (2,3) | 4.4 (4,5) | **p < 0.001** |
| **Abnormal UOS function** | 4 (3,5) | 5 (4,7) | p = 0.104 |
| **Significance (t-test)** | **p <0.001** | p = 0.154 | |

1.      Omari, T., et al., Using high resolution manometry impedance to diagnose upper esophageal sphincter and pharyngeal motor disorders. Neurogastroenterology & Motility, 2022. **35**(1).

# Disorders of gut-brain interaction: more than anxiety and depression, an unmet need

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### Introduction

In disorders of gut-brain interaction (DGBI), treatment outcomes are better in multidisciplinary (MDT) clinics incorporating mental health specialists. There is a high prevalence of comorbid psychiatric disorders, oversimplified as anxiety or depression in research and questionnaires. We are making scientific advances in understanding the molecular basis of DGBI, however targeted gut-brain therapies will remain central to successful treatment. Unfortunately, mental health clinicians remain chronically underfunded in neurogastroenterology services in Australia. We aimed to define the need within our tertiary service.

### Methods

Retrospective audit of 50 consecutive new referrals seen in our neurogastroenterology clinic 2021-2022. A 10-year audit of medical and mental health notes was performed. Patients were assessed for diagnoses, need and receipt of psychological and psychiatric care as per criteria (Table 1); demographic data, gastrointestinal diagnoses, and progress at 6-9months.

### Results

Of 50 patients, all fulfilled Rome IV diagnostic criteria for 1+ DGBI, most commonly defecation disorders, functional dyspepsia, nausea and vomiting disorders, and irritable bowel syndrome. On initial consultation, 45/50 (90%) needed psychology, 12/45 (27%) had community psychology, and 9/45 (20%) were referred to our psychologist (0.1FTE capacity), leaving 24/45 **(53%) unmet need for psychology**. 34/50 (68%) needed psychiatry, 10/34 (29%) had community psychiatry, there is no public outpatient psychiatry, leaving 24/34 **(70%) unmet need for psychiatry**. Prior to clinic assessment, 42% had 3+ psychiatric diagnoses, 27% two, 31% one; still a significant underestimate (Figure 1).

### Conclusions

This study elucidates a substantial unmet need for mental health clinicians within Neurogastroenterology. A shift in funding is needed.

**Table 1:** Assessment criteria for mental health clinician referral:

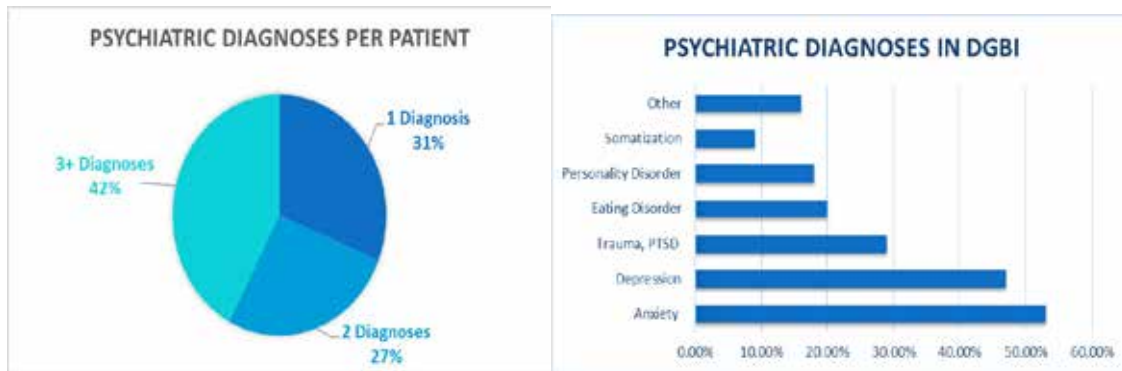| Need for Clinical Psychology | Need for Psychiatry |
| --- | --- |
| Diagnosed disorder of gut-brain interaction | Eating disorder, Disordered eating with malnutrition |
| Symptoms of psychological disorder, plus psychosocial distress | Complex mental health comorbidity |
| | Psychotropic medication – commence or review |

**Figure 1:** Pre-existing psychiatric diagnoses in patients with disorder of gut-brain interaction

## How the gut communicates with the brain, via the vagus nerve

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### Introduction
How the gut communicates with the brain is a major unresolved question. Vagal afferent neurons represent one of the major sensory pathways from the gut wall to the brainstem. Enterochromaffin (EC) cells in the gut mucosa release large quantities of serotonin, but how EC cells communicate with the brain is uncertain. In this study we modified our established anterograde tracing technique from dorsal root ganglia (DRG) to anterogradely label vagal afferent sensory nerve endings in the mucosa of small bowel and colon of control C57Bl/6 mice.

### Methods
In C57 mice, nodose ganglia were injected with 1ul biotinylated dextran amine (BDA, 20% soln.;MW 10,000; Molecular Probes Cat #D1956 via glass micropipette (tip diameter: 5um.

### Results
Anterograde labelling from nodose ganglia revealed single vagal afferent axon terminals in the mucosa of the small intestine and colon. In colon, labelling whole mount preparations revealed that the mean distance between any single vagal afferent mucosal nerve ending and the nearest 5-HT containing EC cell was $29\pm 19\mu m$ (107 endings, N=5 animals). Similar results were found in the mouse small intestine, with EC cells a mean distance of $33\pm14\mu m$ (56 endings N=7) from single vagal axon terminal endings in the mucosa.

### Conclusions
The findings suggest that ECs are randomly distributed through the mucosa and do not show preferential spatial bias to communication with 5-HT containing EC cells.

## Optimizing Colonic Structure Using Short-Chain Fatty Acids in Hirschsprung Disease Model Mice

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[2]Dept. Anatomy and Physiology, University of Melbourne, Parkville, VIC, Australia 3010.

### Introduction
Preliminary research has demonstrated that short-chain fatty acids (SCFAs) possess neurogenic and neuroprotective properties. Moreover, SCFAs appear to have a beneficial impact on optimizing colonic structure in a murine of Hirschsprung disease (HSCR). This study aimed to investigate the effects of SCFAs on colonic structure in $Ednrb^{s-l/s-l}$ HSCR model mice.

### Methods
$Ednrb^{s-l/s-l}$ HSCR model mice were administered daily rectal doses of SCFAs for one week. Subsequently, all colons from the SCFAs-treated $Ednrb^{s-l/s-l}$ HSCR model mice were collected for immunohistochemical analysis. The analysis focused on evaluating colonic structural changes, including inflammatory cell infiltration, crypt structure, mucosal basal hyperplasia and tight junction protein ZO1.

### Results
In the colons of $Ednrb^{s-l/s-l}$ HSCR model mice, several notable observations included the presence of infiltrated inflammatory cells, disordered crypt structures, and hyperplastic basal mucosal layers. These abnormalities were alleviated following SCFAs treatment. Additionally, $Ednrb^{s-l/s-l}$ HSCR model mice displayed reduced staining of the tight junction protein ZO1, while SCFAs treatment led to increased and continuous ZO1 staining. These findings indicate that SCFAs have the potential to ameliorate the disordered colonic structure observed in $Ednrb^{s-l/s-l}$ HSCR model mice.

### Conclusions
This study demonstrates that SCFAs can optimize the colonic structure, and in addition to the previously mentioned neurogenesis and neuroprotection effects, may offer a multifaceted approach to cell therapy in HSCR treatment.

