The Barcelona Debates on the Human Microbiome

June 25-26, 2026
CosmoCaixa, Barcelona

ABSTRACT BOOK

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ABSTRACT BOOK

We are pleased to present the abstract book of the **12th edition** of **The Barcelona Debates on the Human Microbiome**, an international meeting dedicated to showcasing the latest advances in microbiome research.

This year, a total of **56 abstracts** have been accepted, reflecting the breadth and excellence of innovative work conducted by researchers worldwide. All submissions were evaluated through a rigorous blind peer-review process carried out by the Scientific Committee, composed of 10 distinguished experts. This careful selection process ensures that the contributions included represent the highest standards of scientific quality, advancing our understanding of the human microbiome in health and disease.

All accepted abstracts will be presented as **flash communications** during the meeting. Furthermore, the Scientific Committee has identified **six outstanding abstracts** of exceptional merit; among these, one will be distinguished with the **Best Abstract Award** in recognition of its scientific excellence and potential impact.

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ABSTRACTS BY TOPIC

Methods

1. A Customizable Consensus Framework for Microbiome Differential Abundance

Martina Cardinali[1], Toni Gabaldón[2]

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Differential abundance (DA) analysis in microbiome studies is highly method-dependent, and different tools can produce discordant results because of differences in statistical assumptions, normalization strategies, sparsity handling, and false discovery control. This variability complicates interpretation, reduces confidence in the final set of candidate taxa and makes it difficult to compare the results across studies.

We are developing a consensus-based framework to combine multiple DA methods and improve the robustness of microbiome findings.

Several DA methods were assessed on simulated microbiome datasets with known ground truth and varying signal characteristics. Their performance was summarized using false discovery rate (FDR) and F1 score, taking into account both taxon identity and direction of effect. These results were then used to define consensus strategies that combine evidence across methods and prioritize taxa showing more consistent support. Consensus rules and thresholds were calibrated on the simulated data and then applied to the real dataset. The biological plausibility of the resulting taxa was further explored by comparison with condition-associated microbial signatures reported in BugSigDB.

In preliminary analyses, the consensus framework showed a more favorable balance between FDR control and F1 score in the simulated datasets than individual methods considered separately. The approach also reduced disagreement across methods and yielded a more interpretable set of candidate taxa in the real dataset. Comparison with BugSigDB suggested that the consensus-derived findings were biologically plausible and consistent with previously reported condition-associated microbial patterns.

This work presents a simulation-calibrated consensus framework for microbiome DA analysis that aims to improve robustness, interpretability, and transparency in integrating multiple methods.

Back

Consensus tool for robust and comparable results in microbiome differential abundance analysis

Martina Cardinali-Benigni[1,2,3], **Toni Gabaldón**[1,2,3,4]

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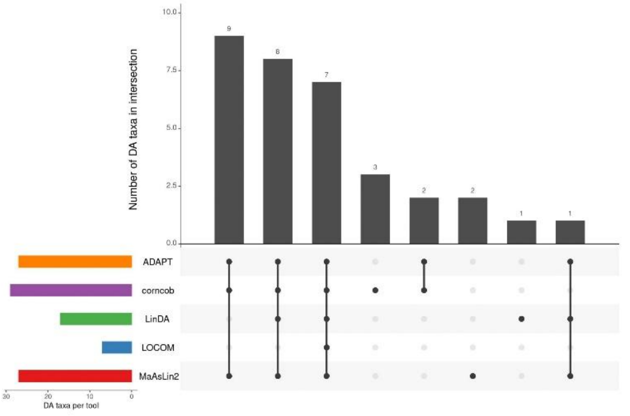
Background

Differential abundance (DA) analysis is central to identifying microbial signatures of health and disease. Yet, multiple statistical tools exist for this task, each relying on different assumptions, and they produce **discordant results on the same dataset**.

No single tool is consistently optimal: performance depends on the community structure, sample size, and effect size of each study. This makes any fixed method choice **arbitrary** and **undermines reproducibility** across studies, calling for a **data-adaptive consensus strategy**.



Aim

- Develop a consensus approach for differential abundance analysis that **integrates multiple tools** and **adapts to each dataset**
- Produce robust, comparable results and reduce the misidentification of DA taxa inherent to single-method analyses

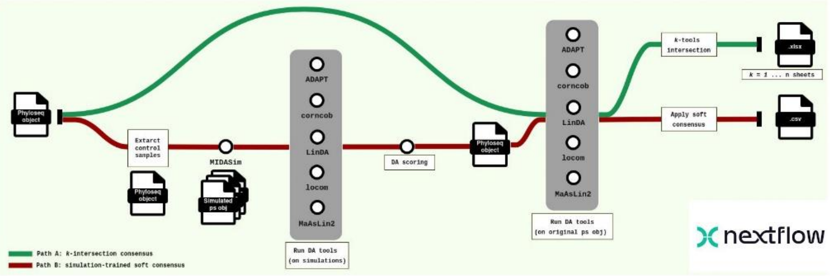
Methodology

- Five complementary tools for differential abundance analysis
- Consensus call based on *k*-tools (Path A) or on weighted scores (Path B)
- Simulation-based (MIDASim) ground-truth
- Reproducible Nextflow workflow, fully containerised (Docker/Singularity), single Phyloseq RDS input

Data

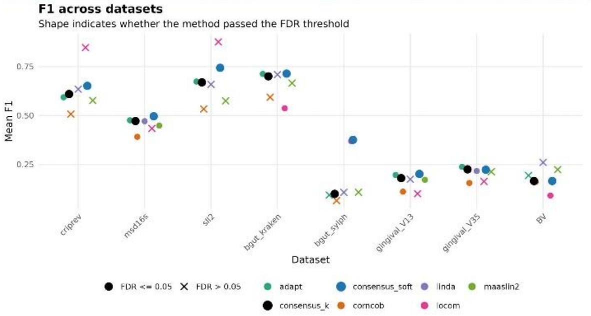
In-house datasets	BGUT	
	CRIPREV	
	SLL1	
	SLL2	
Public datasets **MicrobiomeBenchmarkData**	Gingival_V13	
	Gingival_V35	
	BV	

Workflow



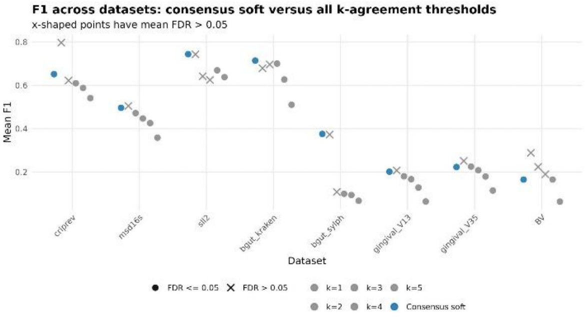
Results

Consensus approach Vs. single tools


F1 across datasets

Shape indicates whether the method passed the FDR threshold

Path A Vs. Path B


F1 across datasets: consensus soft versus all k-agreement thresholds

x-shaped points have mean FDR > 0.05

Conclusions

- Best **single tool** is **dataset-dependent**
- Consensus approach **removes the arbitrary method choice**
- Based on simulated ground truth
  - Consensus strategies **outperform any stand-alone tool** across datasets
  - The weighted score achieves **competitive or superior F1 with controlled FDR**, adapting to each dataset's characteristics
- **Fully automated and reproducible** Nextflow pipeline
- Boosts **robustness and trust** in differential abundance results
- Consistent use across studies make microbial signature findings **comparable**

2. Benchmarking the Identification of Mobile Genetic Elements from de Novo Gut Metagenome Assemblies

Arnau Noguera-Segura[1], Marc Pons-Tarín[1], Sara Vega-Abellaneda[1], Zaida Soler[1], Chaysavanh Manichanh[1]

[1] Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain

As shotgun sequencing becomes more widely available, taxonomic and functional profiling of bacterial communities has become increasingly possible. However, characterizing mobile genetic elements (MGEs) such as plasmids and bacteriophages remains challenging, although they have an important role in microbiome community dynamics and, particularly, in horizontal gene transfer. Accurate detection and quantification of MGEs in metagenomic datasets are hindered by their high diversity and variability, and their frequent sequence similarity with host genomes.

In this work, we employ simulated reads from mock communities composed of bacteria, archaea, plasmids, and viruses to determine the optimal bioinformatic workflow for assembly-based MGE analysis. Bacterial and archaeal compositions reproduce those observed in real faecal samples, while plasmids and phages are randomly selected among those with bacterial hosts present in the community. This framework enables direct benchmarking against a known truth.

We explore a range of assembly-based pipelines, including strategies to improve assemblies such as binning and contig grouping based on gene coabundance among several samples. Performance is assessed at several points of the pipeline through standard metrics like precision, recall, F-score and the completeness and correctness of recovered sequences. Additionally, we explore the effects of sequencing depth and community complexity on MGE recovery.

By providing a reproducible benchmarking framework, this study aims to identify strengths and limitations of current approaches and to inform the development of best-practice workflows for MGE profiling in complex metagenomes. Ultimately, this work seeks to support more robust investigations of microbial ecology, evolution, and horizontal gene transfer using shotgun metagenomic data.

Back

Benchmarking the identification of Mobile Genetic Elements from *de novo* gut metagenome assemblies

Arnau Noguera-Segura[1, 2], Marc Pons-Tarín[1], Sara Vega-Abellaneda[1], Zaida Soler[1], Chaysavanh Manichanh[1, 3]*

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[3] CIBER of Hepatic and Digestive Diseases, Instituto de Salud Carlos III, Spain

*Corresponding author
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Background

The gut microbiome is a highly complex ecosystem that includes not only bacteria, archaea and fungi, but also mobile genetic elements (MGEs), such as **plasmids**, **viruses** and **transposons**, that are capable of **horizontal gene transfer**.

MGEs are of special interest because they facilitate microbial adaptation to selective pressures, potentially by carrying **antimicrobial resistance (AMR) genes**, and because they can disseminate rapidly on a global scale.

However, their high variability and complexity make them hard to identify and distinguish from host genomes in metagenomic assemblies.

Objectives

- To build ***in silico* mock communities** to simulate gut metagenome sequencing.
- To benchmark **assembly**, **binning** and **MGE identification** strategies on the simulated data.
- To design an optimized bioinformatic pipeline for the recovery and characterization of **MGEs** from human gut metagenomic datasets.

Methods

1 *In silico* mock communities

Bacteria and archaea

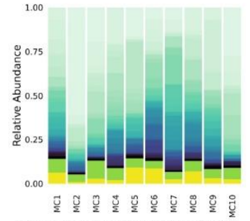
Mock compositions following real metagenomes (as profiled by MetaPhlAn4)

Complete/chromosome-level genome assemblies downloaded from NCBI

Plasmids and viruses

Sequences with known hosts obtained from UHGV and PLSDB

Random log-normal distributions for MGEs with bacterial hosts in the samples

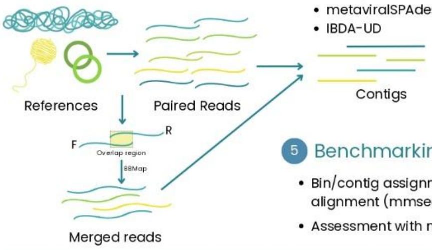


Composition of the 10 simulated samples.

2 Reads simulation

Paired Illumina reads generated with Metagenomic Sequence Simulator (MeSS)

~6Gbp/community



3 Assembly

Tested software:

- MEGAHIT (+ meta-large)
- SPAdes
- metaSPAdes
- metaplasmidSPAdes
- metaviralSPAdes
- IBDA-UD

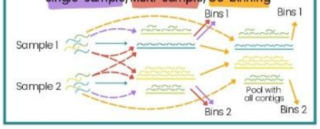
4 Binning

Tested software:

- COMEBin
- SemiBin 2
- MetaBAT 2
- MaxBin 2
- Vamb

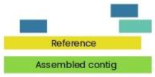
Coverage strategies

Single-sample/Multi-sample/Co-binning



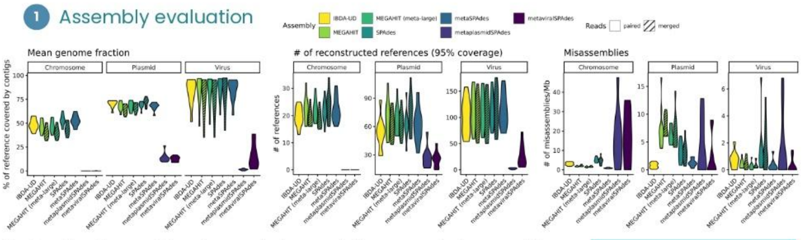
5 Benchmarking

- Bin/contig assignment to reference through alignment (mmseq2) → identity + coverage
- Assessment with metaQUAST and BinBencher



Results

1 Assembly evaluation

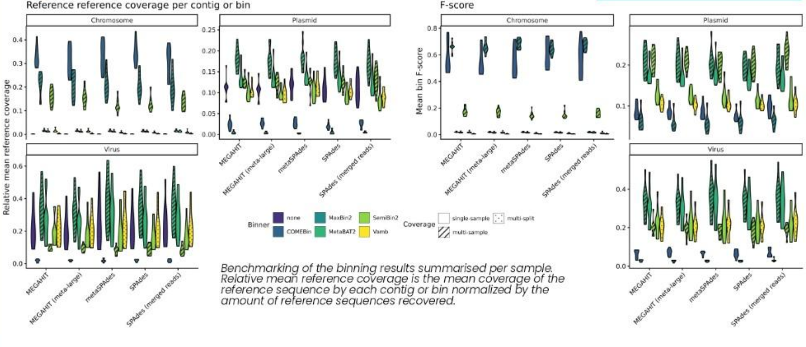


Benchmarking of the assembly results summarised per sample. Mean genome fraction: mean of the percentage of each reference recovered for a sample (in any number of contigs); # of reconstructed references: amount of reference sequences found at least with a coverage of 95% among the assembled contigs; misassemblies: number of misasseblies per length of the recovered references.

Best-performing assemblers:
SPAdes (merged), metaSPades

Best-performing binner:
MetaBat2 (multi-coverage)

2 Binning evaluation



Benchmarking of the binning results summarised per sample. Relative mean reference coverage is the mean coverage of the reference sequence by each contig or bin normalized by the amount of reference sequences recovered.

Conclusions

Short-read metagenomic sequencing can be applied to study the **mobile** through an **assembly-based** approach.

This work evaluates several **assembly** and **binning** software alternatives and shows that the optimal pipelines for MGEs may differ from those that perform best for bacterial chromosome assemblies. In addition, binning, particularly when based on **multi-sample coverage**, can improve performance, although the effect is substantially more pronounced for chromosomal sequences than for MGEs.

Future steps include evaluating alternative contig-grouping approaches and **bin-refinement** algorithms.

Acknowledgements

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References

1. Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: The agents of open source evolution. *Nature Reviews. Microbiology*, **3**(9), 722–732. https://doi.org/10.1038/nrmicro1235
2. Han, H., Wang, Z., & Zhu, S. (2025). Benchmarking metagenomic binning tools on real datasets across sequencing platforms and binning modes. *Nature Communications*, **16**(1). https://doi.org/10.1038/s41467-025-57957-6
3. Kieser, S. (2023, August 11). *Binning with more than one Sample*. Silas Kieser. https://silask.github.io/post/cobinning/

3. Funomic3: Optimized Pipeline and Databases for Mycobiome Profiling

Sara Vega-Abellaneda[1], Zixuan Xie[1], Arnau Noguera-Segura[1], Gerard Serrano-Gomez[1], Maria Tarrat-Castells[1], Chaysavanh Manichanh[1]

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Despite the critical role of the gut mycobiome in human health, research has been predominantly centred on bacteria, leaving fungal communities understudied, primarily due to technical limitations in current methodologies. To address these challenges, we previously developed FunOMIC, a tool comprising both taxonomic and protein databases alongside a pipeline for profiling the mycobiome in shotgun metagenomic samples, and FunOMIC2, an enhanced version of the original tool featuring updated databases and an improved analysis pipeline. Here we present FunOMIC3, with the intention to refine fungi detection in low fungal biomass samples. This tool presents an improved database creation pipeline, which allows its expansion from around 2M marker sequences to around 13M marker sequences, as well as including 1640 more fungal species. The new taxonomy profiling pipeline has been refined to improve the fungal species quantification and avoid overrepresentation of most studied species. Some parameters have been optimized to improve species detection while avoiding false positives. Simulated mock communities were employed to compare the performance of the new FunOMIC3 pipeline and databases with the previous FunOMIC2 (FunOMIC1 could not be included in the analysis due to lack of maintenance). This comparison demonstrates that FunOMIC3 is capable of increasing the average F-score from 0.55 to 0.74, primarily by decreasing the false positives by 56%. The new pipeline also shows an improvement on the fungal quantification, assessed by the root mean square error between the expected and predicted relative abundance of the true positives.

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FunOMIC3

Optimized pipeline and databases for mycobiome profiling

Contact us!

Sara Vega-Abellaneda[1, 2], Zixuan Xie[1], Arnau Noguera-Segura[1], Gerard Serrano-Gomez[1], Chaysavanh Manichanh[1, 2, 3]*

[1] Microbiome Lab, Vall dHebron Institut de Recerca , Spain [2] Departament de Medicina, Universitat Autonoma de Barcelona, Spain
[3] CIBER of Hepatic and Digestive Diseases, Instituto de Salud Carlos III, Spain * Corresponding author: **cmanicha@gmail.com**

INTRODUCTION

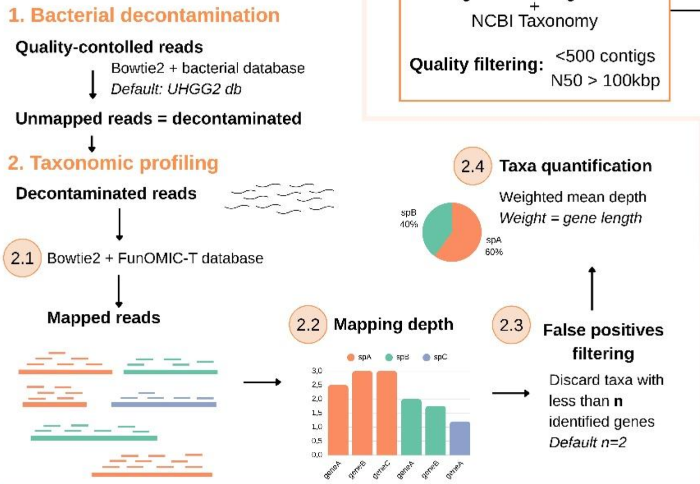
Due to **technical limitations** in current methodologies, microbiome research has been predominantly centred on bacteria, leaving **fungal communities understudied,** particularly in ecosystems with low fungal biomass.
We previously developed **FunOMIC**, a tool comprising both taxonomic and protein databases, along with a pipeline for profiling the mycobiome in shotgun metagenomic samples, and **FunOMIC2**, an enhanced version of the original tool featuring updated databases and an improved analysis pipeline.
Here, we present **FunOMIC3** to further refine fungal detection in **low fungal biomass samples** across diverse ecosystems.

