Centre of Research Excellence in Digestive Health

Differentiation and capture of the human duodenal mucosa-associated microbiota by a novel ex-vivo combination of microbe culture and metagenomic sequencing

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Introduction

Functional dyspepsia (FD) is the generic term used for the "diagnosis" of functional upper abdominal pain and discomfort, with further stratification based on subjective criteria[1]. An altered microbiome referred to as "dysbiosis", is considered a hallmark and trigger for the onset and relapse of many digestive diseases and disorders. However, the functional basis and implications of such dysbiosis within the mucosa-associated microbiota in gastrointestinal diseases and disorders remain largely undefined. While advances in DNA sequencing technologies support the functional and taxonomic assessment of stool microbiota, it is challenging to use these methods with DNA extracted from gut tissue samples, with limited microbial density and/or rich in human DNA.

Research objectives

This study used a combination of microbial culture and (meta)genomic sequencing[2] to characterise the duodenal mucosa-associated microbiota (d-MAM) using biopsies from functional dyspepsia (FD) and non-FD "control" subjects.

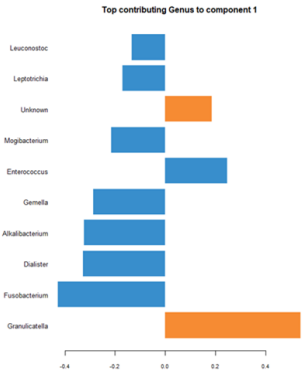
Methods

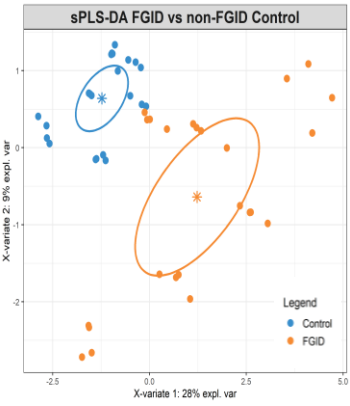
Biopsies were collected from FD and non-FD control subjects (n=6 per group) using the Brisbane aseptic biopsy device and stored anaerobically. Biopsies were transferred to habitat-simulating medium, incubated at 37°C and microbial growth monitored. These cultures were sampled and stored anaerobically. Stocks were then used on two occasions to inoculate fresh media and cultured as described above. The microbial biomass was harvested, the DNA extracted, and used for 16S rRNA sequencing. The resulting data was subjected to our standard workflow for bioinformatics analyses.

Results

The community profiles were patient-specific and both replicates from the same patient's sample were very similar to each other. The Figure below shows the sparse partial least squares differential analysis of the d-MAM profiles from the FD and non-FD "control" groups could be separated. The d-MAM communities from the non-FD "control" subjects appear to possess a greater degree of similarity to each other, whereas the d-MAM profiles recovered from different FD subjects are more variable to each other.

The panel top right shows the discriminatory bacterial taxa between the two patient groups include *Prevotella, Streptococcus, Granulicatella, Veillonella, Enterobacter,* and *Enterococcus*.





Limitations

This is pilot research, performed with a small number of samples (n=6 per group) to validate the method. While these findings are interesting and encouraging, the sample size will be need to be increased for more powerful comparisons and conclusions.

Conclusion

The d-MAM can be recovered and characterized using combined microbial culture and (meta)genomic sequencing. This approach will provide a deep, functional understanding of the d-MAM, and assist with patient phenotyping to advance the diagnosis and treatment of FD and other digestive disorders.

Selected references

1 Lacy, B.E. & Patel, N.K. Rome Criteria and a Diagnostic Approach to Irritable Bowel Syndrome. J Clin Med 6, 99 (2017).
2 Teh, J.J. et al. Novel strain-level resolution of Crohn's disease mucosa-associated microbiota via an ex vivo combination of microbe culture and metagenomic sequencing. The ISME Journal (2021).

Acknowledgments

The financial support from MSH Research Support Scheme, Steigerwald Arzneimittelwerk GmbH, and the NHMRC CRE for Digestive Health is gratefully acknowledged. We also thank the patients and nursing staff of PAH Department of Gastroenterology and Hepatology.