**Investigating whether damage to the gut barrier increases susceptibility to rotenone-induced Parkinson's disease-like symptoms in mice.**

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**INTRODUCTION**

Exposure to environmental pollutants, such as pesticides, is known to increase the incidence of developing Parkinson's disease (PD). In addition, pre-existing chronic GI disorders, such as IBD and IBS, are known to increase risk of PD onset later in life. Given that increased intestinal permeability is a prominent feature of IBD/IBS, we aimed to determine whether damage to the gut barrier increases the susceptibility to the PD-inducing pesticide rotenone.

**METHOD**

Lipopolysaccharide (from E.*coli*, 1mg/kg) was orally administered for 7 days to 8 week-old mice to induce increased intestinal permeability. Following confirmation of intestinal permeability using the *in vivo* FITC permeability assay, mice were orally administered rotenone (30 mg/kg, n=16) for 7-days. Motor deficits were assessed using the rotarod and ledge beam tests, while GI function was assessed via faecal pellet output and bead expulsion tests. Behavioural experiments were performed every 4 weeks up to 12 weeks-post rotenone treatment. All data was compared to the appropriate control groups (n=5-16).

**RESULTS**

A significant increase in intestinal permeability was observed ($P<0.05$) in mice treated for 7 days with LPS when compared to vehicle mice, alongside an increase in C-reactive protein in plasma, indicating systemic inflammation. Mice treated with LPS + rotenone showed signs of motor dysfunction with an increase in foot faults observed at 12-weeks post-treatment ($P<0.05$). No motor deficits were observed in the other groups. No changes in GI function were observed.

**CONCLUSION**

Increased intestinal permeability may increase the susceptibility to developing PD-like symptoms following exposure to certain environmental toxins.

## A gut feeling about protective mechanisms for brain cancer: lessons from the enteric nervous system

Amelia Nash[1], Devy Deliyanti[1], Gunes Yildiz[1], Rachel Gwynne[1], Annette Bergner[1], Yvette Wilson[1], Katharine Drummond[2], Montana Spiteri[3], Saskia Freytag[3], Theo Mantamadiotis[4], Lincon A. Stamp[1], <u>Marlene M. Hao[1]</u>

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### Background

Gliomas are the most common and aggressive form of brain cancer, accounting for 80% of malignant primary brain tumours. Although there have been many improvements in diagnostic and therapeutic options, survival rates remain low for brain cancers with only 1 in 5 patients surviving 5 years beyond their initial diagnosis. Gliomas arise from mutations that affect glial cells and neural stem cells in the brain. In the gut, enteric glial cells have many similarities to CNS glia, and in addition, act as the neural stem cells of the gut. Interestingly, gliomas in the gut are very rare, and over 95% of tumours are benign. Our study aims to investigate a key question: why are enteric glial cells protected from developing aggressive cancers?

### Methods:

To assess this we have developed 2 mouse models with oncogenic overactivation of the PI3K pathway in both CNS and enteric glial cells, driven by either the Nestin or Sox10 promoters.

### Results and Conclusions

In PI3K mutant mice, significant increases in the proliferation of enteric glia were identified both using *in vitro* cultures, and through *in vivo* incorporation of EdU. While there was increased proliferation of enteric glia, no tumour formation in the gut was observed. *Sox10-PI3K* mutant mice also had defects in gut motility, exhibited enlarged caeca, suggesting alterations in the microbiome, and changes in the profiles of immune cells compared to control mice. RNA-seq analysis showed that PI3K mutant enteric glia had upregulation of neurodegenerative disease pathways and metabolic pathways compared to control enteric glia.

## Increased failure of peristaltic reserve function during multiple rapid swallowing in ineffective oesophageal motility patients with ageing

<u>Charles Cock</u> [1,2], James Hunt[1], Richard Heddle[1], Alison Thompson[1], Laura Besanko[1], Carly Burgstad[1], Mistyka Schar[1,2], Taher Omari[2]

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### Introduction

Intact peristaltic reserve function (PRF) following multiple rapid swallowing (MRS) in ineffective oesophageal motility (IEM) is regarded as indicative of intact peristaltic neuromuscular mechanisms. The commonest application of this finding in practice is in deciding on anti-reflux surgery in patients with IEM. The effects of ageing on PRF have not been investigated and we conducted a study of the effects of ageing on PRF during MRS.

### Methods

IEM patients who successfully competed MRS as part of their manometry were included in the following groups: group 1: 20-39 years (n=20), group 2: 40-59 years (n=28), group 3: 60-79 years (n=71), group 4: 80 plus years of age (n=16). MRS was administered as 5x2ml liquids administered in rapid succession (<10 sec). Intact PRF was defined as the distal contractile integral (DCI) in mmHg.s.cm following MRS exceeding the average DCI of 10 liquid swallows (i.e., DCIMRS: DCILiquidsAve > 1). Proportions of intact MRS were compared by group using Fisher's exact test with a p-value of < 0.05 significant.

### Results

The results are displayed in Figure 1. Significantly more IEM patients in group 1 (20-39 years) had intact PRF, compared to group 3 (p < 0.01) and group 4 (p < 0.001). Significantly more individuals in group 4 (80 years plus) had failed PRF, compared to those in group 1 (p < 0.001) and 2 (p < 0.05).

### Conclusions

There is increasing failure of peristaltic reserve function with ageing, congruent with neurodegeneration of peristaltic mechanisms. These findings have implications for the use of MRS for clinical decision making.
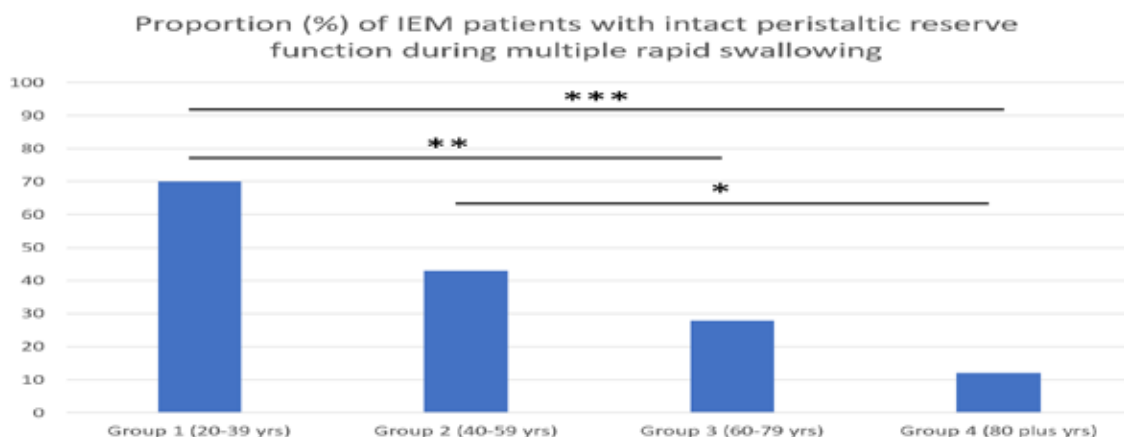
## Contractile Segment Impedance in Eosinophilic Esophagitis

Charles Cock [1,2], James Hunt[1], Richard Heddle[1], Alison Thompson[1], Laura Besanko[1], Carly Burgstad[1], Mistyka Schar[1,2], Taher Omari[2]

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**Introduction**

Eosinophilic oesophagitis (EoE) is an allergic, inflammatory condition commonest in young men, associated with dysphagia and food bolus impaction. While diagnosis and treatment outcomes are currently based on histopathology, mucosal changes in EoE are also measurable through mucosal impedance (MI). We studied a novel MI metric, contractile segment impedance (CSI), measured during high-resolution manometry with impedance (HRM-I) in EoE patients and controls.