OBJECTIVES

- **Expand** the taxonomy database
- Refine fungal detection in **low fungal biomass samples**, such as faecal samples
- Improve the accuracy of **fungal quantification**
- Benchmark performance using **simulated mock-communities**

METHODS

FunOMIC3 pipeline



1. Bacterial decontamination

Quality-contolled reads
↓ Bowtie2 + bacterial database
Default: UHGG2 db
Unmapped reads = decontaminated
↓

2. Taxonomic profiling

Decontaminated reads
↓
2.1 Bowtie2 + FunOMIC-T database
↓
Mapped reads
→
2.2 **Mapping depth**
→
2.3 **False positives filtering**
Discard taxa with less than **n** identified genes
Default n=2
↑
2.4 **Taxa quantification**
Weighted mean depth
Weight = gene length

Database creation: FunOMIC-T v3

1. Data collection
Fungal Genbank genomes
+
NCBI Taxonomy
Quality filtering: <500 contigs
N50 > 100kbp

→

2. BUSCO v6
Extract **single-copy ortholog** genes
MetaEuk + OrthoDB v12

→

3. Sequence clustering
CD-HIT **99% identity**
Remove non-specific sequences

Performance assessment

Simulated mock communities
InSilicoSeq software
Illumina Novaseq error model:
paired 150 bp reads

	Low fungi	High depth	High fungi
Total reads	50 000 000	100 000 000	50 000 000
Bacterial reads	49 500 000	99 000 000	49 050 000
Fungal reads	50 000 (0.1%)	100 000 (0.1%)	500 000 (1%)
Other reads	450 000	900 000	450 000

Table 1. Composition of the 3 simulated mock community variants. Number of simulated reads.

RESULTS

Database expansion

	FunOMIC-Tv1	FunOMIC-Tv2	FunOMIC-Tv3
Total sequences	1 698 953	2 003 720	13 642 558
Mean sequence length	2601.3	2713.3	1738.0
Total species	1897	3011	3673

Table 2: Size comparison of the three versions of the FunOMIC-T database

Improved quantification

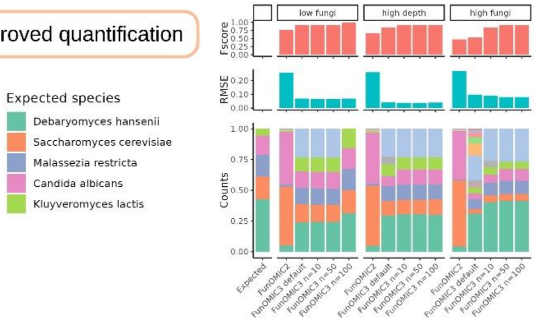


Figure 1: Overview of the composition of the simulated mock-community Mock1. Bottom panel shows expected relative composition in comparison with the composition predicted by FunOMIC2 and FunOMIC3 pipeline. Top panels show Fscore and RMSE (root mean square error) values for these predictions.

Performance increase

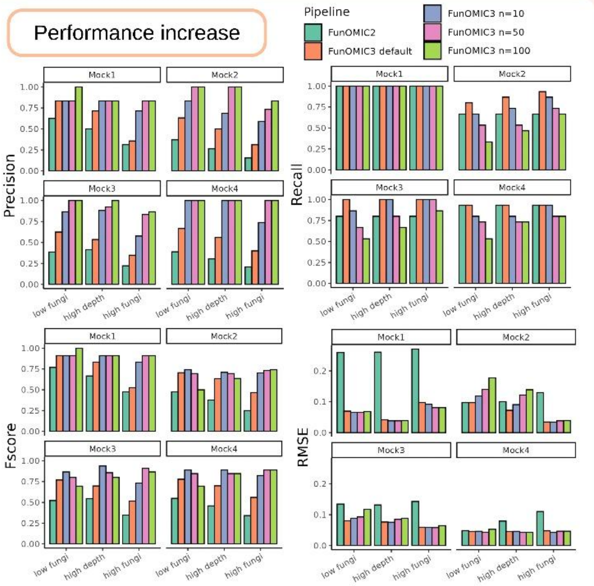


Figure 2: Performance scores of the FunOMIC2 and FunOMIC3 pipelines across 4 different simulated mock-communities. Mock-communities were tested with 3 variants: low fungi, high fungi and high depth. FunOMIC3 pipeline was tested with 4 different values for the *n* parameter: 2 (default), 10, 50 and 100.

CONCLUSIONS

1. The FunOMIC-T v3 database has been expanded by **increasing the number of single-copy orthologs** extracted from each genome.
2. The FunOMIC3 pipeline shows **better overall performance** than FunOMIC2, particularly through a **reduction in false-positive assignments.**
3. The FunOMIC3 pipeline yields a **more accurate quantification** than FunOMIC2, avoiding the overrepresentation bias of some species like *S. cerevisiae* or *C. albicans.*

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CoFunded by the European Union

4. Implementing a Robust, Scalable Pipeline for VHIO's Fecal-Sample Biobank Metagenomic Analyses

Ángel García de la Torre García[1], Lara Nonell[1], Stefania Napoli[1], Alba Santiago[1], Laura Boleda[1], Diego Arregui[1], Mónica Aguilera[1], Garazi Serna[1], Lidia Alonso[1], Paolo Nuciforo[1]

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Introduction: At VHIO we have a growing repository of fecal patient samples, the pan-cancer stool biobank, to better understand the role of the gut microbiome in cancer. Since shotgun metagenomics provides more comprehensive and quantitative insights into microbial communities, we adopted shotgun sequencing as the standard approach for these stool samples. Consequently, a robust, scalable bioinformatics pipeline is required to (i) process sequencing data under optimal conditions and (ii) handle the increasing volume of samples.

Methods: We built a reproducible pipeline for shotgun metagenomics analysis based on the nf-core taxprofiler workflow and a suite of custom downstream scripts and applied it to 294 stool sequencing runs from 175 longitudinally collected samples.

The pipeline was configured for VHIO's high-performance computing cluster with the following steps: (i) initial pre-processing, quality control and host read. (ii) taxonomic classification of non-human reads using Kraken2, followed by abundance re-estimation with Bracken. Bracken outputs are converted into Phyloseq-compatible format, filtered and normalized for downstream analyses.

Results: The pipeline was successfully deployed at VHIO's HPC and applied to the pilot dataset. Quality control of raw sequencing data indicated high overall read quality. Over 85% of the samples had less than 20% of reads that map to the human genome, with an average of 9%. The pipeline efficiently classified 82% of all non-host reads as bacterial taxa. Downstream analyses included exploratory compositional profiling, alpha- and beta- diversities and differential abundance testing.

Conclusions: We have assembled a standardized, scalable and reproducible bioinformatics pipeline for the analysis of shotgun metagenomic data from VHIO biobank stool samples. The framework is integrated in the internal HPC and can be extended to incorporate additional sample types and other cancer indications..

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IMPLEMENTING A ROBUST, SCALABLE PIPELINE FOR VHIO'S FECAL-SAMPLE BIOBANK METAGENOMIC ANALYSES

A. García de la Torre[1], S. Napoli[2], A. Santiago[2], L. Boleda[2], D. Arregui[2], M. Aguilera[2], G. Serna[2], L Alonso[2], P. Nuciforo[2], L. Nonell[1]

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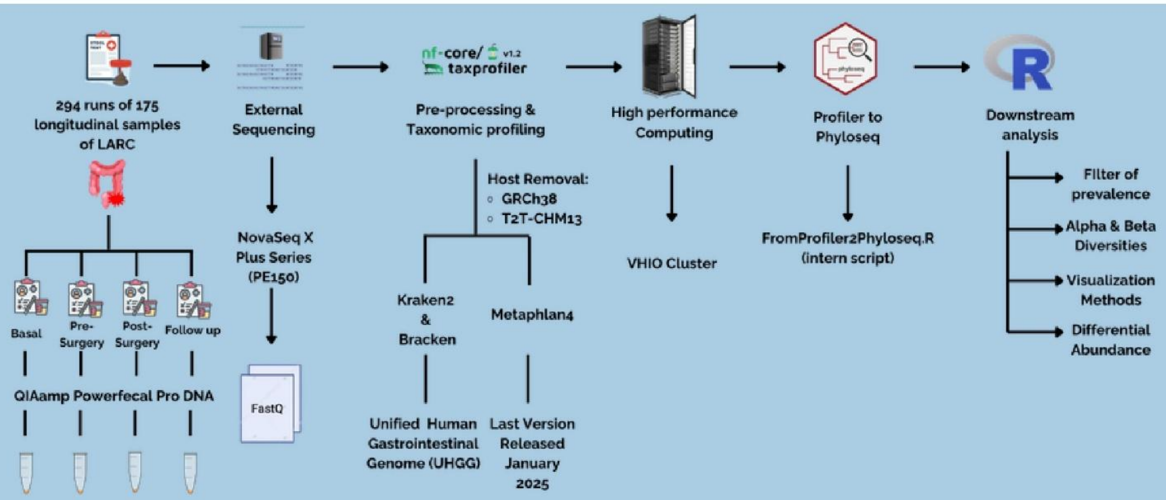
INTRODUCTION

At VHIO we have a growing repository of fecal patient samples, the pan-cancer stool biobank, that can be screened routinely to better understand the role of the gut microbiome in cancer. Since shotgun metagenomics provides more comprehensive and quantitative insights into microbial communities compared to 16S rRNA amplicon sequencing, we have decided to adopt shotgun sequencing as the standard approach for these stool samples. Consequently, a robust, scalable bioinformatics pipeline is required to (i) process sequencing data under optimal conditions and (ii) handle the increasing volume of samples stored in the biobank.

METHODS



294 runs of 175 longitudinal samples of LARC

Basal

Pre-Surgery

Post-Surgery

Follow up

QIAamp Powerfecal Pro DNA

External Sequencing

NovaSeq X Plus Series (PE150)

FastQ

nf-core/ taxprofiler v1.2

Pre-processing & Taxonomic profiling

Host Removal:
- GRCh38
- T2T-CHM13

Kraken2 & Bracken

Unified Human Gastrointestinal Genome (UHGG)

Metaphlan4

Last Version Released January 2025

High performance Computing

VHIO Cluster

Profiler to Phyloseq

FromProfiler2Phyloseq.R (intern script)

Downstream analysis

Filter of prevalence

Alpha & Beta Diversities

Visualization Methods

Differential Abundance

Figure 1. Experimental design and bioinformatic workflow for shotgun metagenomics analysis. 294 stool sequencing runs from 175 longitudinally collected LARC samples from the VHIO stool biobank were processed. The nf-core taxprofiler (Stamouli et al., 2023). pipeline was implemented on VHIO's high-performance computing cluster using Kraken2 (Wood and Salzberg, 2014). and MetaPhlAn4 (Blanco-Míguez et al., 2023) tools. Taxonomic profiler outputs were subsequently converted into a Phyloseq-compatible (McMurdie and Holmes, 2013) format, enabling filtering and normalization for downstream analyses.

RESULTS

The pipeline was successfully deployed at VHIO's HPC and applied to the pilot dataset. Quality control of raw sequencing data indicated high overall read quality. Over 85% of the samples have fewer than 20% of reads mapping to the human genome, with an average of 9% host reads per sample. After host removal, the taxprofiler workflow efficiently classified 82% of all non-host reads as bacterial taxa. Downstream analyses, implemented in R, include exploratory compositional profiling, alpha- and beta-diversity, and differential abundance testing using ANCOMBC2 and LinDA.

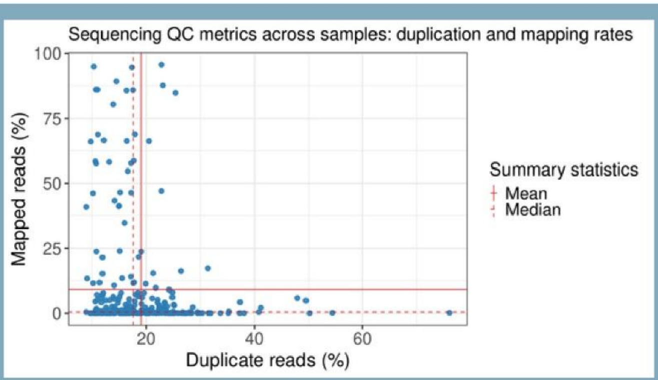


Figure 2. Sequencing quality assessment based on duplication and reads that mapped to human rates. Scatterplot showing the percentage of duplicated reads (x-axis) and mapped reads (y-axis) across all shotgun metagenomic sequencing runs.

CONCLUSIONS

We have assembled a standardized, scalable and reproducible bioinformatics pipeline for the analysis of shotgun metagenomic data from VHIO biobank stool samples. The framework is integrated in the internal HPC and can be extended to incorporate additional sample types (e.g. biopsies, oral swabs) and other cancer indications.

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5. Benchmarking Fungal Taxonomic Assignment Using ITS2 Sequences

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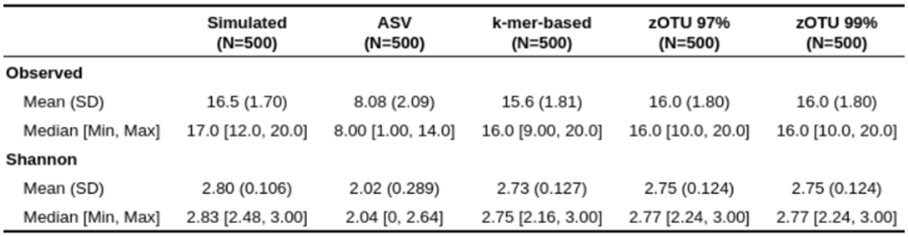
Background: Fungal taxonomic profiling based on ITS2 remains challenging due to the high sequence variability of this marker and the limited benchmarking available for different analytical strategies. While ASV-based approaches are widely used in 16S rRNA studies, their suitability for ITS2 fungal profiling is still uncertain. We compared ASV, k-mer–based and zOTU approaches to evaluate their ability to recover the expected composition of simulated ITS2 communities.

Methodology: A total of 500 benchmarking samples were generated from the UNITE database by randomly extracting ITS2 regions. Taxonomic classification was performed using DADA2 (ASV inference), Centrifuger (k-mer–based) and VSEARCH (zOTU clustering at 97% and 99% similarity) at the genus level using the UNITE database. Alpha and beta diversity, sensitivity, positive predictive value (PPV) and Spearman's correlation coefficient (ρ) were used as performance metrics.

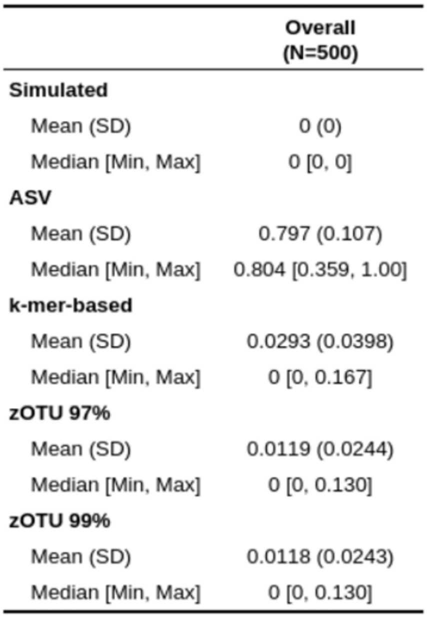
Results: ASV showed the lowest performance across alpha metrics (Table 1), markedly underestimating richness (mean 8.08 vs 16.5 expected, Fig. 1) and Shannon index (Fig.2). ASV also showed the highest Bray-Curtis mean dissimilarity (0.797, Table 2), the lowest mean sensitivity (0.555), lower mean PPV (0.768), and a negative correlation (-0.426) to the theoretical profile (Table 3). In contrast, k-mer–based and both Zotu strategies remained much closer to theoretical values. zOTU approaches at both 97% and 99% similarity showed the best overall performance; no differences were observed between them.

Conclusions: Under these benchmark conditions, ASV inference was less suitable for reconstructing ITS2 fungal communities than k-mer–based and zOTU approaches. zOTU clustering revealed higher sensitivity than k-mer-based approaches. Both zOTU strategies recovered more accurately the expected composition, supporting their use as more robust alternatives for ITS2-based fungal profiling studies.



	Simulated (N=500)	ASV (N=500)	k-mer-based (N=500)	zOTU 97% (N=500)	zOTU 99% (N=500)
Observed					
Mean (SD)	16.5 (1.70)	8.08 (2.09)	15.6 (1.81)	16.0 (1.80)	16.0 (1.80)
Median [Min, Max]	17.0 [12.0, 20.0]	8.00 [1.00, 14.0]	16.0 [9.00, 20.0]	16.0 [10.0, 20.0]	16.0 [10.0, 20.0]
Shannon					
Mean (SD)	2.80 (0.106)	2.02 (0.289)	2.73 (0.127)	2.75 (0.124)	2.75 (0.124)
Median [Min, Max]	2.83 [2.48, 3.00]	2.04 [0, 2.64]	2.75 [2.16, 3.00]	2.77 [2.24, 3.00]	2.77 [2.24, 3.00]

Table 1. Comparison of alpha diversity metrics across methodological approaches. Richness (referred as Observed) and Shannon index were calculated for the simulated reference, ASV inference, both zOTU methodological groups and k-mer-based methods.