**Methods**

EoE patients (n = 30, 24 men, aged 31±8 years) with histologically proven EoE (>15 eosinophils per high power field) who underwent manometry were compared with asymptomatic healthy volunteers as controls (n = 60, 30 men, aged 42±16 years). CSI was measured at every 1cm above the lower oesophageal sphincter (LES) for an average of five 5ml normal saline swallows (MMS Solar GI system, 36P,16I catheter). CSI compared in EoE patients and controls, using t-tests and area under receiver operating curve – AUROC (per 1cm) (Prism 10 for Windows). P-value < 0.05 was considered significant.

**Results**

CSI was significantly lower in EoE patients, compared to controls at all levels above the LES (Figure 1A; 502±33 ohms vs. 1054±41 at 2cm; p < 0.001). CSI, measured during HRM-I, had excellent diagnostic utility for a diagnosis of histologically proven EoE. Diagnostic performance was best at 2cm above the LES with an AUROC of 0.937, sensitivity 90% and specificity 93% for a diagnosis of EoE (Figure 1B).

**Conclusions**

CSI distinguished EoE patients from controls, with maximum accuracy at 2cm above the LES.
CSI has clinical utility to diagnose unsuspected EoE or monitor treatment response.
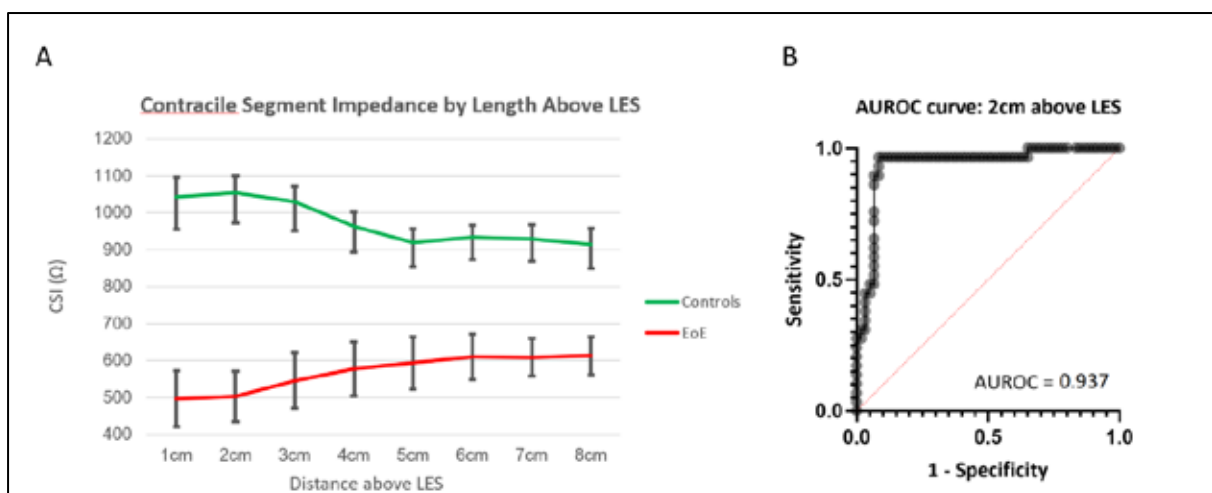


Figure 1 Contractile segment impedance (CSI) Fig. 1A: comparison between EoE Patients (red) and controls (green); Fig1B Area under receiver operating curve (AUROC) for diagnoses of histologically confirmed EoE

## Ineffective oesophageal motility and elevated acid exposure in 31 patients post sleeve gastrectomy

*Ross Hansen*[1,2]*, May Wong*[1,2]*, Jenny Ottey*[1],

[1]Neurogastroenterology Unit, Royal North Shore Hospital, Sydney [2]Sydney Medical School, The University of Sydney

**Introduction**

Limited oesophageal function findings are available on patients following sleeve gastrectomy.

**Methods**

We audited a series of 31 patients, median age 50 (range 22-75) years, referred to our Unit 40 (range 12-115) months after sleeve gastrectomy.

**Results**:

High Resolution Manometry detected Ineffective Oesophageal Motility (IOM) in 13 patients (5 males), incomplete lower oesophageal sphincter (LOS) relaxation in 3; 15 (1 male) exhibited normal peristalsis (N). There was a higher proportion of males in the IOM subset compared to N (p=0.04). No significant differences in age, body mass index, ?me since surgery or resting LOS tone existed between IOM and N. IOM reported heartburn, regurgitation and dysphagia as the primary presenting symptom, whereas N reported heartburn and chest pain. 8 IOM and 11 N underwent 24-hour pH-impedance monitoring. Only one patient in each subset had normal acid exposure (<4.2%). Median total and supine acid exposures were higher in IOM (22.1% and 25.6%) than in N (11% and 14.7%); these differences were not significant (p=0.16 and 0.11). Six IOM (75%) and nine N (83%) had a positive Symptom Association Probability.

**Conclusions**

IOM and gastro-oesophageal reflux are common findings in patients referred for investigation of oesophageal symptoms following sleeve gastrectomy. A surgically induced increase in intragastric pressure has been proposed as the mechanism for increased reflux in such patients [1]. The findings highlight the importance of pre- and post-surgery manometry and reflux monitoring to guide both the bariatric surgery approach and the ongoing management for these patients.

[1]Tolone, S et al. J Gastrointestinal Surgery 24:1-7, 2020.

# Programming enteric neuron differentiation for the treatment of digestive diseases

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**Abstract text:**

Oesophageal achalasia, characterized by the inability of the lower oesophageal sphincter to relax, and gastroparesis, a chronic condition often referred to as "paralysed stomach," are two highly debilitating disorders affecting the upper gastrointestinal tract. Both involve local loss of the nitrergic inhibitory motor neurons from the enteric nervous system, which are characterised by expression of neuronal nitric oxide synthase (nNOS). These diseases are poorly managed with current pharmacological and surgical interventions, which only address the symptoms but not the cause of the disease.

Previous studies from our laboratory have shown that transplantation of a mixed population of enteric neural progenitors restores the nitrergic inhibitory communication to the gastric smooth muscle cells. In this current study, we aim to direct iPSC differentiation specifically to a nNOS inhibitory neuron phenotype using transcription factor directed differentiation prior to transplantation. iPSCs have been directed to a naïve enteric neuron precursor phenotype using a previously published protocol, and we will drive the expression of a variety of transcription factors that have been shown to be upregulated in nNOS neurons.

The ability to produce a specific subset of enteric neurons will be highly important for future stem cell therapy approaches.

# Enteric neuroprotection as a therapeutic strategy to prevent gastrointestinal side effects of chemotherapy.

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## Background

Oxaliplatin (OXL) is a third-generation platinum-based chemotherapeutic agent clinically used for treatment of colorectal cancer (CRC) and other cancers. Nonetheless, beneficial treatment outcomes are truncated by its undesirable gastrointestinal (GI) side effects. OXL induces immunogenic cell death by provoking the presentation of damage associated molecular patterns. As such, this study proposes a novel concept to target, a high mobility group protein B1 (HMGB1), to alleviate enteric neuropathy and gastrointestinal dysfunction. HMGB1 presents in the nucleus of cells, however, upon cellular damage, such as that induced by chemotherapeutics, HMGB1 translocates to the extracellular space where it demonstrates chemoattractant and mitogenic activity. Glycyrrhizin (GR) – a HMGB1 inhibitor, effectively impedes upon the translocation of HMGB1, ultimately resulting in neuroprotection.

## Method

Orthotopic models of CRC were used to investigate whether *in vivo* co-administration of GR will attenuate adverse effects and potentiate the anti-tumour efficacy of OXL. In vivo and ex vivo experiments were performed to assess tumour growth, immune cell infiltration, and structural and morphological changes to the enteric nervous system (ENS).

## Results

Targeting HMGB1 chemoattractant activity with GR improved disease severity, reduced immune cell infiltration, restored GI function, ultimately providing neuroprotection to the ENS. The inhibition of HMGB1 signalling mitigated against OXL-induced neuronal loss, normalising colonic motility, and reducing symptoms of GI dysfunction.

## Conclusion

The outcome of this study demonstrates that the co-administration of GR with OXL can be considered as a potential therapy to reduce OXL-induced GI adverse effects and provide avenues for safe long-term treatment at the most effective tumour-suppressing doses for many cancers.
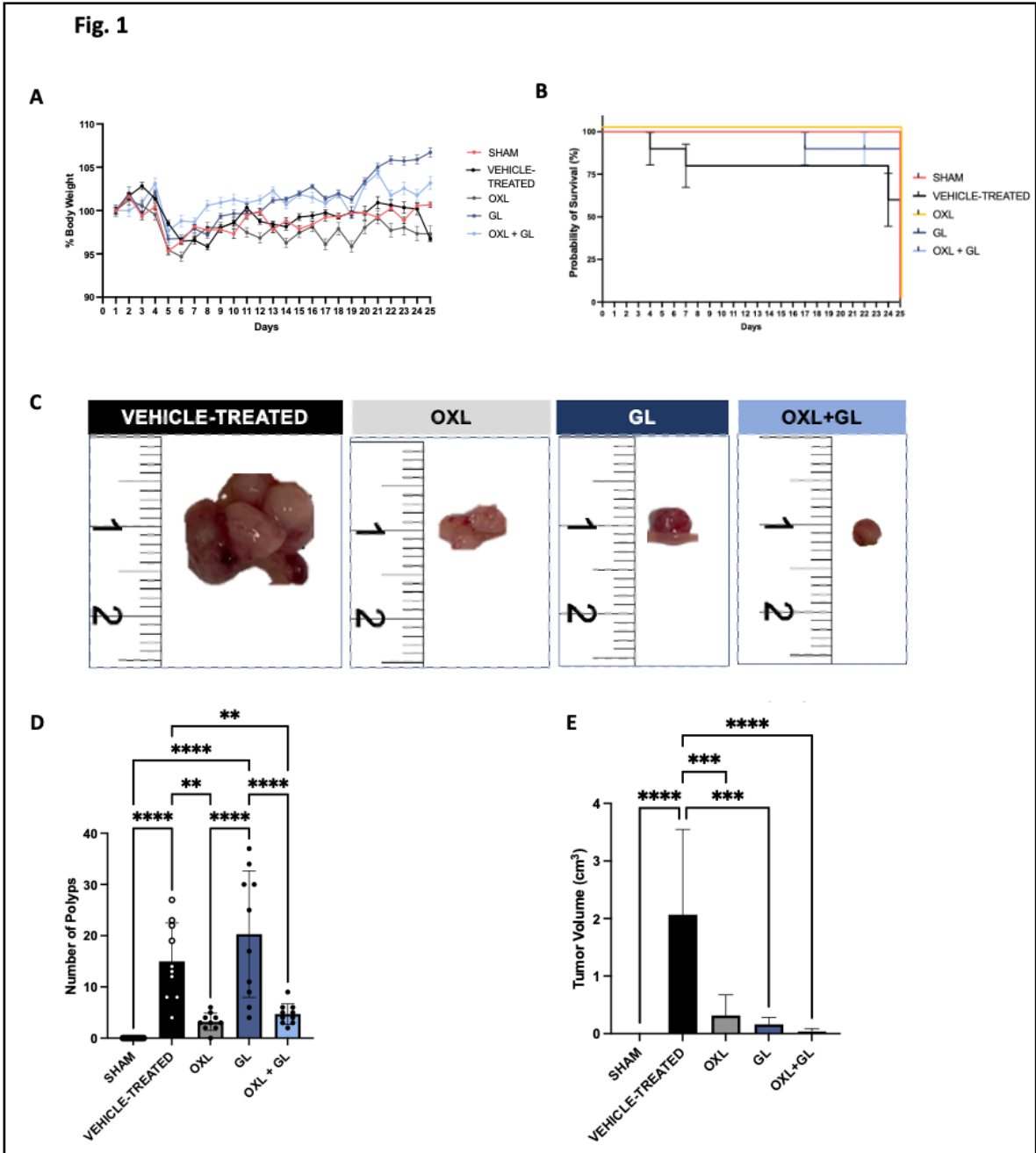
**Fig. 1**

**FIGURE 1**

Effects of treatments on the body weight, survival rate, and tumour growth in Balb/c mice with orthotopic CRC. **A** Body Weight loss or gain **B** Kaplan–Meier survival plot representing the percentage of survival of mice after treatment **C** Representative images of orthotopic tumours excised from different treatment groups. **D** The mean number of polyps **E** The mean tumour volume following various treatments. N = 10 mice per group. Data expressed as mean ± SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ compared with sham group.

## Application of Classification Scheme for Pharyngeal Motility Assessments at a Tertiary Referral Centre

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### Introduction
Pharyngeal high-resolution manometry with impedance (P-HRM-I) provides biomechanical swallowing outcomes that, when interpreted according to a recently proposed classification scheme [1] denote upper oesophageal sphincter (UOS) dysfunction, pharyngeal weakness, "other" and normal swallowing (Table 1). The Gastroenterologist's expertise in oesophageal HRM-I provides the foreseeable extension to P-HRM-I; however current clinical uptake is limited. The study aims to describe P-HRM-I outcome metrics and the classification scheme relevant to pharyngeal dysphagia.

### Methods
Retrospective audit of the clinical database from the Flinders Medical Centre Motility Service (September 2021- 2023) to identify P-HRM-I studies. Patients were stratified according to a clinical diagnosis based on a classification scheme. Three illustrative case examples of UOS dysfunction, pharyngeal weakness, and reduced UOS basal pressures are described and compared to a normal swallow.
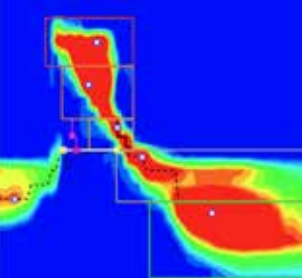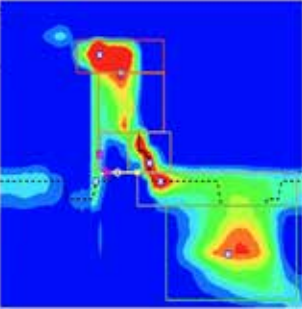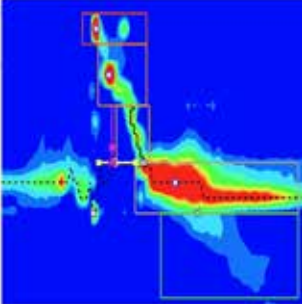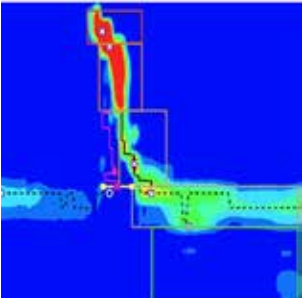
### Results
Of the 61 P-HRM-I studies were identified (mean age 64 (range 17-84) years, 26 male) and classified as follows: 34% (21) had pharyngeal weakness, 28% (17) had UOS dysfunction, 30% (18) were normal, and 8% (5) defined as other. The patient example of UOS dysfunction with bulbar-type Motor Neurone Disease demonstrates elevated UOS relaxation pressures and increased intra-bolus pressures. The patient example of pharyngeal weakness with post-polio syndrome demonstrates reduced hypopharyngeal peak pressures. The example of "other" demonstrates normal pharyngeal contractility and UOS relaxation and opening; however, reduced UOS basal pressure was observed.