	Overall (N=500)
Simulated	
Mean (SD)	0 (0)
Median [Min, Max]	0 [0, 0]
ASV	
Mean (SD)	0.797 (0.107)
Median [Min, Max]	0.804 [0.359, 1.00]
k-mer-based	
Mean (SD)	0.0293 (0.0398)
Median [Min, Max]	0 [0, 0.167]
zOTU 97%	
Mean (SD)	0.0119 (0.0244)
Median [Min, Max]	0 [0, 0.130]
zOTU 99%	
Mean (SD)	0.0118 (0.0243)
Median [Min, Max]	0 [0, 0.130]

Table 2. Distance between simulated data and alternative methodological approaches. Distances were calculated using Bray–Curtis dissimilarity for ASV inference, k-mer-based methods, and both zOTU groups, using the simulated dataset as the reference.

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	Simulated ASV (N=500)	Simulated k-mer-based (N=500)	Simulated zOTU 97% (N=500)	Simulated zOTU 99% (N=500)
Sensitivity				
Mean (SD)	0.555 (0.0315)	0.975 (0.0356)	0.992 (0.0205)	0.992 (0.0202)
Median [Min, Max]	0.552 [0.500, 0.688]	1.00 [0.850, 1.00]	1.00 [0.917, 1.00]	1.00 [0.917, 1.00]
PPV				
Mean (SD)	0.768 (0.0641)	1.00 (0)	0.998 (0.0116)	0.998 (0.0119)
Median [Min, Max]	0.762 [0.609, 1.00]	1.00 [1.00, 1.00]	1.00 [0.929, 1.00]	1.00 [0.929, 1.00]
Correlation				
Mean (SD)	-0.426 (0.248)	0.941 (0.107)	0.974 (0.0797)	0.974 (0.0796)
Median [Min, Max]	-0.451 [-0.876, 0.509]	1.00 [0.372, 1.00]	1.00 [0.447, 1.00]	1.00 [0.447, 1.00]

Table 3. Performance metrics across methodological approaches. Sensitivity, positive predictive value (PPV), and Spearman correlation coefficient (ρ) for the simulated reference, ASV inference, k-mer-based methods, and both zOTU methodological groups.

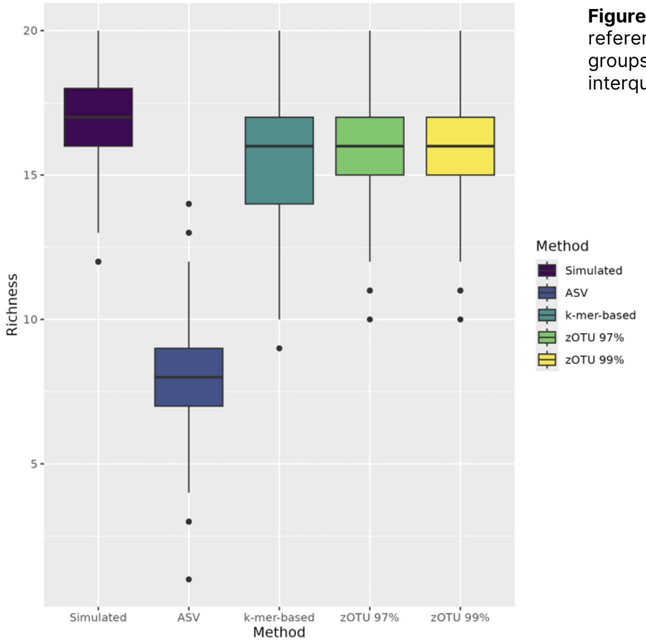

Figure 1. Richness (y-axis) distribution boxplots across the simulated reference, ASV inference, k-mer-based and both zOTU methodological groups (x-axis). The horizontal line indicates the median and boxes show the interquartile range (IQR).

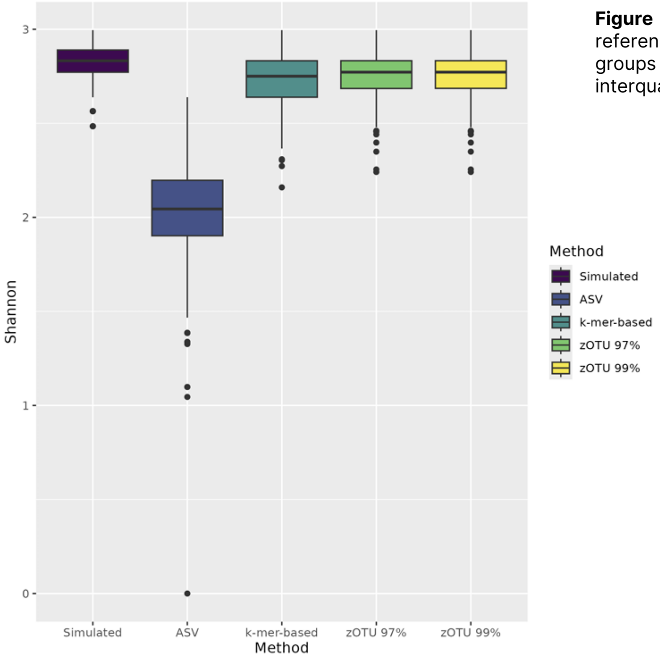

Figure 2. Shannon index (y-axis) distribution boxplots across the simulated reference, ASV inference, k-mer based and both zOTU methodological groups (x-axis). The horizontal line indicates the median and boxes show the interquartile range (IQR).

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BENCHMARKING FUNGAL TAXONOMIC ASSIGNMENT USING ITS2 SEQUENCES

Nerea Carrón · Antonio Sanchez-Baos · Adrià Cruells · Manuel Garcia-Ulloa · Pedro González-Torres

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Small things that matter.

BACKGROUND

Fungal taxonomic profiling using the ITS2 marker remains challenging due to high sequence variability and limited benchmarking for different analytical strategies. While ASV-based approaches are widely used in *16S rRNA* studies, their suitability for ITS2 fungal profiling is still uncertain. We compared ASV, k-mer–based and zOTU approaches to evaluate their ability to recover the expected composition of simulated ITS2 communities.

METHODOLOGY

500 Simulated Samples

Randomly extracted ITS2 regions using forward primers from the UNITE database

Classification Methods

DADA2 (ASV) + Bayesian classifier

Centrifuger (k-mer)

VSEARCH (zOTU 97% and 99%) + Similarity-based search

Performance Metrics

Alpha

Sensitivity

Predictive Positive Value (PPV)

Spearman's ρ

Bray-curtis dissimilarity

RESULTS

ASV showed the lowest performance across alpha metrics, markedly underestimating richness and Shannon index (**Fig. 1**). ASV also showed the highest Bray-Curtis mean dissimilarity, the lowest mean sensitivity, lower mean PPV, and a negative correlation to the theoretical profile. In contrast, k-mer–based and both zOTU strategies remained much closer to theoretical values. zOTU approaches at both 97% and 99% similarity showed the best overall performance; no differences were observed between them (**Table 1**).

Table 1. Comparison of ASV, k-mer, zOTU (97%), and zOTU (99%) methods based on sensitivity, PPV, Spearman correlation (ρ), and Bray-Curtis dissimilarity.

Metric	ASV	k-mer	zOTU (97%)	zOTU (99%)
Sensitivity	0.555	0.975	0.992	0.992
PPV	0.768	1.000	0.998	0.998
Spearman ρ	−0.426	0.941	0.974	0.974
Bray-Curtis	0.797	0.0293	0.0119	0.0118

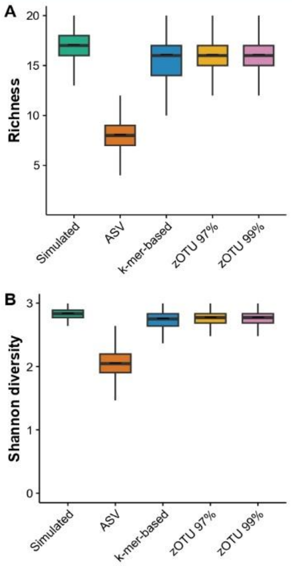

Fig 1. Boxplots of (A) species richness and (B) Shannon diversity.

CONCLUSIONS

- ASV inference was less suitable for reconstructing ITS2 fungal communities than k-mer-based and zOTU approaches.
- zOTU clustering revealed higher sensitivity than k-mer-based approaches.
- zOTU approaches (97% & 99%) showed the best overall performance, supporting their use as more robust alternatives for ITS2-based fungal profiling studies.

6. GAIA Clinical RUO: a Metagenomics Pipeline for Pathogen and Antimicrobial Resistance Detection

Erik Malinverni[1], Guillermo Andrés Reig[1], Roberto Chumaceiro[1], Daniel Julian[1], Jair Dilmó Capó[1], Riccardo Aiese Cigliano[1], Andreu Paytuví-Gallart[1]

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Background: The rising threat of pathogenic bacteria and antimicrobial resistance (AMR) necessitates rapid diagnostic tools. Metagenomic next-generation sequencing (mNGS) offers a hypothesis-free method for simultaneous pathogen detection and resistance profiling. We validated GAIA Clinical RUO v1.0, a bioinformatics pipeline for comprehensive pathogen identification, AMR, and virulence factor (VF) genotyping in clinical research.

Methods: GAIA Clinical integrates alignment-based taxonomic classification with dedicated AMR and VF profiling modules. Performance was evaluated using an in silico validation strategy with simulated Illumina paired-end reads. Three datasets were used: (A) 28 bacterial pathogens with defined AMR/VF profiles; (B) negative controls; and (C) Escherichia coli pathotypes. Samples were simulated at varying read depths, and performance metrics (sensitivity, specificity, PPV, NPV, and LOD) were calculated against ground-truth data.

Results: For pathogen detection, the pipeline demonstrated robust species identification (sensitivity 1.00, specificity 0.987). The limit of detection (LOD) was determined for all targets, ranging from 479 to 24,281 reads. E. coli pathotyping achieved high performance (sensitivity 1.00, specificity 0.997). For AMR/VF gene detection, high specificity (0.962 in Dataset A) and moderate sensitivity (0.855 in Dataset A) were achieved. AMR/VF sensitivity was strongly depth-dependent, typically requiring >25,000 reads for saturation, while specificity remained consistently high (>0.95).

Conclusions: GAIA Clinical RUO v1.0 offers robust performance for bacterial pathogen identification with low limits of detection. Accurate AMR and VF genotyping is achievable with high specificity, although it requires higher sequencing depth than taxonomic identification. This tool is a reliable solution for comprehensive metagenomic analysis of complex bacterial infections.

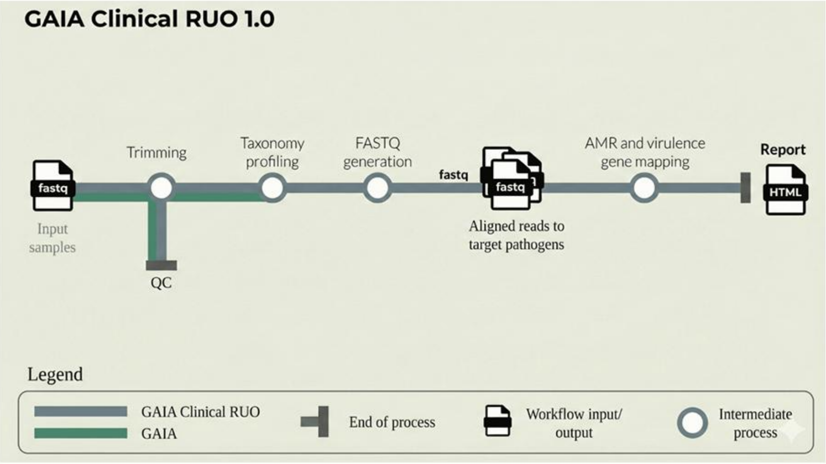

Figure 1. Schematic overview of the GAIA Clinical RUO 1.0 bioinformatics pipeline. The workflow processes raw input reads (FASTQ) through an initial phase of trimming, quality control (QC), and taxonomy profiling, which together comprise the foundational GAIA pipeline (highlighted in green). The extended GAIA Clinical RUO workflow (grey) subsequently isolates reads aligned to target pathogens into intermediate FASTQ files. These filtered reads undergo targeted mapping to identify antimicrobial resistance (AMR) and virulence genes, culminating in the generation of a comprehensive HTML report.

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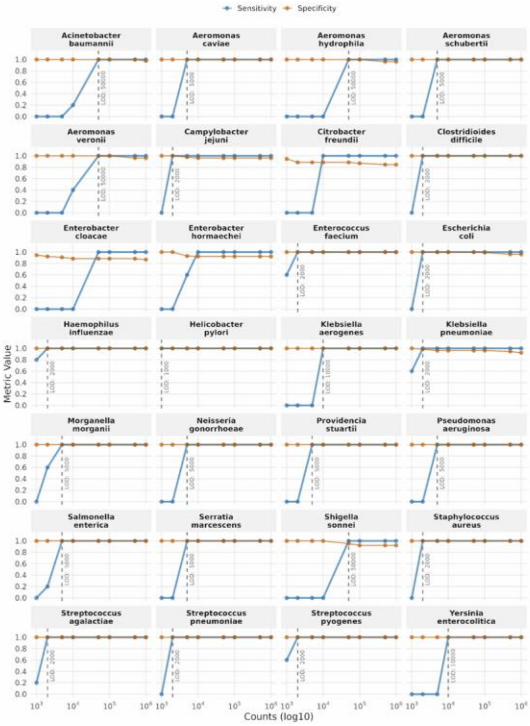

Figure 2. The multi-panel plot assesses the analytical sensitivity (blue) and specificity (orange) of antimicrobial resistance (AMR) gene detection across 28 bacteria as a function of input read depth (log10 counts). Specificity is robust, while sensitivity sharply increases with depth, consistently reaching optimal performance (1.0) at the species-specific Limit of Detection (LOD). Vertical dashed lines mark this empirically established LOD, the minimum sequence count needed for reliable AMR determinant identification.

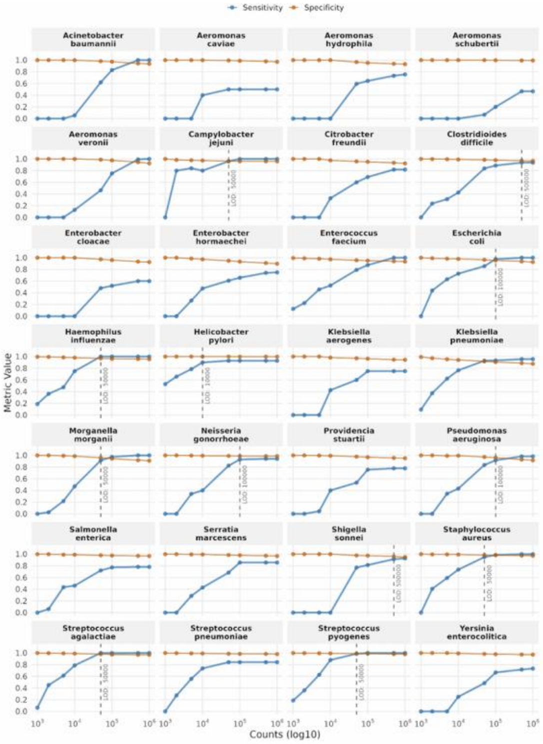

Figure 3. The multi-panel plot assesses the analytical sensitivity (blue) and specificity (orange) of virulence gene detection across 28 bacteria as a function of input read depth (log10 counts). Specificity is robust, while sensitivity sharply increases with depth, consistently reaching optimal performance (1.0) at the species-specific Limit of Detection (LOD). Vertical dashed lines mark this empirically established LOD, the minimum sequence count needed for reliable AMR determinant identification.

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GAIA Clinical RUO

a metagenomics pipeline for pathogen and antimicrobial resistance detection

Riccardo Aiese Cigliano

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Introduction

The Global Threat of AMR

Bacterial infections and AMR are one of the most significant challenges to global public health, causing ~4.95M deaths annually.

Critical Need for Speed

Rapid pathogen identification is vital for sepsis cases, where every hour of delayed therapy increases mortality rates.

Culture Limitations

Traditional culture methods are the gold standard but suffer from slow turnaround and low sensitivity for unculturable organisms.

The Metagenomic Solution

NGS offers a "hypothesis-free" shotgun sequencing alternative but requires bioinformatics expertise and informatics infrastructure.

GAIA Clinical RUO: The Bioinformatics Solution

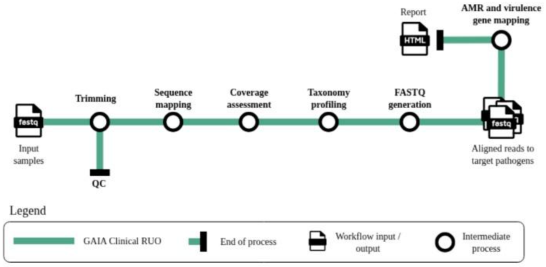
We developed GAIA Clinical RUO, a new bioinformatics solution, to help bridge the gap in pathogen detection, offering comprehensive genotyping for Antimicrobial Resistance (AMR) and Virulence Factors (VF).

Broad Compatibility

Compatible with Illumina and ONT Whole-Genome Sequencing (WGS), as well as Illumina 16S amplicon sequencing.

SequentiaHub Ecosystem

Deployed within our platform for easy execution and secure storage of all omics data.



GAIA CLINICAL RUO: IN SILICO VALIDATION FRAMEWORK

Experimental Design & Dataset Strategy

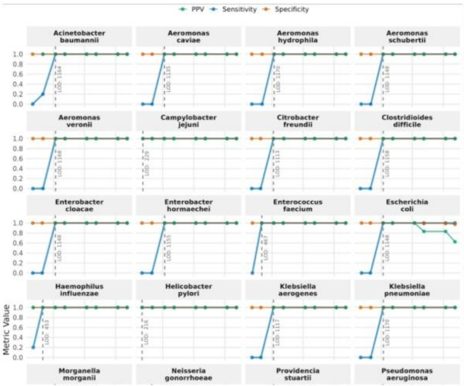
- **Healthy Control:** 5 Replicates (10M reads). Negative baseline.
- **Dataset A:** 1,120 samples: 28 pathogens with defined AMR.
- **Dataset B:** 20 samples: Pathogens lacking AMR/VF profiles.
- **DAtaset C:** 400 samples: *E. coli* pathotypes (EPEC, ETEC).