### Conclusion
P-HRM-I is a feasible and valuable addition to standard HRM investigations in dysphagia assessment. The pharyngeal classification scheme enables quantification and definition of swallowing biomechanics, which may inform management.

1.        Omari, T., et al., Using high resolution manometry impedance to diagnose upper esophageal sphincter and pharyngeal motor disorders. Neurogastroenterology & Motility, 2022. **35**(1).

**Table 1:** P-HRM-I Classification Criteria (based on the mean of a 10 ml thin liquid bolus)

| Category Title | Criteria | Pharyngeal Pressure Topography Plot Example |
|---|---|---|
| **Normal** | Hypopharyngeal PeakP 61mmHg UOS IRP < 8 mmHg, and/or IBP was <25mmHg and/or UOS MaxAd >3.7 mS |  |
| **Upper Oesophageal Sphincter (UOS) Dysfunction** | UOS IRP > 8 mmHg, and/or IBP was >25mmHg and/or UOS MaxAd <3.7 mS |  |
| **Pharyngeal Weakness** | Hypoph-PeakP < 61mmHg |  |
| **Other** | Outcome metrics do not meet normal, UOS dysfunction or pharyngeal weakness category criteria. |  |

Abbreviations: UOS, upper oesophageal sphincter, Hypoph-Peak, hypopharyngeal peak pressure, IBP, intra-bolus pressures, UOS MaxAdm, UOS maximum admittance, UOS IRP, UOS integrated relaxation pressure.

## Psychological predictors of symptom and quality of life response to the low FODMAP diet: A 6-month longitudinal study in adults with irritable bowel syndrome

Lauren P Manning[1], Caroline J Tuck[2], Maaike Van den Houte[3], Lukas Van Oudenhove[3], Jessica R Biesiekierski[4]

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[2] Department of Nursing and Allied Health, Swinburne University, Hawthorn, Australia
[3] Translational Research Center for Gastrointestinal Disorders, KU Leuven, Leuven, Belgium
[4] Department of Nutrition, Dietetics & Food, Monash University, Melbourne, Australia

### Introduction

The low FODMAP diet is an effective symptom management for 50-75% of individuals with irritable bowel syndrome (IBS), leaving 25-50% as non-responders. Determining predictors of treatment response at the individual level are lacking. The aim was to investigate predictors of symptom response to the low FODMAP diet.

### Methods

Adult IBS participants underwent a dietitian-led low FODMAP diet. Questionnaires assessing psychological factors (including expectations and behavioural avoidance), symptoms and quality of life (QoL) were collected pre-dietitian (week 0), post-dietitian (week 1), post-FODMAP restriction (week 5), post-FODMAP reintroduction (week 13) and post-personalisation (week 25). Latent class growth analysis identified classes of response trajectories. Linear mixed models tested baseline psychological score effects on symptoms and QoL over time. Cross lag panel models identified directional relationships.

### Results

112 participants (89% female, median 30±17 years) were included. There were three classes of symptom response trajectories, with majority of participants (56.2%) in the intermediate group, characteristic of moderate initial symptom severity and significant improvement. Higher treatment beliefs predicted a stronger initial symptom response (effect on linear slope p=0.036). Lower gut-specific anxiety and higher treatment control at baseline predicted a stronger reduction in symptoms and improved QoL across all time points (Fig 1).

### Conclusions

Moderate initial gut symptom severity, low levels of psychological symptoms, and positive illness perceptions and treatment beliefs predicted better response to the low FODMAP diet in adults with IBS. These findings emphasise the importance of personalised treatment, and highlight potential clinical predictors of response to the low FODMAP diet.
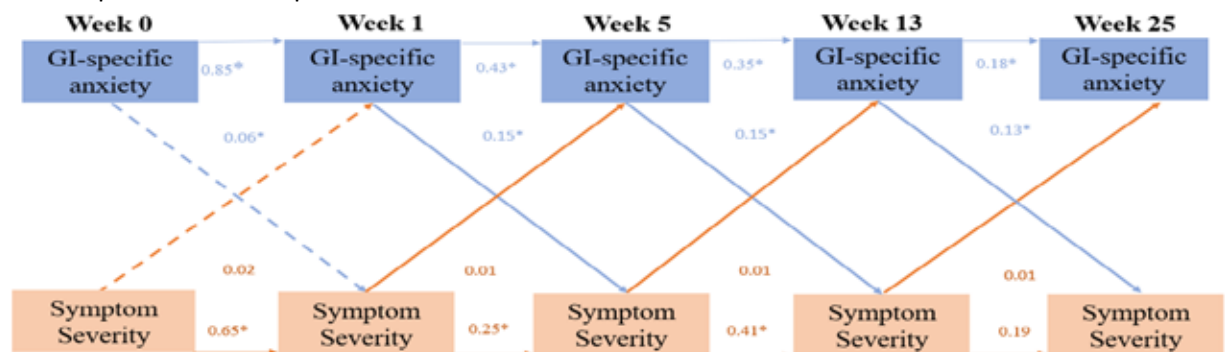


Figure 1. Cross lagged panel model linking gastrointestinal (GI)-specific anxiety with symptom severity. Values represent standardised path coefficients. Asterisks indicate significance (p<0.05).

## Evaluating Hypopharyngeal Post-Swallow Admittance as a Non-Radiological Marker of Pharyngeal Residue in Dysphagia

Alvin Cheah[1,2], Dheeraj Pandey[1,2], Julia Maclean [1,2], Peter Iung-Chiang Wu[1,2], Taher Omari[3], Michal Szczesniak[1,2]

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[2] St George and Sutherland Clinical School, University of New South Wales, Sydney, Australia
[3] College of Medicine and Public Health, Flinders University, Adelaide, Australia

**Introduction**

Post-swallow residual is a sign of swallowing impairment and poses aspiration risk. Analysis of post-swallow admittance during pharyngeal high-resolution impedance manometry (PHRIM) offers a potential non-radiological marker of pharyngeal residue. Aim of this study was to compare estimates obtained with post-swallow admittance against radiographically estimated residuals.

**Methods**

Firstly, existing PHRIM data from 48 non-dysphagic control patients were re-analysed to derive upper limit of normal (95th percentile) for hypopharyngeal post-swallow admittance (HpPostAdm). Then HpPostAdm as well as radiographically estimated residuals were independently calculated for 5ml liquid (IDDSI 0) swallows in 22 consecutive PHRIM studies with concurrent videofluoroscopy in patients with oropharyngeal dysphagia of various aetiologies. On radiology total area ($cm^2$) of pharyngeal residue was calculated in the lateral plane immediately post-swallow after spatial calibration using distance between radio opaque PHRIM sensors as reference. HpPostAdm was calculated using SwallowGateway as mean admittance spanning the hypopharyngeal segment within 0.5s after the pharyngeal stripping wave.

**Results**

There was a strong correlation between HpPostAdm and residue area observed radiographically post-swallow in the lateral plane (Spearman's rho = 0.66, p = 0.0008). 10/22 patients had abnormal HpPostAdm (>0.84 mS) and their radiologically estimated residue was higher (median 1.53$cm^2$ IQR [1.4 3.5]) than in those with normal HpPostAdm (median 0.3$cm^2$ IQR [0.08 0.88]), Mann-Whitney z = -2.77, p=0.0056).