Simulation Methodology

- **Genome selection:** High-quality NCBI RefSeq assemblies iteratively selected for max gene diversity.
- **Read Generation:** ART-Illumina (2×100bp) mimicking HiSeq 2000 platform-specific error models.
- **LOD determination:** 8 abundance levels (1K to 1M reads) to find technical detection limits.

Analytical Performance

Accurate detection of clinical pathogens The pathogen detection module of GAIA Clinical RUO demonstrated excellent sensitivity (1.00) and specificity (0.999) across all conditions.



Pathogen detection **sensitivity**, **specificity** and **PPV** values of GAIA Clinical RUO on Dataset A across pathogens with varying supporting counts. The average LOD is marked with a vertical dashed line.

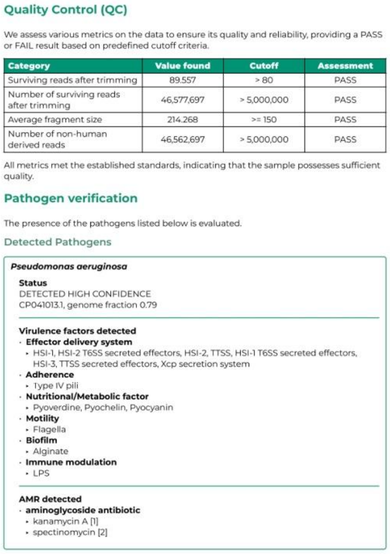
PATHOGEN SENSITIVITY
100.0%

PATHOGEN SPECIFICITY
99.9%

AMR SENSITIVITY
94.0%

AMR SPECIFICITY
98.7%

F1 COMPOSITE
98.1%



Quality Control (QC)

We assess various metrics on the data to ensure its quality and reliability, providing a PASS or FAIL result based on predefined cutoff criteria.

Category	Value found	Cutoff	Assessment
Surviving reads after trimming	89.557	> 80	PASS
Number of surviving reads after trimming	46,577,697	> 5,000,000	PASS
Average fragment size	214.268	>= 150	PASS
Number of non-human derived reads	46,562,697	> 5,000,000	PASS

All metrics met the established standards, indicating that the sample possesses sufficient quality.

Pathogen verification

The presence of the pathogens listed below is evaluated.

Detected Pathogens

Pseudomonas aeruginosa

Status
DETECTED HIGH CONFIDENCE
CP041013.1, genome fraction 0.79

Virulence factors detected

- **Effector delivery system**
  - HSI-1, HSI-2 T6SS secreted effectors, HSI-2, TTSS, HSI-1 T6SS secreted effectors, HSI-3, TTSS secreted effectors, Xcp secretion system
- **Adherence**
  - Type IV pili
- **Nutritional/Metabolic factor**
  - Pyoverdine, Pyochelin, Pyocyanin
- **Motility**
  - Flagella
- **Biofilm**
  - Alginate
- **Immune modulation**
  - LPS

AMR detected

- **aminoglycoside antibiotic**
  - kanamycin A [1]
  - spectinomycin [2]

Samples were evaluated against strict thresholds for **read survival** (>80%), **fragment size** (≥150 bp), and **minimum read depth** (**>5,000,000 total** and **non-human reads**). As established standards were not fully met, sample quality may be insufficient and results should be interpreted with caution.

Conclusions

GAIA Clinical RUO offers a standardized, reproducible, and highly sensitive framework for clinical metagenomics. This study lays the necessary groundwork for subsequent clinical utility trials, moving mNGS from a research capability to a reliable component of the precision infectious disease management toolkit.

GAIA Clinical RUO

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7. Benchmark of GAIA 3: A Versatile and Accurate Pipeline for Both Short-/Long-Read Shotgun and Amplicon Metagenomics

Roberto Malinverni[1], Erik Andrés Reig[1], Guillermo Chumaceiro[1], Daniel Julian[1], Riccardo Aiese Cigliano[1], Andreu Paytuví-Gallart[1]

[1] Sequentia Biotech S.L., Barcelona, Spain

Background: Despite advances in sequencing, accurate microbial profiling remains challenging. GAIA 3 was developed to address this with a multi-stage workflow: raw FASTQ reads undergo quality control and trimming, high-quality reads are aligned to curated databases with reliable alignments selected, and for whole-genome shotgun samples, a genome coverage module filters false positives. The purpose of this study was to benchmark GAIA 3 against state-of-the-art tools.

Methods: GAIA 3 was benchmarked on long-read shotgun datasets from PacBio HiFi and ONT R9/R10, compared to 11 established tools including Kraken2, Bracken, MEGAN-LR, and Sourmash. For 16S rRNA profiling, 125 short-read Illumina samples were assessed at the genus level against DADA2, Mothur, QIIME 2, PathoScope 2.0, and Kraken2; 18 long-read ONT samples were tested at the species level against EMU and LAST.

Results: GAIA 3 outperformed alternative tools across all sequencing methods. In long-read shotgun metagenomics, it achieved the highest F1 score (0.949), balancing sensitivity and specificity through its genome coverage module. For 16S rRNA data, GAIA 3 achieved top accuracy for short-read genus-level (F1 = 0.787) and long-read species-level (F1 = 0.532) classification. NCBI and SILVA databases performed similarly for short reads, but NCBI gave higher precision for long-read species classification, while SILVA increased recall at the cost of precision.

Conclusions: GAIA 3 demonstrates state-of-the-art accuracy and versatility across short- and long-read metagenomics. By minimizing false positives and performing robustly across reference databases, it provides a reliable, unified solution for high-resolution microbial characterization.

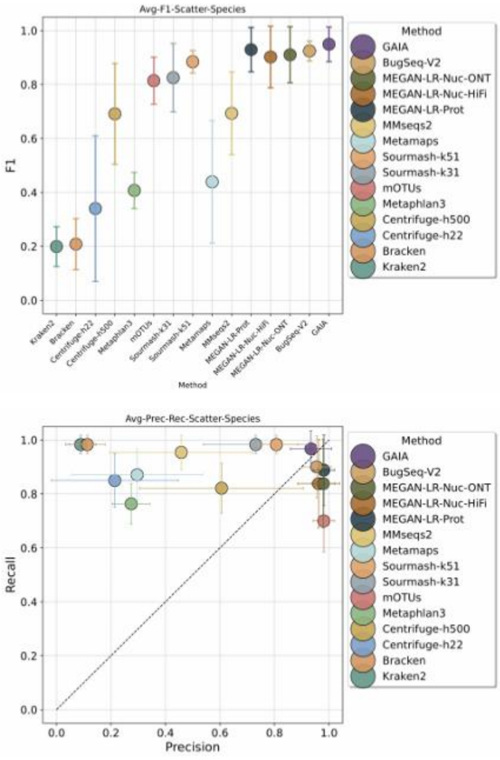

Figure 1. Averaged classification performance of evaluated methods in terms of F1-score (above) and precision and recall (below) for all long-read samples.

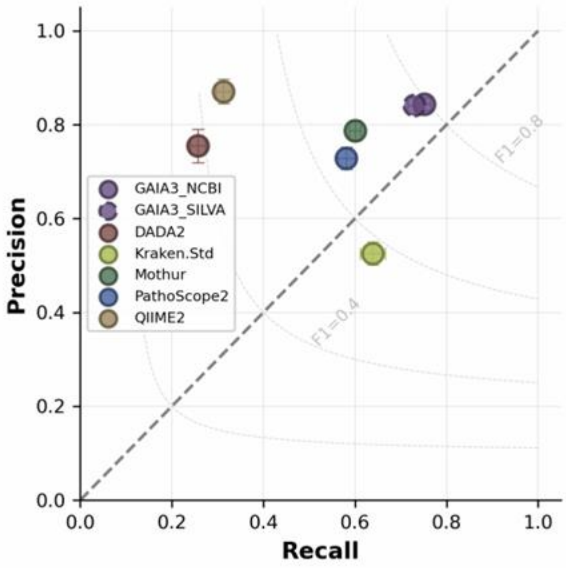

Figure 2. Averaged classification performance of evaluated methods in terms of precision and recall for all short-read samples.

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Benchmark of GAIA 3

a versatile and accurate pipeline for both short-/long-read shotgun and amplicon metagenomics

Jaïr Dilmé Capó

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Introduction

Broad Relevance of Metagenomics

Metagenomics enables culture-independent analysis of complex microbial communities, providing a powerful way to study biodiversity directly from sequencing data.

Impact Across Sectors

Widely used across human microbiome research, environmental, agriculture, food safety and clinical investigation.

A Complex Analytical Landscape

Different sample types, sequencing platforms and data make taxonomic profiling highly heterogeneous.

From Data to Biological Insight

Accurate taxonomic classification is critical to obtain robust, comparable and interpretable microbial profiles.

GAIA3: The Solution

- Input-agnostic taxonomic classification platform
- Supports **amplicon** and **shotgun** metagenomics
- Compatible with **Illumina**, **ONT** and **PacBio**
- Detects **prokaryotes**, **eukaryotes** and **viruses**
- Works with **NCBI**, **SILVA** and **custom** databases

GAIA 3

Taxonomy · **Alpha diversity** · **Beta diversity** · **Report**

BENCHMARK VALIDATION: 16S AND LONG READS

16S rRNA amplicon

Framework: Odom et al. 2023 + Zhang et al. 2023

Datasets: 125 short-read 16S | 18 ONT full-length 16S

Tools: DADA2 · Mothur · QIIME 2 · PathoScope 2.0 · Kraken2 · EMU · LAST+LCA

GAIA DBs: NCBI-based DB | Curated SILVA DB

Long-read shotgun

Framework: Portik et al. 2022.

Datasets: PackBio HiFi & ONT mock communities

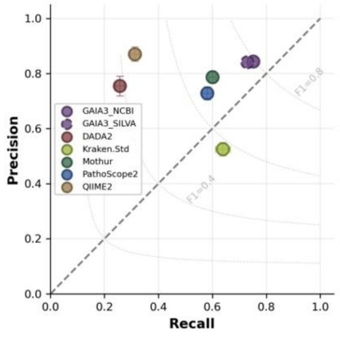
Tools: **Short-read:** Kraken2 · Bracken · Centrifuge · MetaPhlAn3 · mOTUs2

Long-read: MetaMaps · MEGAN-LR · MMseqs2 · BugSeq

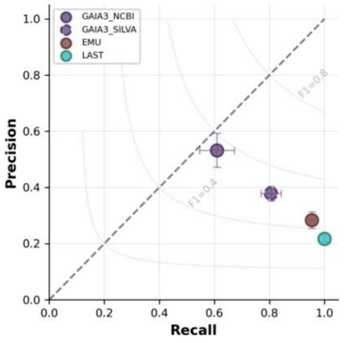
Generalized: Sourmash

PERFORMANCE EVALUATION

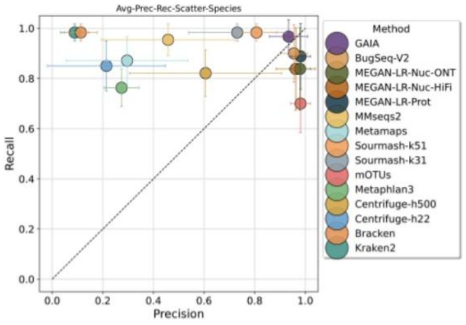
16S short reads



16S long reads



Shotgun long reads



CONCLUSION

GAIA 3 demonstrates state-of-the-art accuracy and versatility across short- and long-read metagenomics. By minimizing false positives and performing robustly across reference databases, it provides a reliable, unified solution for high-resolution microbial characterization.

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BEST ABSTRACT

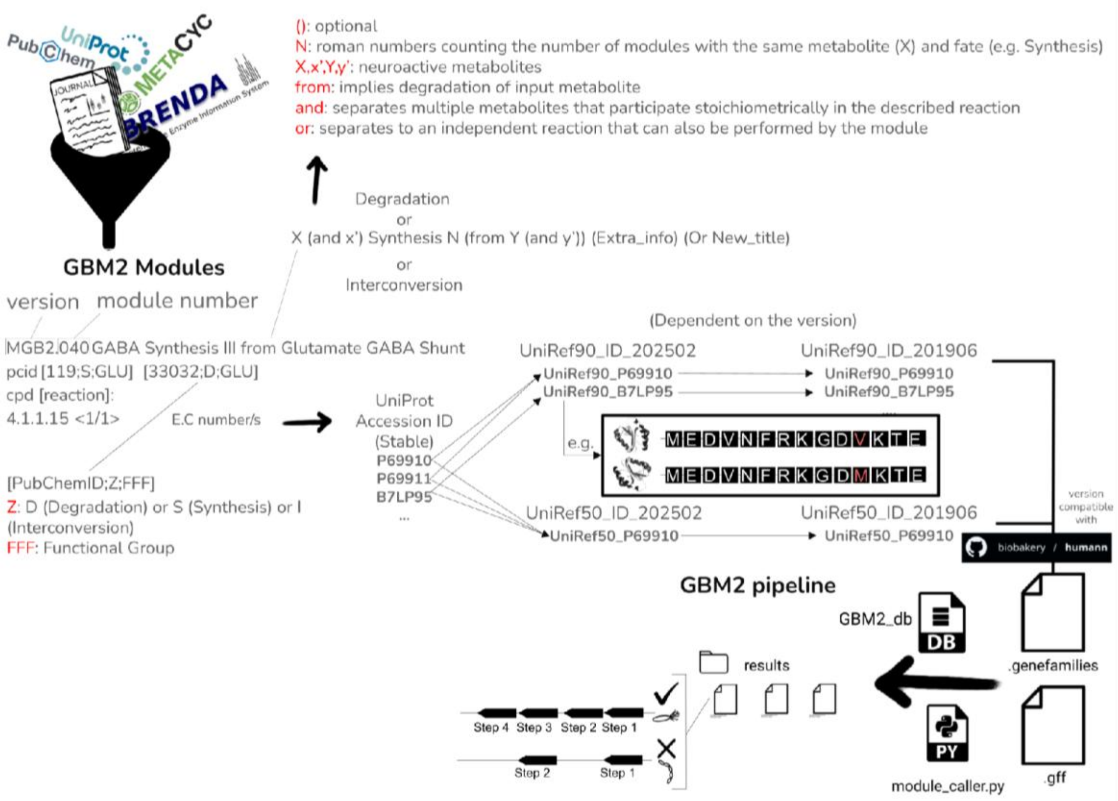
8. GBM2 Expands Functional Interpretation of Microbiome–Gut–Brain Pathways in Genomes and Metagenomes

Albert Garcia-Valiente[1], Viviana Rossi[1], Ismael Vera-Muñoz[1], Samia Squizani[1], Aitor Blanco-Miguez[3,4], Francesco Asnicar[3], Sara Vieira-Silva[2], Gwen Falony[5], Nicola Segata[4,6], Mireia Vallès-Colomer[1]

[1] MELIS Department, Universitat Pompeu Fabra (UPF), Barcelona, Spain; [2] Laboratory of Molecular Bacteriology, Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium; [3] Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy; [4] PreBiomics S.r.l., Italy; [5] Host-Microbe Interactomics Group, Animal Sciences Department, Wageningen University & Research, Wageningen, Netherlands; [6] European Institute of Oncology, IRCCS, Milan, Italy

Microbial metabolites produced in the gut can modulate neurotransmission, immune responses, and behavior, highlighting the microbiome as a relevant component of the gut–brain axis. Despite the growing burden of mental and neurological disorders, the field still lacks a comprehensive view of microbial neuroactive metabolism, in part because current methods do not capture many recently described pathways and compounds and often rely on outdated or proprietary annotation systems.

GBM2 (Gut-Brain Modules 2) is an open-source computational framework for systematic profiling of the neuroactive metabolic potential. It expands previous approaches by manually curating 187 gut-brain metabolic modules, each corresponding to a metabolic conversion spanning short-chain fatty acids, tryptophan-, tyrosine-, arginine-, and glutamate-derived metabolites, as well as bile acid, vitamin, and hormone-related pathways..



1. Valles-Colomer, M. et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol 4, 623–632 (2019)

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BEST ABSTRACT

9. Detecting Parallel Adaptive Changes in the Gut Microbiome

Aina Martinez i Zurita[1], Sharon Greenblum[2], Nandita Garud[1]

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The human gut microbiome contains a diverse ecosystem of microorganisms that impact many aspects of human health. Due to the large population size of bacteria in each human host and their short generation time, it is estimated that an average human microbiome experiences billions of de-novo mutations every day. With such a large mutational input, the same mutation may arise in multiple hosts, and if beneficial, could rise to high frequency in these hosts. Mutations changing in frequency in parallel across many hosts in response to similar selection pressures may be adaptive and thus have important functional relevance to the microbiome. Statistical methods to detect such parallelism have proved insightful in metazoan populations, yet complementary methods that account for the unique features of complex bacterial populations have not yet been developed or rigorously tested. Here we develop a statistical framework to detect cases of parallelism in temporally sampled metagenomic datasets across many hosts. We first demonstrate our method is capable of detecting adaptive loci in a previously identified antibiotic resistance gene, DNA gyrase subunit A (gyrA), using a temporally sampled metagenomic dataset of 60 patients undergoing a 5-day ciprofloxacin antibiotic treatment. We further uncover elevated effect sizes across multiple other genomic loci during the antibiotic exposure and post-exposure in several bacterial species. Our work begins to uncover the dynamics of adaptive variants that may have been previously missed due to subtle yet consistent allele fraction changes.