**Conclusion**

Reasonable correlation can be observed between radiographic estimates of pharyngeal residue and hypopharyngeal post-swallow admittance measured by PHRIM. With further research it may be a useful and clinically relevant non-radiological markers in disordered swallowing.

# Mortality of disorders of brain-gut interaction (DGBI) and motility disorders in 1.3 million people

Guy D. Eslick[1], Michael P. Jones[2], Nicholas J. Talley[1]

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[2] School of Psychological Sciences, Macquarie University, Sydney, Australia

## Introduction

The natural history across the lifespan for disorders of brain-gut interaction (DGBI) and related motility disorders is largely unexplored. Long-term outcomes for some DGBIs are known, but information on whether DGBIs are associated with increased mortality is limited. The aim of this study is to determine if DGBI's and related motility disorders are associated with an increased mortality in a large population-based cohort.

## Methods

General practice electronic medical records were sourced from the United Kingdom (UK) via The Health Improvement Network (THIN). DGBIs and motility disorders were identified through Read codes corresponding to each condition. We conducted a Cox-proportional Hazard model using Hazard ratios (HR) and 95% confidence intervals (CI).

## Results

1.274 million unique patients were available for analysis, including 118,735 deaths. Mean age at first contact: 38.86 (Standard deviation (SD): 20.7). We included cases of constipation (n=15,555), irritable bowel syndrome (IBS, n=173,967), functional dyspepsia (FD, n=267,418), gastroparesis (n=107), gastroesophageal reflux (n=131,378), achalasia (n=540), and diarrhoea (n=213,967).

The analysis of mortality was adjusted for sex. A decreased mortality was observed for constipation (HR: 0.89, 95% CI: 0.85-0.93, p<0.0001), IBS (HR: 0.73, 95% CI: 0.71-0.75, p<0.0001), FD (HR: 0.80, 95% CI: 0.79-0.81, p<0.0001), gastroesophageal reflux (HR: 0.73, 95% CI: 0.72-0.74, p<0.0001), and achalasia (HR: 0.89, 95% CI: 0.71-1.10, p=0.30). However, an increased mortality was observed for diarrhoea (HR: 1.23, 95% CI: 1.21-1.24, p<0.0001), and gastroparesis (HR: 5.05, 95% CI: 3.51-7.27, p<0.0001).

## Conclusions

Only diarrhoea and gastroparesis had an increased mortality in a large community cohort cared for in primary care.

# Gastrointestinal dysfunction in a mouse model of Autism Spectrum Disorder and potential therapeutics

Vic Lin[1], Yuansong Li[1], Mitra Mohsenipour[1], Tanya Abo-Shaban[1], Kevin Mou [1], Rhiannon T Filappone[3], Mohammed Al Amoudi[1], Angela J. Perez[1], Mst Shirajum Munira[1], Joshua K Williams[1], Suzanne Hosie[1], Gayathri K Balasuriya[1], Joel C Bornstein[2], Kulmira Nurgali [3], Ashley E Franks[4], Elisa L. Hill-Yardin[1, 2]

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[2]Department of Anatomy and Physiology, The University of Melbourne
[3]Institute for Health and Sport, Victoria University, Western Centre for Health, Research and Education, Sunshine Hospital.
[4]Department of Microbiology, Anatomy, Physiology and Pharmacology, School of Life Sciences, La Trobe University.

## Introduction

Individuals diagnosed with autism spectrum disorder (ASD; autism) commonly experience gastrointestinal (GI) comorbidities including altered GI motility and permeability. We assessed GI function in the preclinical model of autism, Neuroligin-3$^{R451C}$ (*Nlgn3$^{R451C}$*) mice.

## Methods

Male wildtype and *Nlgn3$^{R451C}$* C57BL/6 mice (8-14-weeks-old, fasted for 18h) were used for *ex vivo* permeability and motility experiments. Permeability was assessed in duodenum, jejunum, distal ileum and colon segments (~4-5cm long), closed using suture thread. Tissue preparations were injected with 1mg/ml 4-kDa FITC (Fluorescein isothiocyanate; 40μL/cm), placed in DMEM and incubated at 37°C for 2h. 10mM caffeine, or 30 or 90mM of L-glutamine was delivered to some preparations. Every 30 min, external solution absorbance was measured via a FlexStation3 microplate reader (490-519nm). We characterised motility patterns in *Nlgn3$^{R451C}$* mice using *ex vivo* organ bath video imaging and our novel edge detection software interface ('GutMap'). During motility experiments, the nitric oxide synthase inhibitor, N-nitro-L-arginine (NOLA) or the GABA$_A$ antagonist, gabazine, were applied to small intestinal and colon preparations, respectively.

## Results

Fasted *Nlgn3$^{R451C}$* mice showed increased GI permeability across gut regions. Caffeine and L-glutamine administration restored permeability in *Nlgn3$^{R451C}$* mice. *Nlgn3$^{R451C}$* mice had shorter ileal contraction durations and more frequent short colonic contractions. NOLA increased jejunal contractions in wildtypes and mutants with no effect in ileal preparations. Gabazine application reduced short contraction frequency in *Nlgn3$^{R451C}$* colons.

## Conclusion

We highlight increased GI permeability and dysmotility in *Nlgn3$^{R451C}$* mice. These changes are modified by treatment with caffeine or L-glutamine (permeability), and NOLA or gabazine (motility).

# Alteration of neuroimmune pathways rescues impaired intestinal permeability and sickness behaviour in a mouse model of colitis.

Samantha M. Matta[1], Chalystha Y. Q. Lee[1], Mohammed U. Alamoudi[1,] Ashley E. Franks[2], Peter J. Crack[3], Elisa L. Hill-Yardin[1]

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[3] Department of Biochemistry and Pharmacology, The University of Melbourne, VIC, Australia

## Introduction

Autistic individuals often suffer from inflammatory disorders and gastrointestinal dysfunction. However, dynamics between the brain, gut, and immune system in autism remain ambiguous. We previously observed heightened immune activity in the $Nlgn3^{R451C}$ mouse model of autism, characterized by increased microglial density and altered macrophage morphology. These mice express a missense mutation in the Neuroligin-3 cell adhesion gene which is located on neuronal synapses. Although mice lacking the interferon-alpha/beta receptor subunit 1 ($IFNAR1^{-/-}$, a major component of inflammatory pathways) exhibit improved behaviour in chronic inflammation models, the combined effect with $Nlgn3$ mutations is unexplored.

## Methods

$Nlgn3^{R451C}xIFNAR1^{-/-}$ mice were generated by crossing $Nlgn3^{R451C}$ with $IFNAR1^{-/-}$ mice. We induced ulcerative colitis using 3% DSS (Dextran-sodium-sulfate) for 7 days and assessed colitis symptoms, colon length, and ileal intestinal permeability and collected faecal samples. Mouse sickness and exploratory behaviour was evaluated through open field and elevated plus maze tests, respectively. Sequencing and analysis of microbial samples is underway.

## Results

All DSS-treated mice developed colitis symptoms, but $Nlgn3^{R451C}xIFNAR1^{-/-}$ mice recovered faster. DSS treatment led to shortened colons in all mouse groups assayed except $Nlgn3^{R451C}xIFNAR1^{-/-}$ mice. $Nlgn3^{R451C}$, $IFNAR1^{-/-}$, and $Nlgn3^{R451C}xIFNAR1^{-/-}$ mice also exhibited resistance to DSS-induced increase in intestinal permeability. Mutants (i.e., $Nlgn3^{R451C}$, $IFNAR1^{-/-}$, and $Nlgn3^{R451C}xIFNAR1^{-/-}$ mice) exhibited reduced sickness behaviour (including locomotor impairments) compared to wild-types. $Nlgn3^{R451C}xIFNAR1^{-/-}$ mice showed increased exploratory behaviour.

## Conclusions

IFNAR1 and $Nlgn3$ genes interact to improve functional and behavioural outcomes in response to DSS-induced colitis, indicating that neuroligin-3 modulation may counteract colitis-induced phenotypes and enhance resistance to inflammatory insults.

# Implications of Oro-Pharyngeal Dysbiosis in Head and Neck cancer: Oral Microbiome and Chemoradiation Related Complications

D Pandey[1,2]*, J Maclean[1,2], M Szczesniak[2], , F Zhang[5], H Yim[1,5], P Graham[1,4], E El-Omar[1,5],  P Callahan[6], R Middleton[6], PI Wu[1,2]

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2. University of New South Wales, St George Clinical School, Sydney, NSW, Australia
3. St George Hospital, Department of Speech Pathology, Sydney, NSW, Australia
4. St George Hospital, Department of Radiation Oncology, Sydney, NSW, Australia
5. St George Hospital, Microbiome Research Centre, Sydney, NSW, Australia
6. Australian Nuclear Science and Technology Organisation, Sydney, NSW Australia

## Introduction

Emerging evidence suggests a potential role of the oropharyngeal microbiota in head and neck cancer (HNC) carcinogenesis and treatment-induced toxicities. This study aimed to: 1) explore the impact of chemoradiotherapy (CRT) on the oropharyngeal microbiome and the trajectory of its dysbiosis during and post-CRT treatment; 2) investigate whether mucositis and associated dysbiosis after CRT are risk factors for long-term dysphagia.

## Methods

Saliva microbiome samples were collected from 47 HNC patients (age 64, SD±12.2) undergoing CRT and 20 age-matched non-HNC participants (age 68, SD±10.5) as controls. HNC patients were followed up for longitudinal microbiome collection and clinical assessment at the end of treatment and 12 months after CRT treatment. Whole-genome sequencing was employed to analyse the microbiome and assess its dysbiosis trajectory, comparing them with clinical measures for mucositis and dysphagia.

## Results

The HNC oral microbiome before treatment exhibited decreased diversity compared to healthy controls and was compositionally different (Figure 1). During treatment, alpha diversity decreased significantly, and beta diversity showed significant compositional changes compared to pre-treatment HNC patients and healthy controls (p<0.05). Twelve months after treatment, microbiome diversity recovered significantly, and composition shifted Severe mucositis during treatment was associated with a higher prevalence of long-term dysphagia.

Microbiome analysis revealed no significant difference in observed species diversity between severe and mild-moderate mucositis groups before treatment but marked differences after CRT. At 12 months, severe group microbiome diversity remained low, while the mild-moderate group recovered significantly. Beta diversity showed a more profound microbiome shift in the severe group at 12 months compared to the mild-moderate group. Dysphagic patients at 12 months had lower observed species diversity than non-dysphagic patients, while the composition measured by beta diversity was not significant.

## Conclusions

This study demonstrates the impact of CRT on the oropharyngeal microbiome in HNC patients and suggests that severe mucositis and associated dysbiosis after CRT may contribute to long-term dysphagia. These findings may help to develop strategies for microbiome-based interventions to improve treatment outcomes and reduce complications in HNC patients.
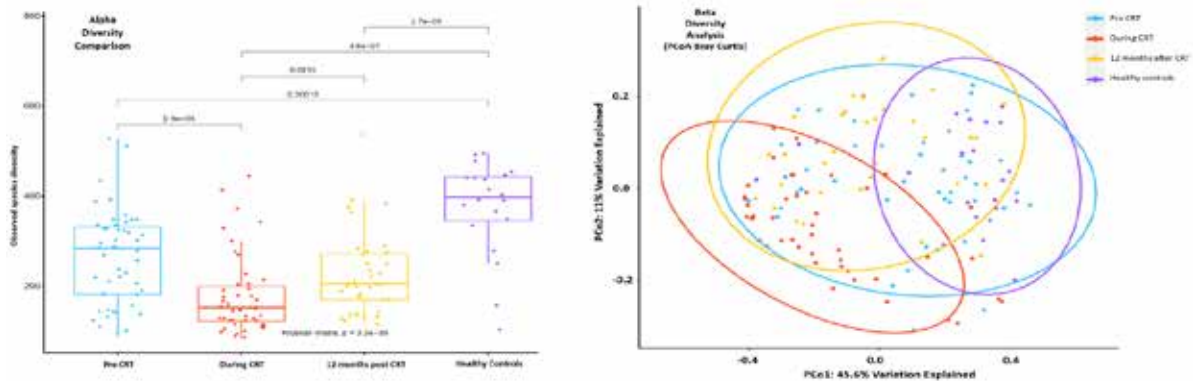
Figure 1: Alpha diversity comparison and Beta diversity analysis between healthy controls and HNC patients at 3 microbiome collection points; before, during and 12 months post CRT
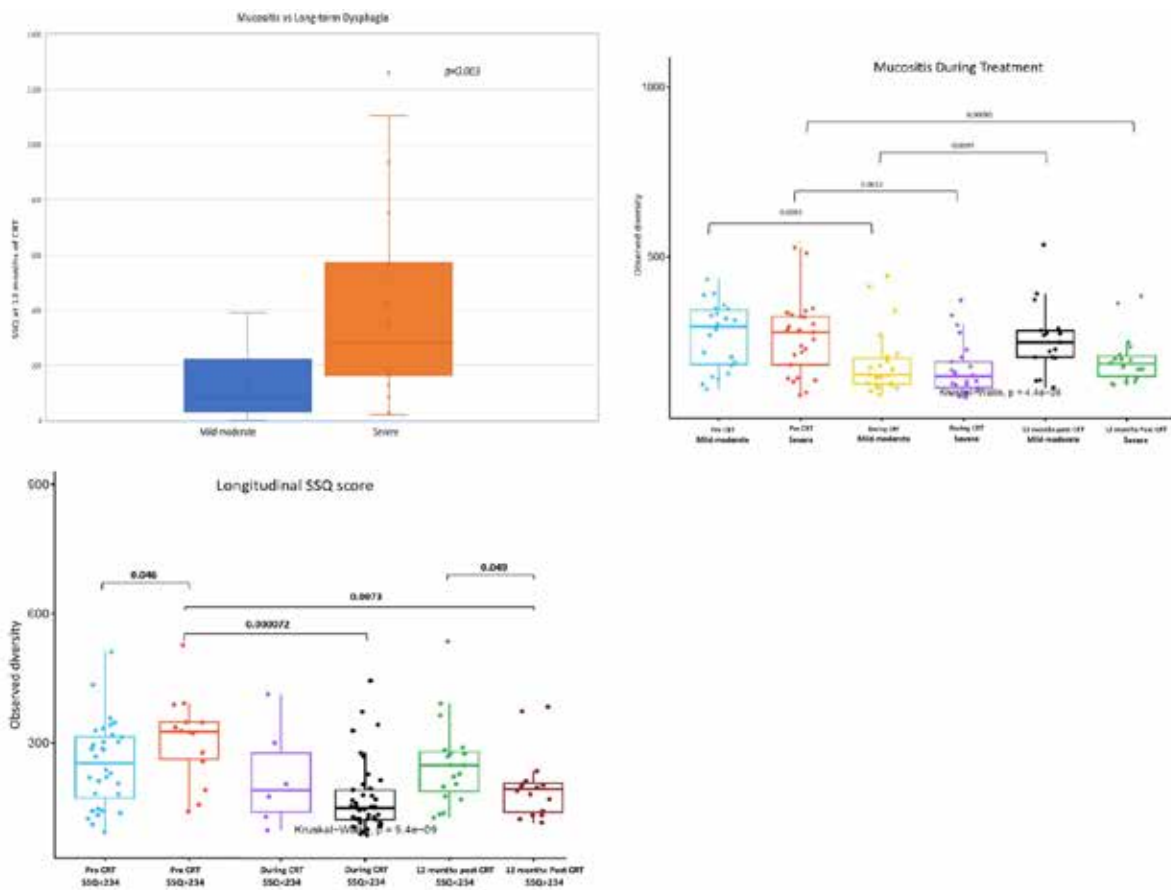