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ABSTRACTS BY TOPIC

Emerging Areas

10. From Birth to Brain: Specific Early Microbes and Perinatal Factors Shaping Cognitive Development. Evidence from a Spanish Child Cohort at Seven Years of Age

Silvia Arboleya[1], Nicole Duhau[1], David Herrero-Morín[2], Sonia González[3], Jorge Luis Arias[4], Natalia Arias[5], Miguel Gueimonde[1]

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Background and Aims: The gut microbiome is increasingly recognized as a key regulator of human health, beginning in early life. Its colonization is a gradual process shaped by perinatal factors such as delivery mode, feeding practices, gestational age, and antibiotic exposure. The microbiota–host cross-talk provides essential stimuli for homeostasis, with both immediate and long-term consequences, making the first 1,000 days a critical “window of opportunity.” Gut and brain development occur concurrently in this period. Over the last decade, significant efforts have focused on deciphering the communication between these two organs, particularly in relation to psychiatric and neurodegenerative disorders, yet the influence of early microbial colonizers on cognitive development remains poorly understood. This study explores the relationship between early-life gut microbiome composition, perinatal factors and infant diet, and their impact on cognitive performance at seven years of age in a cohort of Spanish children.

Methods: A longitudinal cohort of 60 healthy full-term infants from Asturias, Northern Spain, was followed from birth until seven years of age. Gut microbiota composition and derived metabolites during the first four years were analysed using 16S rRNA sequencing, qPCR, and gas chromatography. Infant diet, anthropometric measurements and clinical data were collected prospectively. Cognitive performance at seven years was assessed using the BAS-II and ENFEN tools.

Results: Specific early-life gut microbes were associated with later verbal and non-verbal cognitive scores. Memory and attention outcomes were also correlated with gut microbiome composition. Certain perinatal factors, particularly infant feeding practices and subsequent dietary patterns, significantly shaped the early microbiome and indirectly influenced neurodevelopment.

Conclusions: These findings support the concept that early-life microbiome–gut–brain axis interactions have lasting effects on cognitive development. Promoting healthy microbial and perinatal environments during infancy may represent key modifiable factors influencing neurocognitive outcomes.

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FROM BIRTH TO BRAIN: SPECIFIC EARLY MICROBES AND PERINATAL FACTORS SHAPING COGNITIVE DEVELOPMENT. EVIDENCE FROM A SPANISH CHILD COHORT AT SEVEN YEARS OF AGE

Silvia Arboleya[1,2], Nicole Duhau[1], David Herrero-Morín[3], Sonia González[2,4], Jorge L. Arias[2,5,6], Natalia Arias[2,6,7], Miguel Gueimonde[1,2]

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Introduction

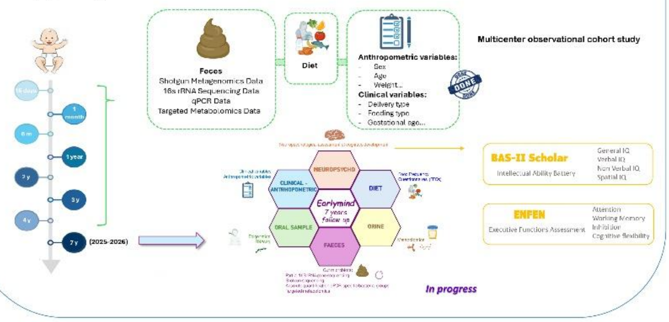
The gut microbiome is increasingly recognized as a key regulator of human health, beginning in early life. Its colonization is a gradual process shaped by perinatal factors. The microbiota–host cross-talk provides essential stimuli for homeostasis, with both immediate and long-term consequences, making **the first 1,000 days a critical "window of opportunity".** During this critical developmental window the brain also undergoes rapid growth and key neurodevelopmental processes occur, including neurogenesis, synaptogenesis, synaptic pruning, and myelination. **This temporal overlap has raised an important question: could early gut microbiota influence cognitive development during childhood?** Over the last decade, significant efforts have focused on deciphering the communication between these two organs, particularly in relation to psychiatric disorders, yet the **influence of early microbial colonizers on cognitive development remains poorly understood**. Furthermore, evidence from human studies is still scarce, especially in pediatric populations, highlighting the need for longitudinal studies and the integration of multiple omics technologies.

Objective

This study explores the relationship among early-life gut microbiota composition, perinatal factors and infant diet, and their impact on cognitive performance at seven years of age in a cohort of Spanish children.

Methodology

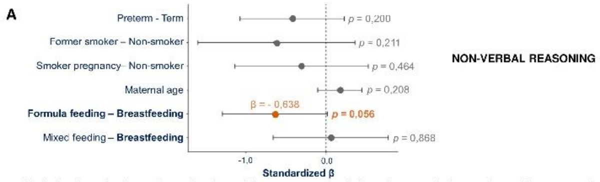
A longitudinal cohort ("*EarlyMicroHealth*") of 72 healthy infants from Asturias (Northern Spain) was followed from birth to seven years of age. Gut microbiota composition and microbiota-derived metabolites during the first four years of life were analyzed using 16S rRNA gene sequencing, shotgun metagenomic sequencing, quantitative PCR (qPCR), and gas chromatography and ultra-performance liquid chromatography techniques. Infant diet, anthropometric measurements, and clinical data were collected prospectively. Cognitive performance at seven years of age was assessed using the standardized BAS-II and ENFEN neuropsychological assessment tools.



Results

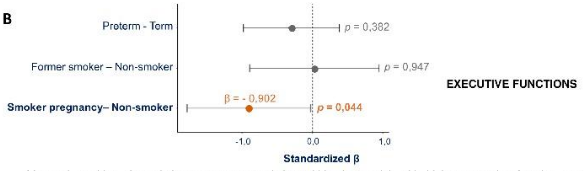
1 Associations Between **Perinatal Factors** and Cognitive Development:

A



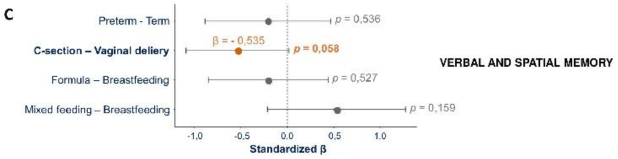
Exclusive breastfeeding at 6 months showed the strongest association with non-verbal reasoning performance at 7 years.

B



Maternal smoking showed the strongest association within the model, with higher executive function scores observed in the absence of tobacco exposure.

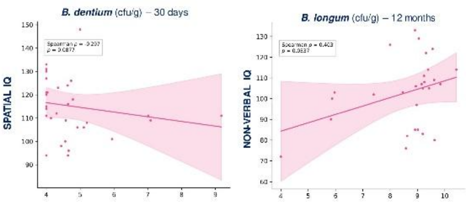
C



Vaginal delivery was the strongest predictor and was associated with better verbal and spatial memory performance at 7 years of age.

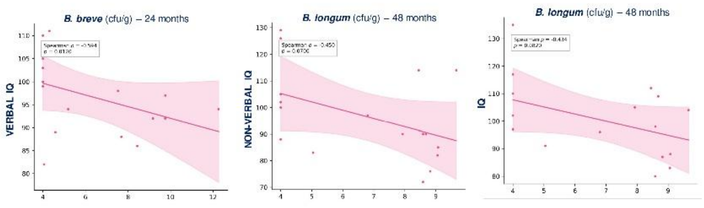
Figure 1: Multiple linear regression analysis. (A) Factor 1: Non-verbal reasoning (BAS-II), 34.7% of the variance (R^2 = 0.347; p = 0.003); (B) Factor 2: Executive functions (ENFEN); (C) Factor 3: Verbal and Spatial Memory (BAS-II), 19.1% of the variance (R^2 = 0.191; p = 0.032). *A subsample of the cohort (n = 54) children aged 7 years.*

2 Associations Between **Specific Gut Microbes** and Cognitive Development:



FIRST MONTHS OF LIFE

A higher abundance of *Bifidobacterium longum*, one of the key early-life gut colonizers, at 12 months of age, together with a lower abundance of *Bifidobacterium dentium* at 1 month, was associated with higher non-verbal and spatial IQ scores.



FROM TWO YEARS OF AGE ONWARDS

However, lower levels of *Bifidobacterium longum* and *Bifidobacterium breve* were associated with higher non-verbal and general IQ scores beyond the age of two years, which may be related to the natural maturation of the gut microbiota and the succession of microbial species occurring during early childhood.

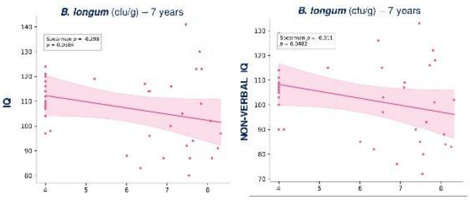


Figure 2: Correlation between General IQ, Verbal IQ, Non-verbal IQ, Spatial IQ (BAS-II Scholar) and *Bifidobacterium* spp (*Bifidobacterium longum, Bifidobacterium breve* and *Bifidobacterium dentium*– qPCR). *A subsample of the cohort (n =41) children aged 7 years.*

Conclusions

- The *EarlyMicroHealth* cohort represents a unique resource for advancing research on the microbiome–gut–brain axis. Its longitudinal design and the integration of clinical, dietary, cognitive, and microbiological data provide an opportunity to address several key limitations of current research on the relationship between the gut microbiota and cognition.
- Perinatal factors were associated with cognitive performance at 7 years of age.
- Preliminary findings revealed associations between early-life gut microbiota composition and different cognitive domains at 7 years of age.
- Ongoing analyses of the complete dataset will further elucidate these relationships and contribute to a better understanding of the long-term role of early-life microbial exposures in neurocognitive development.

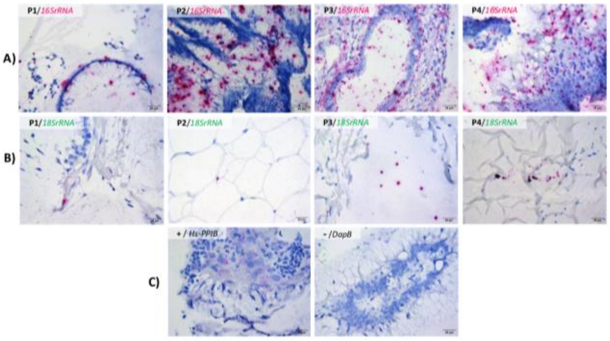
Funding: Grant CNS2023-145550 funded by MICIU/AEI /10.13039/501100011033 and by European Union NextGenerationEU/PRTR

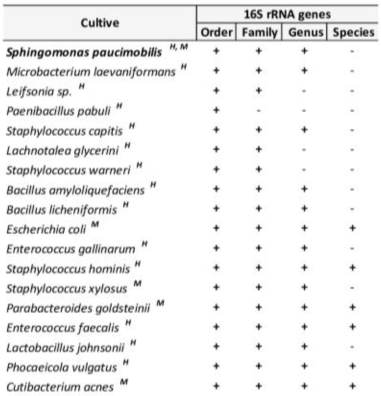
11. Bacterial and Fungal Signatures in the Rare Cancer Pseudomyxoma Peritonei

Rafaela Rocha Pezzopane[1], Álvaro Arjona Sánchez[2], Antonio Romero Ruiz[2], Marina Barbuda Lunar[1], Marí C. Vázquez Borrego[1], Florina I. Bura[1], Melissa Granados Rodriguez[1], Lidia Rodriguez Ortiz[1], Ana Martínez López[1], José Alhama Carmona[1], Carmen Michán Doña[3]

[1] University of Córdoba (UCO); Maimonides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain; [2] Maimonides Biomedical Research Institute of Córdoba (IMIBIC); Surgical Oncology Unit (HURS), Córdoba, Spain; [3] Maimonides Biomedical Research Institute of Córdoba (IMIBIC); Pathology Unit (HURS), Córdoba, Spain

Pseudomyxoma peritonei (PMP) is a rare disease characterized by the accumulation of mucussecreting cells within the peritoneal cavity, typically of appendiceal origin. The only available treatment involves complete cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy. Therefore, finding ways to improve treatment and better understand how the disease develops is required. Microorganisms have previously been detected in PMP using genomic approaches and, because of this, antibacterial treatments have been suggested. However, all these previous studies are centered on the bacteriome without considering any other microorganisms. This work investigates the bacteriome and the mycobiome of PMP using different approaches such as: metagenomics, microbial culture, and staining techniques. In addition, we investigated the potential functional implications of these microorganisms using specific analytical approaches. Experiments with a PMP animal model using antibiotics and antifungals were also conducted to explore heir pathophysiological effects on the disease. For the first time, the presence of fungi in PMP has been demonstrated. We have identified hundreds of bacterial families and dozens of fungal families. Bacterial diversity was significantly higher in tumors compared to controls, particularly across different tumor grades, a trend not observed for fungal diversity. Notably, significant shifts in microbial composition and predicted metabolic pathways were associated with tumor grade, including alterations in immune signaling pathways. Viable microorganisms were also successfully isolated. Our results provide valuable insights and indicate future research directions to elucidate the genesis and development of PMP. (PI22/01213, PRYES223170ARJO).





Cultive	16S rRNA genes			
	Order	Family	Genus	Species
Sphingomonas paucimobilis $^{H, M}$	+	+	+	-
Microbacterium laevaniformans ^{H}	+	+	+	-
Leifsonia sp. ^{H}	+	+	-	-
Paenibacillus pabuli ^{H}	+	-	-	-
Staphylococcus capitis ^{H}	+	+	+	-
Lachnotalea glycerini ^{H}	+	+	-	-
Staphylococcus warneri ^{H}	+	+	-	-
Bacillus amyloliquefaciens ^{H}	+	+	+	-
Bacillus licheniformis ^{H}	+	+	+	-
Escherichia coli ^{M}	+	+	+	+
Enterococcus gallinarum ^{H}	+	+	+	-
Staphylococcus hominis ^{H}	+	+	+	+
Staphylococcus xylosus ^{M}	+	+	+	-
Parabacteroides goldsteinii ^{M}	+	+	+	+
Enterococcus faecalis ^{H}	+	+	+	+
Lactobacillus johnsonii ^{H}	+	+	+	-
Phocaeicola vulgatus ^{H}	+	+	+	+
Cutibacterium acnes ^{M}	+	+	+	+

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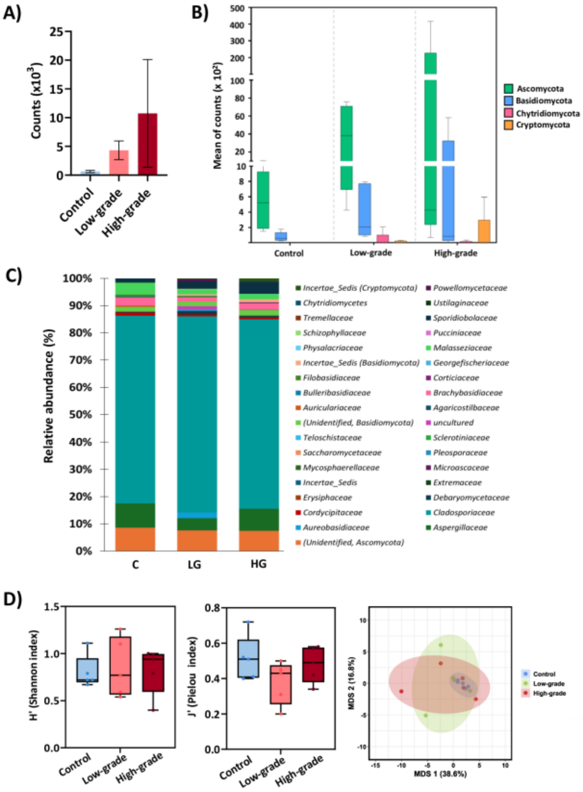
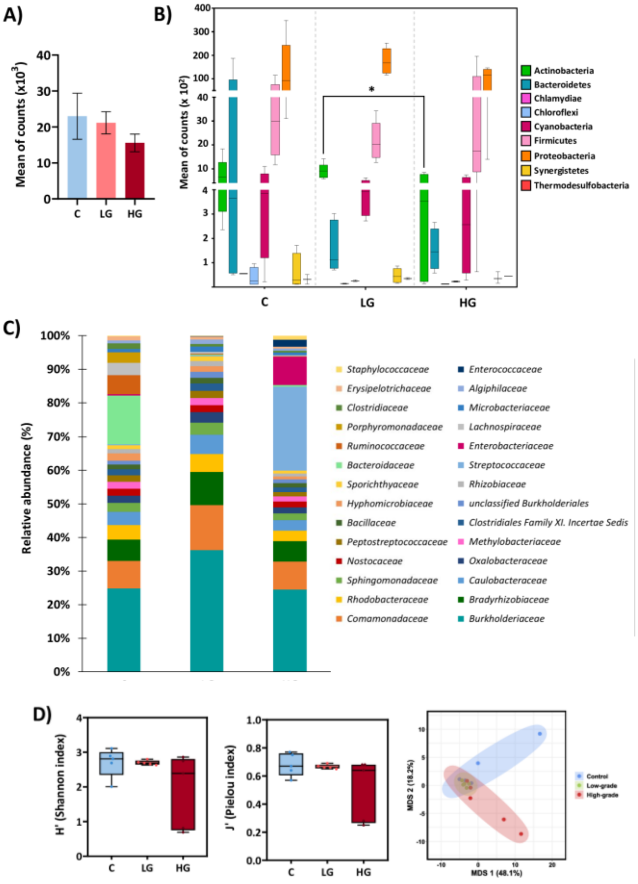