Figure 2. A) Comparison of SSQ score at 12 months between HNC patients with mild-moderate and severe mucositis during treatment. B) Longitudinal microbiome alpha diversity based on mucositis severity. C) Longitudinal microbiome alpha diversity based on longitudinal SSQ score at each microbiome collection.

# Microbiomes, way up north: metagenome analysis of the dorsal tongue microbiome in a Singapore population cohort

Charlene E. Goh[1], Mindia A. S. Haryono[2], Michelle H. Lee[3], Eveline Febriana[3], Maybritte Lim[3,4], Francine Seah[1], Fu Jia Hui[1], Julie K. Yip[5], Tan Kai Soo[1], Philip M. Preshaw[1,6], Sue-Anne Toh[3,4,7], Rohan B. H. Williams[2,8]

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[8] Singapore-MIT Alliance for Research and Technology (SMART)

## Introduction

The human oral microbiome is known to be the most complex after the gut microbiome and is likely to play a role in metabolic and immunological functions in health and disease. Here, the dorsal tongue microbiome was profiled in 472 healthy participants enrolled in a longitudinal cohort study of factors leading to type 2 diabetes in Asians: '*Assessing the Progression to Type-2 Diabetes*' (APT-2D).

## Methods

Dorsal tongue swabs were collected, genomic DNA extracted and shotgun metagenome sequencing performed (Illumina). Taxonomic profiling was performed using MetaPhlAn4. Association statistics were computed against demographic, clinical, dietary and health-related validated instrument data obtained from APT-2D.

## Results

Across all 472 subjects MetaPhlAn4 detected 443 microbial taxa, with average of 172 detected per subject (range: 87-284), with 3.5 and 9.5% accounting for 50% and 75% of cumulative relative abundance. Top ranked taxa included *Neisseria subflava*, *Prevotella melaninogenica*, *Porphyromonas pasteri*, *Rothia mucilaginosa*, *Haemophilus parainfluenzae*, *Veillonella rogosae*, *Fusobacterium pseudoperiodonticum* and *Streptococcus infantis*. Significant associations *(P<0.05)* from PERMANOVA analysis ($R^2$) were salivary pH (3.4%), BMI (1.1%), birth year (1.1%), ethnicity (1.38%) and 3 Perceived Stress Score (PSS-10) items (1.3-1.5%). No significant associations were identified with diabetes related biomarkers.

## Conclusions

In this study population, the dorsal tongue microbiome is diverse but is only of moderate complexity on a per-subject basis. A range of innate and environmental factors modulate community structure with a low degree of association, highlighting a high degree of inter-individual variation. Some high abundant member species hold previous known disease associations. Analysis of this complex data set is ongoing.

# INTRODUCING

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Heartburn
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Stomach cramps
Feeling of fulless
Abdominal pain   Abdominal cramps

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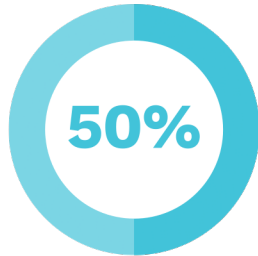
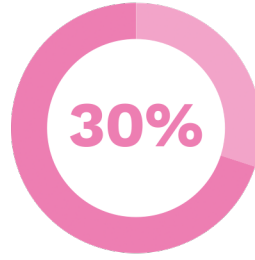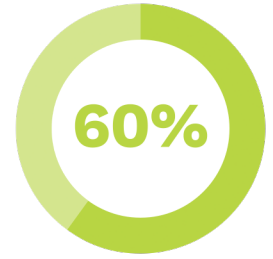Always read the label and follow the directions for use.

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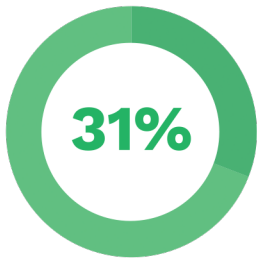
**31%** of Australian's experience digestive symptoms[1]

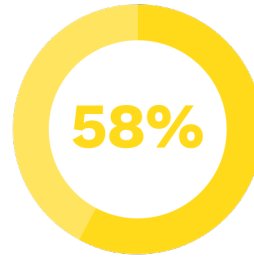**30%** of sufferers say there is nothing that works effectively to control their symptoms[1]

**60%** try and avoid using medicinal products unless symptoms are severe[1]

**50%** have changed their diets significantly to control their digestive symptoms[1]

**58%** of symptom sufferers believe natural/herbal products would be effective at relieving their digestive symptoms[1]

# What is IBS?

**Irritable Bowel Syndrome (IBS)** is a functional gastrointestinal condition that can cause symptoms such as: stomach pain, abdominal cramps, bloating, gas, diarrhoea and constipation. IBS symptoms are unpredictable and can strike at any time and can have an impact on a person's confidence and quality of life.

## IBS Sufferer Profile[1]

6% of Australians suffer from Irritable Bowel Syndrome[1]

- Adults, predominantly female
- 18 – 65 years old, skews to 45-54 year olds
- 68% of IBS sufferers were diagnosed by their GP
- 57% experience anxiety
- Proactive treaters: 2 in 3 IBS Sufferers are proactive and try to improve their digestive health[1]
- Suffer from multiple symptoms, up to 4.2 symptoms monthly
- Most common symptoms are stomach pain, abdominal cramps, bloating and gas.

## Heavy Symptom Sufferer profile[1]

9% of Australians experience more than 3 digestive symptoms monthly[1]

- Adults, predominantly female
- 18 – 65 years old, skews to 25-34 year olds
- Suffer from multiple symptoms, up to 4.9 symptoms monthly
- Most common symptoms are stomach pain, abdominal cramps, constipation, gas and bloating.

## Did you know?

IBS Sufferers and Heavy Symptom sufferers use over 5 methods to treat any digestive symptoms.[1]

**Recommend Iberogast IBS + Digestive Relief for relief of 6 digestive symptoms in one.**

**1.** Bayer Market Research - Fiftyfive 5 Project Button 2021. 2. Madisch A. Treatment of Irritable Bowel Syndrome with herbal preparations: results of a double blind, randomized placebo-controlled, multi-centre trial. Aliment Pharmacol Ther 2004; 19: 271–279. doi: 10.1111/j.1365-2036.2004.01859.x This study was supported by a grant from Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. 3. Madisch A et al. Treatment of Functional dyspepsia with a herbal preparation. A double blind, randomised, placebo-controlled, Multicenter Trial Digestion. 2004; 69:45-52. 4. Rosch W et al. A randomised clinical trial comparing the efficacy of a herbal preparation STW5 with the prokinetic drug cisapride in patients with dysmotility type of functional dyspepsia. German J Gastroenterology. 2002;40:401-408

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