A)
Counts (x10^3)
0
5
10
15
20
25
Control
Low-grade
High-grade
B)
Mean of counts (x 10^2)
Ascomycota
Basidiomycota
Chytridiomycota
Cryptomycota
Control
Low-grade
High-grade
C)
Relative abundance (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
C
LG
HG
Incertae_Sedis (Cryptomycota)
Chytridiomycetes
Tremellaceae
Schizophyllaceae
Physalacriaceae
Incertae_Sedis (Basidiomycota)
Filobasidiaceae
Bulleribasidiaceae
Auriculariaceae
(Unidentified, Basidiomycota)
Teloschistaceae
Saccharomycetaceae
Mycosphaerellaceae
Incertae_Sedis
Erysiphaceae
Cordycipitaceae
Aureobasidiaceae
(Unidentified, Ascomycota)
Powellomycetaceae
Ustilaginaceae
Sporidiobolaceae
Pucciniaceae
Malasseziaceae
Georgefischeriaceae
Corticiaceae
Brachybasidiaceae
Agaricostilbaceae
uncultured
Sclerotiniaceae
Pleosporaceae
Microascaceae
Extremaceae
Debaryomycetaceae
Cladosporiaceae
Aspergillaceae
D)
H' (Shannon index)
J' (Pielou index)
Control
Low-grade
High-grade
MDS 1 (38.6%)
MDS 2 (16.8%)
Control
Low-grade
High-grade


A)
Mean of counts (x10^3)
0
10
20
30
40
C
LG
HG
B)
Mean of counts (x 10^2)
*
C
LG
HG
Actinobacteria
Bacteroidetes
Chlamydiae
Chloroflexi
Cyanobacteria
Firmicutes
Proteobacteria
Synergistetes
Thermodesulfobacteria
C)
Relative abundance (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Staphylococcaceae
Erysipelotrichaceae
Clostridiaceae
Porphyromonadaceae
Ruminococcaceae
Bacteroidaceae
Sporichthyaceae
Hyphomicrobiaceae
Bacillaceae
Peptostreptococcaceae
Nostocaceae
Sphingomonadaceae
Rhodobacteraceae
Comamonadaceae
Enterococcaceae
Algiphilaceae
Microbacteriaceae
Lachnospiraceae
Enterobacteriaceae
Streptococcaceae
Rhizobiaceae
unclassified Burkholderiales
Clostridiales Family XI. Incertae Sedis
Methylobacteriaceae
Oxalobacteraceae
Caulobacteraceae
Bradyrhizobiaceae
Burkholderiaceae
D)
H' (Shannon index)
J' (Pielou index)
C
LG
HG
MDS 1 (48.1%)
MDS 2 (18.2%)
Control
Low-grade
High-grade

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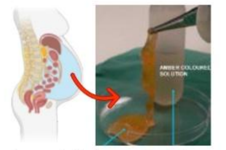
BACTERIAL AND FUNGAL SIGNATURES IN THE RARE CANCER PSEUDOMYXOMA PERITONEI

Rafaela Rocha-Pezzopane[1,2], **Marina Barbudo-Lunar**[1,2], **Mari C. Vázquez-Borrego**[1,2], **Florina I. Bura**[1,2], **Melissa Granados-Rodriguez**[1,2], **Lidia Rodriguez-Ortiz**[2,3], **Ana Martínez-López**[2,4], **Javier López-Jiménez**[1,2], **Verónica Luna-Guerrero**[1,2], **José Alhama-Carmona**[1,2], **Carmen Michán-Doña**[1,2], **Álvaro Arjona-Sánchez**[2,3], **Antonio Romero-Ruiz**[1,2]

[1] Dept. of Biochemistry and Molecular Biology, University of Córdoba, Córdoba, Spain. [2] Maimonides Biomedical Research Institute of Córdoba (IMIBIC), Córdoba, Spain. [3] Surgical Oncology Unit, Surgery Department, Reina Sofía University Hospital, Córdoba, Spain. [4] Pathology Unit, Reina Sofía University Hospital, Córdoba, Spain

INTRODUCTION



Representative image of a mucinous tissue sample (Pillai, Krishna et al., 2017)

Pseudomyxoma peritonei (PMP) is a rare disease characterized by the **accumulation of mucus-secreting cells** within the peritoneal cavity, typically of appendiceal origin. The only available treatment involves complete **cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy**. Therefore, finding ways to improve treatment and better understand how the disease develops is required. **Microorganisms** have previously been detected in PMP using genomic approaches and, because of this, **antibacterial treatments** have been suggested. However, all these previous studies are centered on the bacteriome **without considering any other microorganisms**. This work investigates the **bacterial and fungal communities of PMP** using **metagenomics**, **microbial culture**, and **histological staining**. It also explores the **potential functional roles** of these microorganisms through analytical methods.

MATERIALS AND METHODS

Sample collection »» *Pre-treatment and DNA extraction* »» *PCR and sequencing* »» *Bioinformatic analysis* »» *Microbiological culture* »» *Microscopy*

RESULTS

Bacteriome and mycobiome characterization of PMP - Taxonomy

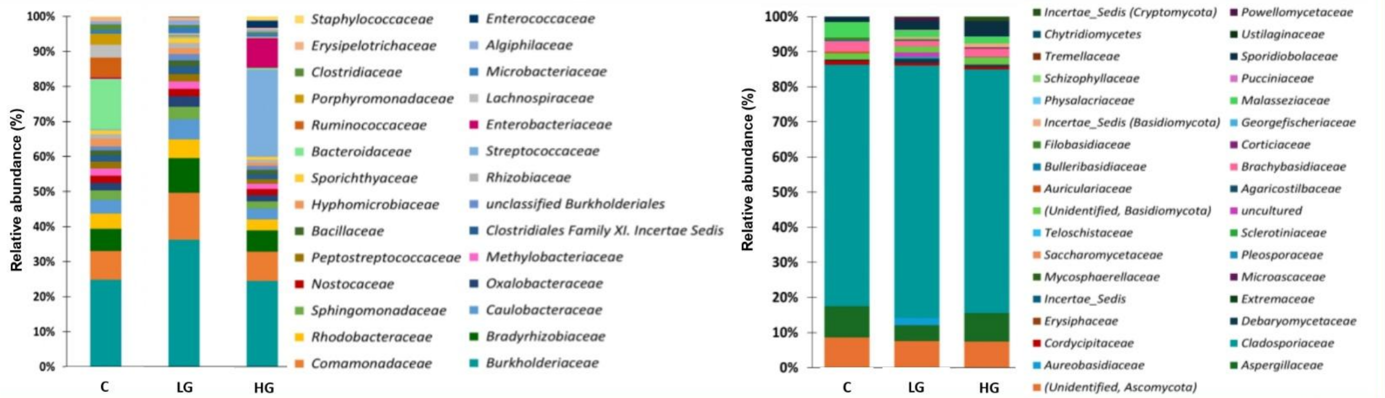


Fig. 1. Bacterial and fungal composition at the family level (≥1% in at least one group) for C, LG and HG determined by 16S rRNA gene sequencing. Significant changes were: Chromatiaceae (LG > HG; $p < 0.05$); Legionellaceae (C > LG, HG > LG; $p < 0.05$); Rhizobiaceae (LG > HG; $p < 0.05$).

16S rRNA Analysis and Diversity

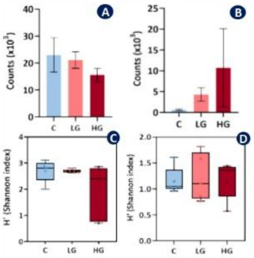


Fig. 2. Average number of (A) bacterial and (B) fungi identifications across different samples, categorized by tumor grade: control (C), low-grade (LG) ad high-grade (HG). **Alpha diversity** (*H´*, Shannon-Wienner) index values for **(C)** bacterial and **(D)** fungi communities in C, LG, and HG.

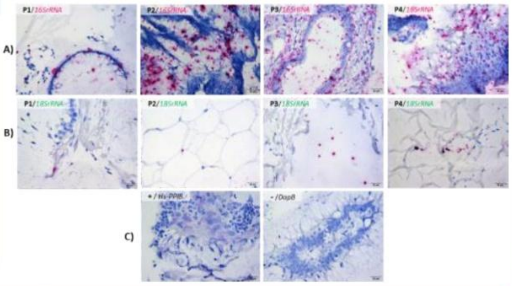
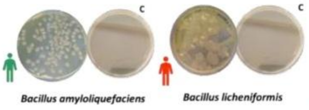


Figure 3. RNAscope-CISH detection of microbial rRNA in FFPE PMP tissues. [A–B] Bacterial 16S [A] and fungal 18S [B] rRNA (red punctate signals) in epithelial and mucinous compartments (patients P1–P4). [C] Positive (Hs-PPIB) and negative (DapB) assay controls. Counterstained with hematoxylin. Scale bar = 25 µm (x400).

Strains of bacteria cultured and their identification by genomics

Cultive	16S rRNA genes			
	Order	Family	Genus	Species
Sphingomonas paucimobilis $^{H, M}$	+	+	+	-
Microbacterium laevaniformans ^{H}	+	+	+	-
Leifsonia sp. ^{H}	+	+	-	-
Paenibacillus pabuli ^{H}	+	-	-	-
Staphylococcus capitis ^{H}	+	+	+	-
Lachnotalea glycerini ^{H}	+	+	-	-
Staphylococcus warneri ^{H}	+	+	-	-
Bacillus amyloliquefaciens ^{H}	+	+	+	-
Bacillus licheniformis ^{H}	+	+	+	-
Escherichia coli ^{M}	+	+	+	+
Enterococcus gallinarum ^{H}	+	+	+	-
Staphylococcus hominis ^{H}	+	+	+	+
Staphylococcus xylosus ^{M}	+	+	+	-
Parabacteroides goldsteinii ^{M}	+	+	+	+
Enterococcus faecalis ^{H}	+	+	+	+
Lactobacillus johnsonii ^{H}	+	+	+	-
Phocaeicola vulgatus ^{H}	+	+	+	+
Cutibacterium acnes ^{M}	+	+	+	+

Table 1. Cultured bacteria identified in PMP samples. ^{H}Species identified exclusively in human mucin samples; ^{M}Species identified exclusively in mouse mucin samples.



Bacillus amyloliquefaciens *Bacillus licheniformis*

Colonies of mucus-secreting bacteria

Fig 4. Mucous colonies of irregular shape were observed after 72h incubation after incubation in liquid LB medium at 37 °C.

Functional profiles prediction from 16S metagenomic rRNA data

KEEG PATHWAY	METABOLIC PATHWAY	LG *vs.* C	HG *vs.* C	HG *vs.* LG
Amino acid metabolism	Arginine and proline metabolism			🟩 *
	Phenylalanine metabolism			🟩 *
Carbohydrate metabolism	Glycolysis / Gluconeogenesis			🟥 *
	Fructose and mannose metabolism			🟥 *
	Starch and sucrose metabolism			🟥 *
	Pyruvate metabolism			🟩 *
	Glyoxylate and dicarboxylate metabolism			🟩 *
	Propanoate metabolism			🟩 *
	Butanoate metabolism			🟩 *
Cellular community - eukaryotes	Focal adhesion			🟥 *
Circulatory system	Cardiac muscle contraction			🟩 *
D-Aminoacid metabolism	D-Alanine metabolism			🟥 *
Digestive system	Carbohydrate digestion and absorption			🟥 *
Endocrine and metabolic disease	Type II diabetes mellitus			🟥 *
Glycan biosynthesis and metabolism	Peptidoglycan biosynthesis			🟥 *
Immune system	NOD-like receptor signaling pathway			🟥 *
Infectious disease: bacterial	Bacterial invasion of epithelial cells			🟥 *
Lipid metabolism	Steroid biosynthesis			🟩 *
	Steroid hormone biosynthesis			🟩 *
	Glycerolipid metabolism			🟥 *
	Glycerophospholipid metabolism			🟥 *
	Biosynthesis of unsaturated fatty acids			🟩 *
Metabolism of cofactors and vitamins	Lipoic acid metabolism			🟥 *
Metabolism of other amino acids	beta-Alanine metabolism			🟩 *
	Taurine and hypotaurine metabolism			🟩 *
	Phosphonate and phosphinate metabolism			🟩 *
	Selenocompound metabolism			🟥 *
Metabolism of terpenoids and polyketides	Geraniol degradation			🟩 *
	Polyketide sugar unit biosynthesis		🟩 *	
	Biosynthesis of type II polyketide products			🟩 *
Neurodegenerative disease	Parkinsons disease			🟩 *
Nucleotide metabolism	Purine metabolism			🟥 *
	Pyrimidine metabolism			🟥 *
Replication and repair	Mismatch repair			🟥 *
	Homologous recombination			🟥 *
Signal transduction	Two-component system			🟩 *
Signaling molecules and interaction	ECM-receptor interaction			🟥 *
Transcription	RNA polymerase			🟥 *
Translation	Aminoacyl-tRNA biosynthesis			🟥 *
	Ribosome			🟥 *
Xenobiotics biodegradation and metabolism	Benzoate degradation			🟩 *
	Naphthalene degradation			🟩 *
	Drug metabolism - other enzymes			🟥 *

Table 2. Tax4Fun analysis. Metabolic pathways with significant differences in abundance which are shown in green boxes indicate a decrease, while red cells indicate an increase relative to the reference group (C for LG and HG comparisons, or LG for HG comparisons). * ($p < 0.05$).

CONCLUSIONS

For the first time, fungal presence in PMP has been demonstrated. We identified hundreds of bacterial families and dozens of fungal families across samples. Bacterial diversity was **significantly higher in tumors** compared to controls, especially across different tumor grades; In contrast, **fungal diversity remained stable** across groups; **Microbial composition** and **predicted metabolic pathways** (notably immune signaling) **shifted** significantly **with tumor grade**; **Viable bacteria and fungi** were successfully **isolated** from PMP samples.

These findings offer novel insights into the PMP-associated microbiome and highlight new avenues to investigate the role of microbes in PMP genesis and progression.

Acknowledgments: Instituto de Salud Carlos III (ISCIII; Ref.: PI22/01213 y PI25/00696), co-funded by the European Union and from Asociación Española contra el Cáncer (Ref.: PRYES223170ARJO). Projects (MICINN PID2022-139807OB-100; Junta de Andalucía, PCM_00118, ISCIII PI22/01213). Fundings to BIO187 (PAIDI, UCO) and RNM306 (PAIDI, CSIC) groups. Predoctoral contract of Rafaela Pezzopane (FPU22). Research Support Services (SCAI) of the University of Córdoba and UCAIBs of IMIBIC.

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12. Gut Microbiome Functional Reprogramming Induced by Chronic Social Stress: A Porcine Metaproteomic Perspective

Yuliaxis Ramayo-Caldas[1,2], Raquel Río-López[1], Judith Guitart-Matas[3], Magnus Øverlie Arntzen[4], Adrià Clavell-Sansalvador[2], Ioanna-Theoni Voularki[2], Olga González-Rodríguez[2], Librado Jesús García-Gil[5], Xavier Xifró[6], Antoni Dalmau[1]

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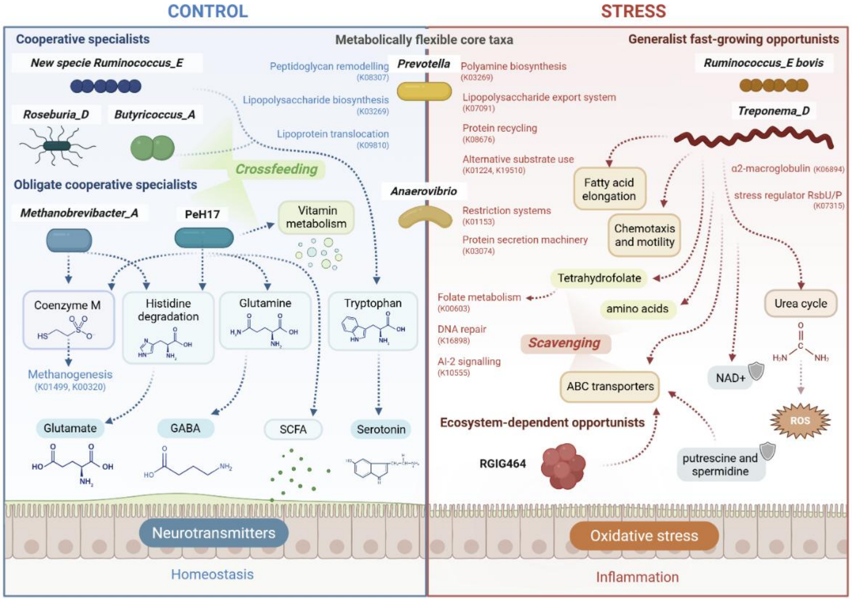
Chronic social stress (CSS) is a risk factor for metabolic, inflammatory and neuropsychiatric disorders, with increasing evidence highlighting the microbiota–gut–brain axis as a key mediator. However, metaproteomic approaches to study CSS in pig models remain largely unexplored. This study aimed to characterise functional shifts in the pig gut microbiome under CSS and identify protein biomarkers.

At the end of the growing period, hair and faecal samples were collected from 60 two-month-old Duroc pigs exposed to CSS (n=30; restricted space and repeated mixing) or control conditions (n=30). Faecal samples were analysed using genome-resolved metaproteomics (nanoLC-MS/MS). Proteins were identified against a custom database comprising 911,040 microbial genes from 484 metagenome-assembled genomes (MAGs), enabling linkage of expressed proteins to specific microorganisms. Discriminant and differential analyses identified CSS-responsive taxa and functions, achieving up to 99.51% classification accuracy based on individual proteins. Functional enrichment and pathway reconstruction revealed distinct metabolic strategies among MAGs.

Hair cortisol levels differed significantly between groups ($p<0.05$). Stress-associated MAGs included Treponema_D, Catenibacterium and Erysipelotrichaceae members, whereas control-associated MAGs included Roseburia, Butyricicoccus, PeH17, and novel Christensenellales and Lachnospiraceae taxa. Control microbiomes were enriched in short-chain fatty acid production, vitamin biosynthesis, stress resistance and metabolic interdependencies. In contrast, CSS microbiomes showed increased resource competition, nutrient scavenging, motility and diverse transport systems.

Overall, CSS functionally reshaped the pig gut microbiome, shifting it from a cooperative to a competitive community. Identified metaproteomic signatures may serve as accurate biomarkers of pig welfare and provide translational insights into human stress-related dysbiosis.



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Gut Microbiome Functional Reprogramming Induced By Chronic Social Stress: A Porcine Metaproteomic Perspective

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BACKGROUND

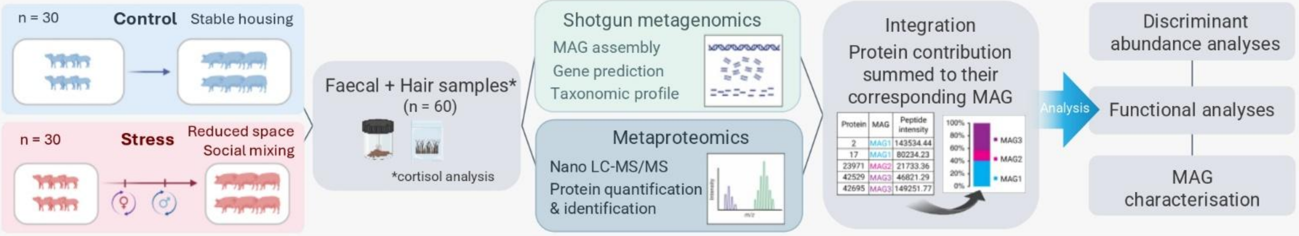
Chronic social stress (**CSS**) is a recognised risk factor for developing metabolic, inflammatory and neuropsychiatric diseases. However, the application of metaproteomics to study the effects of CSS on the pig **gut-brain axis** has not yet been widely explored.

OBJECTIVES

- Use the pig as a valuable translational model
- Characterise how CSS reshapes the functional activity of the gut microbiome
- Identify potential protein biomarkers of stress exposure

METHODOLOGY

At the end of the growing period, hair and faecal samples were collected from 60 two-month-old Duroc pigs exposed to CSS (n=30) or control conditions (n=30). Faecal samples were analysed by genome-resolved metaproteomics (**nano LC-MS/MS**). Protein identification was performed against a custom database of 911,040 genes from 484 metagenome-assembled genomes (**MAGs**), linking expressed proteins to microorganisms.



n = 30 **Control** Stable housing

n = 30 **Stress** Reduced space Social mixing

Faecal + Hair samples* (n = 60)
*cortisol analysis

Shotgun metagenomics
MAG assembly
Gene prediction
Taxonomic profile

Metaproteomics
Nano LC-MS/MS
Protein quantification & identification

Integration
Protein contribution summed to their corresponding MAG

Protein	MAG	Peptide intensity
2	MAG1	143534.44
17	MAG1	80234.23
23971	MAG2	21733.36
42529	MAG3	46821.29
42695	MAG3	149251.77

Discriminant abundance analyses
Functional analyses
MAG characterisation

Partial Least Squares Discriminant Analysis (**PLS-DA**) was employed to detect subtle changes that are only observable with multivariate approaches. Finally, a functional analysis was performed using enrichment analysis, metabolic module completion analysis, and pathway reconstruction.

RESULTS & DISCUSSION

Genomic & Proteomic catalogues

A multi-level catalogue was generated from faecal metagenomic and metaproteomic data.

- 483 MAGs
  >70% compl. & <10% cont
  413 species (408 bact + 5 arch)
- 911,040 genes
  Sample-specific metagenomic protein sequence database
- 26,868 proteins
  Filtered by 10% freq in control or stress from 42,696 proteins
- 1,783 functions
  Based in KEGG Orthology Identifiers (KO_IDs) from 1,825 total functions

Similarity between omics & PLS-DA

Discriminant analyses identified CSS-responsive taxa and functions.

- RV = 0,43
  Similarity between metagenomics and metaproteomics tables
- MAG abundance
  84.4% accuracy
  Control n = 38 Stress n = 21
- MAG protein abundance
  75.5% accuracy
  Control n = 21 Stress n = 18
- Individual protein abundance
  99.5% accuracy
  Control n = 345 Stress n = 656

Functional analyses

Functional enrichment and pathway reconstruction showed distinct **metabolic strategies** among MAGs (Fig. 1).

1st approach – Protein-centric
Significant overrepresentation of KO_IDs among the discriminant proteins (n = 1001)
→ Hypergeometric test
30
enriched functions
Control n = 14 Stress n = 16

2nd approach – Function-centric
Protein contribution summed to their corresponding KO_ID (filtered by 10% freq)
→ PLS-DA
251
Discr. functions
Control n = 93 Stress n = 158

MAG characterisation

Metabolic potential of discriminant MAGs:

Module Completion Fractions
Fraction of essential KO_IDs detected per KEGG module for each discriminant MAG
→ KEGG Mapper Reconstruct
Inspect the metabolic pathways of each relevant MAG manually

CONCLUSIONS

CSS triggers a fundamental functional restructuring of the porcine gut microbiome, shifting it from a **cooperative** to a **competitive** community. The identified metaproteomic signatures serve as highly accurate **biomarkers** for assessing pig welfare and provide **translational insights** into human stress-related dysbiosis.

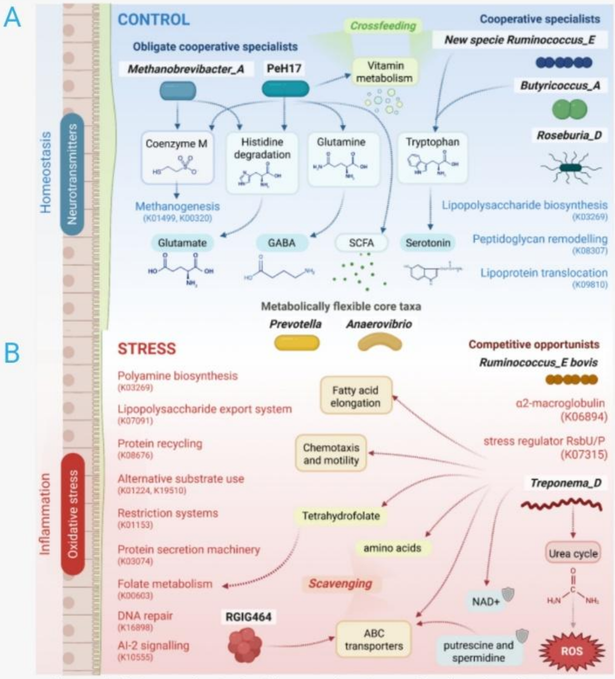


Figure 1. Patterns of gut microbiome and proteome functional profiles between A) Control condition and B) Stress condition.

Funding:

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BEST ABSTRACT

13. Fecal Microplastics are Associated with a Bacterial Stress-response Signature and Selective Depletion of Short-chain Fatty Acid Producers in the Human Gut Microbiome

Emma Calikanzaros[1], Cristina M. Villanueva[1], Jianshu Zhao[2], Lucas Patel[2], Yuhan Weng[2], Rafael de Cid[3], Marta Llorca[4], Marinella Farré[4], Mariona Bustamante[1], Rob Knight[2]

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Background: Micro- and nanoplastics (MNPs) are ubiquitous contaminants detected in human biological matrices. Experimental studies report MNP-associated gut microbiome disruption, but human evidence remains scarce and limited by low-resolution amplicon sequencing. This study characterised gut microbiome composition and function in relation to directly measured fecal MNP concentrations and their metabolic correlates.

Methods: Cross-sectional analysis in 49 adults from Barcelona, Spain. Fecal MNP concentrations (0.7–20 µm) were quantified by HPLC(SEC)-HRMS. Microbiome composition and function were characterised by shotgun metagenomics and metagenome-assembled genome (MAG) assembly. Differential abundance of taxa (OGUs) and Gene Ontology (GO) functional terms was estimated using BIRDMAn — a hierarchical Bayesian framework — adjusting for age, sex, BMI, and diet. Fecal metabolomics included SCFA, bile acid, and indole quantification.

Results: MNPs were detected in 52% of fecal samples. We identified 18 differentially abundant OGUs (16 depleted, 2 enriched) and 15 GO terms in MNP-positive participants. Depleted taxa included SCFA-producing anaerobes (Mitsuokella multacida, Megasphaera spp., Catenibacterium spp., Latilactobacillus sakei); two strains of Enterococcus faecalis were consistently enriched. Functionally, MNP-positive participants showed depletion of SCFA biosynthesis pathways and enrichment of osmotic stress tolerance pathways — notably ectoine biosynthesis and trehalose metabolism — consistent with abiotic stress adaptation rather than acute dysbiosis.

Conclusions: This study provides the first high-resolution human evidence linking real-world MNP body burden to gut microbiome disruption: depletion of SCFA producers, enrichment of a stress-tolerant pathobiont, and upregulation of stress-response pathways. Fecal metabolomics will determine whether these shifts reflect measurable metabolic consequences.

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Fecal Microplastics & the Human Gut Microbiome

Diversity, taxa, function, and metabolites in a community cohort — adjusted for age, sex, and vegetarian diet

Emma Calikanzaros*1,2,3; Jianshu Zhao4; Lucas Patel4; Yuhan Weng4; Mariona Bustamante1,2,3; Rob Knight4; Cristina M. Villanueva1,2,3

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Research Question & Cohort

Does the presence of fecal MNPs associate with differences in gut microbiome composition, function, or metabolite output?

Cohort 49 healthy adults from Barcelona (omnivore + vegetarian/vegan)

Groups MP+ (n=25, MNPs detected in stool) vs. MP− (n=24)

Adjustment GLM. Models adjusted for age, sex, and vegetarian diet

MNP analysis HPLC-SEC-HRMS, 0.7–20 µm, stool

Methods Shotgun metagenomics · BIRDMAn DA (taxa + GO terms) · Targeted metabolites measurements (SCFA, indoles, bile acids) by LC-MS/MS

Results 1 — Community Composition & Differential Abundance

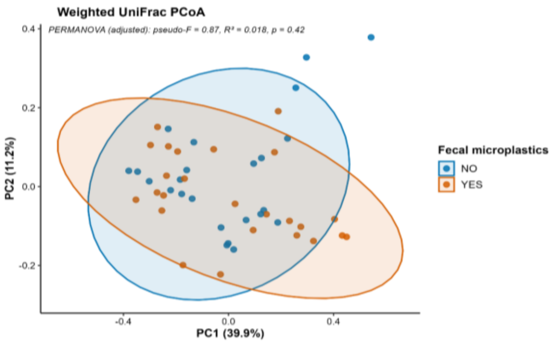

Fig 1. Gut microbiome beta diversity by micro-nanoplastics exposure status

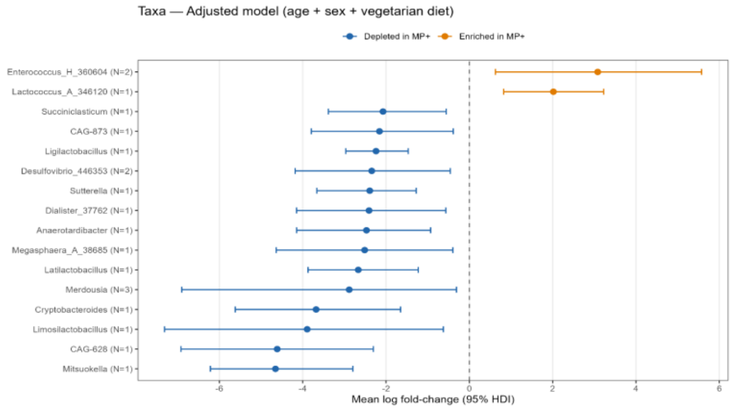

Fig 2. BIRDMAn differential abundance on OGU-level taxonomy · 14 genera depleted, 2 enriched (95% HDI)

Results 2 & 3 — Gene Ontology-Term Differential Abundance · Fecal Metabolites concentrations

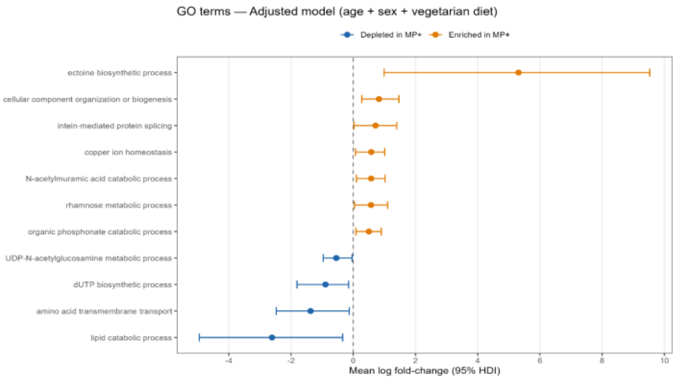

Fig 3. BIRDMAn differential abundance on Gene Ontology-terms · Depleted: lipid catabolism, amino acid transport · Enriched: ectoine (stress), copper homeostasis · Dot = mean; bar = 95% HDI

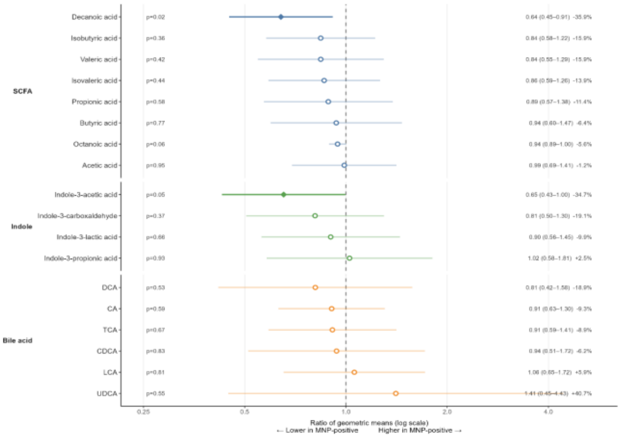

Fig 4. Fecal metabolites ratio of geometric means (MP+ vs MP−) · Diamonds = p<0.05; open circles = p≥0.05; bars = 95% CI

Conclusions

- First high-resolution human evidence linking real-world MNP body burden to gut microbiome disruption
- No significant shift in overall gut microbiome structure (alpha- and beta-diversity) in MP+ individuals
- 14 genera credibly depleted in MP+, including known SCFA producers; enriched bacterial abiotic stress-response pathways (ectoine biosynthesis, copper ion homeostasis)
- The pattern is consistent with a community-level adaptation to persistent environmental stress rather than classical inflammatory dysbiosis
- Decanoic acid (−35%, p=0.02) and 7/8 SCFAs directionally lower, consistent with depletion of SCFA-producing genera · Cross-sectional, n=49, hypothesis-generating

BEST ABSTRACT

14. A Vaginal Microbiome Pharmacology Foundation Model for Microbiome-Based Drug Development and Reproductive Health Insights

Douglas Brubaker[1], Alex Bergendorf[1]

[1] Case Western Reserve University, Cleveland, United States

Introduction: Engineering microbiome therapies requires an organizing principle for the mechanisms by which microbiome factors impact the host. We previously developed the “pharmacobiome”, a framework that uses mechanisms of drugs to quantify the host impact of microbiome factors. Here, we generalized this framework into a Microbiome Pharmacology Foundation Model (MPFM) for mechanism-informed design of microbiome therapeutics.

Methods: We used correlation analysis of vaginal multi-omics data to generate host gene signatures for vaginal microbiome factors and compared the signatures to drug treatment host transcriptomics to construct microbiome factor-drug similarity scores. This Vaginal Microbiome Pharmacology database of 6,719,800 training instances enabled us to train the MPFM, a deep autoencoder model, and to derive experimentally testable predictions of individual microbiome factors and designed combination therapeutic properties.

Results: The MPFM training converged with very strong performance ($r = 0.57$, $MSE= 0.0058$) and the latent space formed a continuous manifold. We analyzed cisplatin in the MPFM to identify anti-cancer metabolites and experimentally confirmed that taurine was Lactobacillus-derived and cytotoxic against cancer cell lines ($p<0.0005$). We also analyzed metronidazole to identify antibiotic metabolites and experimentally showed hydroxyisocaproate was Lactobacillus-derived and selectively killed vaginal pathogens ($p<0.05$). Finally, we used the model to explore patient-specific microbiome pharmacology and design probiotic combinations to support in vitro fertilization. We compared our results with a new, prospective human cohort.

Conclusions: We demonstrated consistent performance of the MPFM and experimentally supported the validity of predictions. Our continued work simulates probiotic, prebiotic, and postbiotic combinations for patient-specific in vivo sample analysis to develop tailored microbiome therapies for gynecologic conditions.

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A Vaginal Microbiome Pharmacology Neural Network Model for Analysis of Combinatorial Patient-Specific Microbiome Signatures for Therapeutic Development and Reproductive Health Insights

Alexander M. Bergendorf[1], Raymond Krajci[1], Alexis Heath[1], Jordan Small[3], Debjyoti Thakur[3], Ashley Yoon[1], Jason E. Elliott[3, 4], Romel D. Mackelprang[8], Elizabeth Irungu[9], Timothy R. Muwonge[10], Elly Katabira[10], Florian Hladik[11,12,13], Jairam Lingappa[8,11,14], Adam D. Burgener[1,3,5], Alicia R. Berard[1,3,6,7], Christina Farr Zuend[1], and Douglas K. Brubaker[1, 2‡]

Patent: U.S. Application No. 63/747,418

CASE WESTERN RESERVE UNIVERSITY

Background

A foundational challenge in microbiome engineering is being able to predictably modulate host biology through microbiome-based ingredients.

Structuring host-microbiome associations within a modeling framework would support discovery of designed microbiome therapeutics.

Our lab previously proposed such a framework in the *Pharmacobiome*, a method that quantifies similarity of host gene signatures caused by a drug or small molecule stimulus to microbiome factors. This method produced experimentally validated results to determine anti-cancer and anti-microbial properties of metabolites.

Here, we extend this framework to develop a comprehensive map of inferred therapeutic actions of the vaginal microbiome through a *Microbiome Pharmacology Foundation Model* using a deep autoencoder, to derive testable microbiome-based therapeutic hypotheses.

Methods

LINCS L1000

- The LINCS L1000 database has drug transcriptomic response signatures for 201 cell lines spanning 35 tissue types[1]
- We can derive consensus perturbation signatures for each of the 35,000+ drugs to determine their average effect on the host transcriptome

Partners pre-exposure prophylaxis (PrEP) study

- Performing a Spearman correlation between 16S rRNA and bulk transcript vaginal epithelial expression from PrEP, a transcriptomic signature is derived[2]
- This same calculation may be performed for vaginal metabolomics data to derive metabolite gene expression signatures

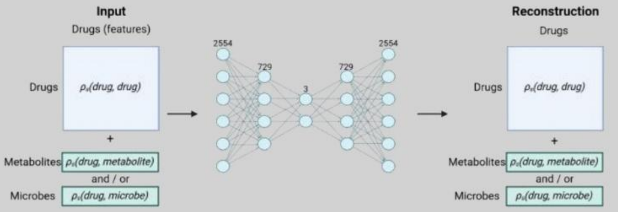

Figure 1: An autoencoder training framework to learn associations between drugs and vaginal microbiome factors

Microbiome Pharmacology Foundation Model

- Performing a Spearman correlation between drug signatures and themselves and drug signatures with microbiome signatures puts factors in a "host-effect" space
- Resultant matrix is fed through a deep learning architecture called an autoencoder to comprehensively learn drugs and microbes host impacts

Results

Health-associated and dysbiosis taxa orient separately within drug-space

- Training the model with gene expression signatures from drugs that have specific targets, vaginal taxa, and vaginal metabolites reveals distinct groupings of taxa by impact on host[3]
- *Lactobacillus spp.* group to the upper right of the latent space while facultative anaerobes like *Gardnerella vaginalis* group towards the bottom left

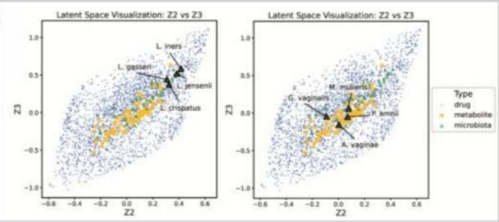

Figure 2: Health-associated and dysbiosis-associated taxa visualized on latent variables Z2 and Z3

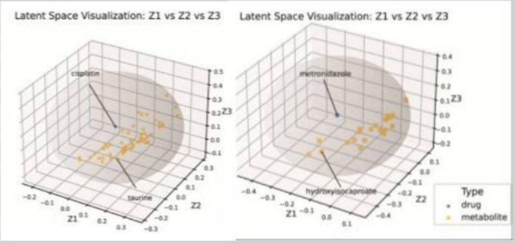

Figure 3: Cisplatin and taurine (left) and metronidazole and hydroxyisocaproate (right) visualized within 3D bottleneck

Experimentally validated anti-cancer and anti-biotic metabolites group near drugs with concordant properties

- Taurine, a metabolite experimentally determined to be cytotoxic against cancer cell lines[4], grouped near the chemotherapy medication cisplatin
- Hydroxyisocaproate, a metabolite experimentally determined to selectively kill vaginal pathogens[5], grouped near the antibacterial drug metronidazole

Identifying simulated probiotic combinations to support *in vitro* fertilization (IVF) success

- By creating weighted combinations of microbial input signatures and feeding through the trained autoencoder, we can predict the pharmacological properties of probiotic combinations[6]
- Taking patient microbial proportions, we can also project patient pharmacological profiles within the latent space
- Averaging the profile of individuals achieving IVF success, we can see which probiotic communities are closest to individuals achieving success with IVF

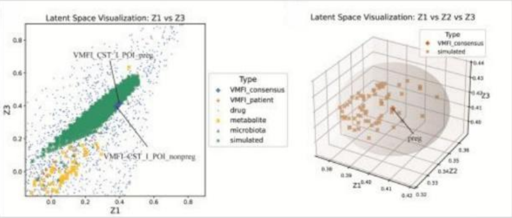

Figure 4: Simulated probiotic communities and consensus pregnancy signatures (left) and simulated probiotic communities near consensus signature for IVF success

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Discussion

Implications

- Identification of microbiome-based communities that have similar functional effects as drugs allows for therapeutic hypothesis generation
- Investigation of patient-specific microbiome pharmacology
- Novel microbiome-based solutions for vaginal cancer, infections, and fertility support

Future Directions

- Model shown includes a subset of drugs + microbes + metabolites. This can be expanded initially train on drug data, and fine-tune to other microbiome systems to better embed drugs and microbiome factors within a shared space
- This framework is largely applicable to other systems where the microbiome is implicated in health and disease

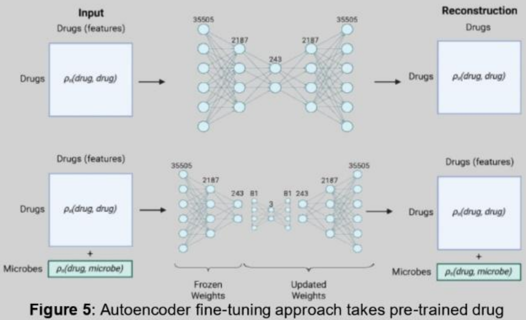

Figure 5: Autoencoder fine-tuning approach takes pre-trained drug latent variable weights and adapts them to microbiome context

Affiliations and Acknowledgements

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The authors acknowledge use of BioRender for Figure 1 and 5.

Clinical Associations

15. Exploring the Gut Microbiome as a Predictive Biomarker of Neoadjuvant Response in Locally Advanced Rectal Cancer

Nel Marín-Sánchez[1], Miguel Ángel Peinado[2], Roger Paredes[1], Alessandra Borgognone[1], Núria Mulet Margalef[2], Berta Martín Abad[2], Mireia Obón-Santacana[3], Mariona Parera[1], Maria Casadellà[1], Mar Muñoz[1], José Luís Manzano[2], Andrea Plaja Salarich[2], Elisabet Mompradé-Olivé[2]

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Locally advanced rectal cancer (LARC) incidence is rising in younger patients without a clear cause. Despite advances in neoadjuvant therapy, predictive biomarkers of treatment response remain lacking. Evidence suggests the gut microbiome may influence colorectal cancer (CRC) prognosis and therapy response. This pilot study explores associations between the gut microbiome, treatment response, epigenomic patterns and clinical/exposome factors in LARC patients.

Pre- and post-neoadjuvant samples were collected from 22 LARC patients with T3/T4 tumours and/or lymph node metastases at the Catalan Institute of Oncology (ICO-Badalona). Patients achieving complete clinical response (cCR) were classified as responders (Rs, n=8), while those undergoing surgery and/or experiencing regrowth were classified as non-responders (NRs, n=14). Stool samples underwent shotgun metagenomic sequencing (MetaPhlAn4). A CRC-enriched bacterial index was calculated using Fusobacterium nucleatum (<0.5%), Bacteroides fragilis (<5%) and Parvimonas (<5%). DNA methylation was assessed using QUAlu.

No differences in microbial gene richness or beta diversity were observed between Rs and NRs. At baseline, Rs were enriched in short-chain fatty acid-producing Clostridia —Agathobaculum butyriciproducens, Fusicatenibacter saccharivorans and Blautia faecicola—, while Adlercreutzia equolifaciens was more abundant in NRs ($p<0.05$). Among Rs with detectable CRC-enriched taxa, 83.3% showed decreased bacterial index, whereas all NRs without a cCR showed increased F. nucleatum post-treatment. Increased index values were observed in five patients, including three NRs who later developed tumour regrowth. One showed the highest hypermethylation alongside increased Fusobacterium.

Despite the small sample size, faecal enrichment of CRC-associated bacteria during neoadjuvant treatment may identify LARC patients with poor prognosis. Larger studies are needed to validate these findings and assess clinical relevance.

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Exploring the Gut Microbiome as a Predictive Biomarker of Neoadjuvant Response in Locally Advanced Rectal Cancer

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BACKGROUND

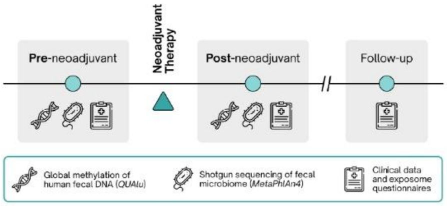
Locally advanced rectal cancer (LARC) incidence is increasing in younger patients without a clear cause[1]. Evidence suggests that the gut microbiome and other lifestyle-related factors may influence colorectal cancer (CRC) prognosis and therapy response[2]. However, the identification of predictive biomarkers of neoadjuvant therapy response is an unmet clinical need in LARC[3].

The **EMELARC** project aims to **identify predictive biomarkers** of response to neoadjuvant therapy in LARC by investigating the complex interplay between the microbiome, epigenome and exposome.

METHODS

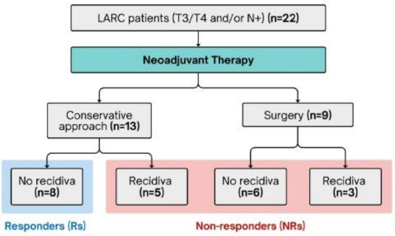
Pre- and post-neoadjuvant samples were collected from 22 LARC patients (T3/T4 and/or N+). Stool samples were analyzed using shotgun metagenomic sequencing (Fig. 1).

Figure 1. *Study design and sample collection*



All patients were Caucasian and of Spanish origin (45.4% female; 42-86 years of age). Patients were classified according to neoadjuvant therapy response as responders (Rs) or non-responders (NRs) (Fig. 2).

Figure 2. *Patient classification by response to the neoadjuvant therapy*



LARC patients (T3/T4 and/or N+) **(n=22)**

Neoadjuvant Therapy

Conservative approach **(n=13)**

Surgery **(n=9)**

No recidiva (n=8)

Recidiva (n=5)

No recidiva (n=6)

Recidiva (n=3)

Responders (Rs)

Non-responders (NRs)

RESULTS

- Metagenomic profiling revealed marked inter-individual variability, with microbiome composition showing greater similiarity within patients than across any assessed demographic, clinical or exposome factors (Fig. 3).
- Heterogeneous microbial community compositions were identified across samples, including *Bacteroides*-, *Ruminococcus*- and *Bifidobacterium*-dominated profiles (Fig. 4).

Figure 3. *Microbial community structure of patients pre- and post-neoadjuvant therapy*

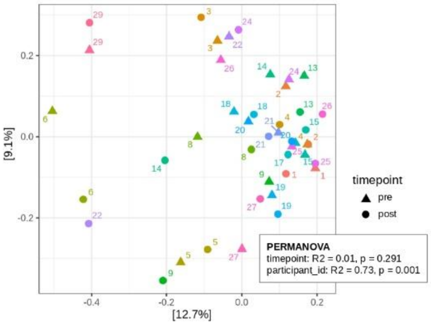
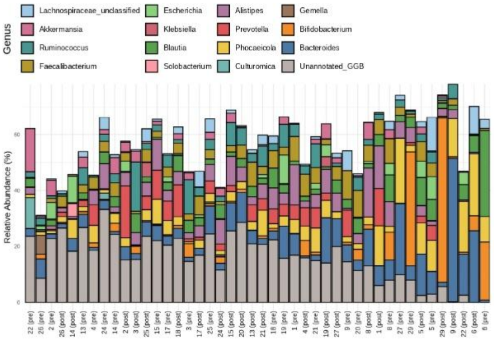


Figure 4. *Gut microbiota composition (top most abundant genera) pre- and post-neoadjuvant therapy*



- No significant differences in microbial gene richness or beta diversity were observed between Rs and NRs.
- At baseline, Rs showed an enrichment in species *Agathobaculum butyriciproducens*, *Fusicatenibacter saccharivorans* and *Blautia faecicola*, while *Adlercreutzia equolifaciens* was more abundant in NRs (Fig. 5).
- Among Rs with detectable levels, 83.3% exhibited a decrease in a calculated index based on the abundance of CRC-associated bacteria[4,5], including *Fusobacterium nucleatum*[6], *Bacteroides fragilis* and *Parvimonas* (Fig. 6).
- In contrast, 100% of NRs without a complete clinical response showed increased *F. nucleatum* levels post-neoadjuvant therapy (Fig. 6).

Figure 5. *Differentially abundant bacterial species between Rs and NRs at baseline (pre-neoadjuvant) using MaAsLin2*

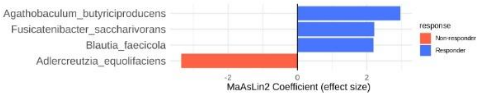
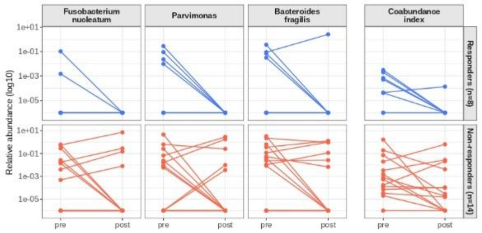


Figure 6. *Longitudinal changes in CRC-associated bacteria and coabundance index by treatment response*



DISCUSSION AND CONCLUSION

Responders were enriched in short-chain fatty acid-producing members of the class *Clostridia* (phylum *Bacillota*)[7], including ***A. butyriciproducens***, ***F. saccharivorans***, and ***B. faecicola***. Conversely, the equol-producing bacterium ***A. equolifaciens*** (phylum *Actinomycetota*)[8] was depleted, suggesting distinct microbial metabolic profiles between response groups.

An increase in the **combined CRC-associated bacterial index** was observed in 5 patients, including 3 NRs who subsequently developed post-surgical tumour regrowth, indicative of rapid progression. Notably, one of these patients exhibited the highest level of hypermethylation.

Despite the small sample size, fecal enrichment of ***F. nucleatum*** **and other CRC-associated bacteria during neoadjuvant therapy may support the identification of LARC patients**. Larger studies are needed to validate these findings and assess their clinical relevance.

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ADDITIONAL KEY INFORMATION

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16. Influence of Maternal Mental Health on Lactation and Infant Microbiome

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General objective: To investigate the relationship between maternal mental health on breastfeeding and infant microbiome.
Specific objectives:
1.- To explore the impact of maternal stress during the perinatal period on the type and duration of breastfeeding.
2.- To compare microbiome changes on human milk in relation withchildbirth indicators and perinatal mental health on lactant women from Hospital del Mar.
3.- To evaluate the association between breastfeeding and infant microbiome during the first year of life, compared to other forms of lactation and postnatal complications.
Discussion: This study is a observational study, completing health surveys during pregnancy until 24 months postpartum.As inclusion and exclusion criteria, our participants are:- Pregnant women- Over 18 years old- Recruited between 12-16 weeks of gestation- Receiving antenatal care at the research centers.
Our sample size is 1200 pregnant women from the research centers, approximately 300 women per center.The study begins with prenatal monitoring starting at 12-14 weeks of gestation and following families during postpartum, meeting at 4 months and 24 months after childbirth.Inside the PhD research, I design different variables and surveys in relation to maternal health,breastfeeding on the redcapdatabase. In relation to the first objective, I will analize the results from the validated questionnaires about mental health and their expectative about breastfeeding and how evolves during postpartum.For breastfeeding rates, I will analyze milk and stool samples in relation to birth(Type of birth, antibiotic use) and mental health (diet, exercise, meditation adherence), collected by validated questionnaires completed on redcap.
Evidence where relevant: Postpartum is a period of special vulnerability related to maternal mental health
Key message: This proposal further explore the interactions between breastfeeding, maternal mental health, and infant microbiome.

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INFLUENCE OF MATERNAL MENTAL HEALTH ON BREASTFEEDING AND INFANT MICROBIOME

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BACKGROUND

Postpartum is known as a period of special vulnerability related to maternal mental health. Globally, one in five women is estimated to develop postpartum depression[1] Elevated cortisol levels change composition in gut microbiota too, particularly a reduction in beneficial bacteria, and Higher cortisol levels in breast milk were associated with maternal stress and anxiety[2]

OBJECTIVES

This proposal will explore the interactions between breastfeeding, maternal mental health and infant microbiome, proposing new directions for research :

MENTAL HEALTH AND BREASTFEEDING

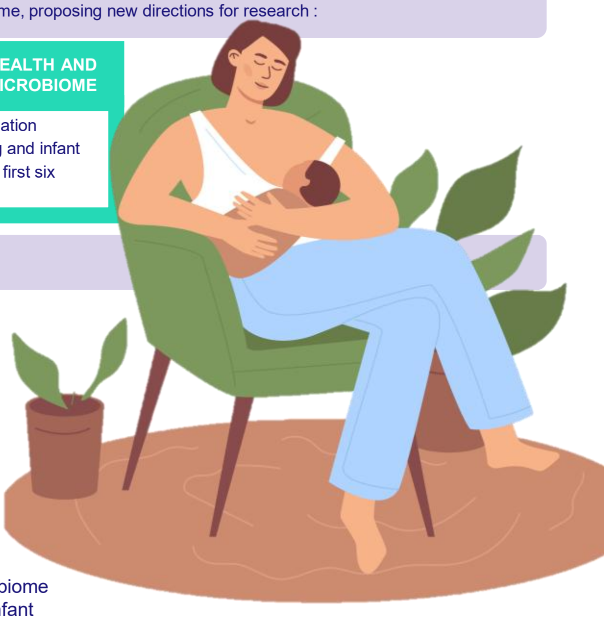
To explore the impact of maternal stress during the perinatal period on the type and duration of breastfeeding.

MENTAL HEALTH AND UMAN MILK MICROBIOME

To study changes on human milk in relation with childbirth indicators and perinatal mental health on breastfeeding mothers from Hospital del Mar.

MENTAL HEALTH AND INFANT MICROBIOME

To evaluate the association between breastfeeding and infant microbiome during the first six months of life.



HYPOTHESIS

Maternal stress and depression during the perinatal period affect negatively on breastfeeding, exclusively breastfeeding comes down, increasing the practice of introducing commercial milk formula (CMF) and stopping breastfeeding sooner.

Mental health disorders as postpartum depression, dystocic childbirth, traditional separation and mixed breastfeeding methods, change milk composition, variates predominant species on human milk and have an effect on mother and infant health during postpartum.

Breastfeeding, especially when complemented by a supportive caregiving environment and high maternal satisfaction, is associated with changes in microbiome in infants and mums during the first months of life, in contrast to other forms of infant feeding or situations involving maternal mental health challenges.

METHODS

STUDY DESIGN

This study is inside Mindmum projec a multicenter longitudinal study about mental health during pregnancy and postpartum.

POPULATION

Pregnant women over 18 years old, recruited between 12-14 weeks, and their offspring up to 24 months postpartum.

Participating centres:

VARIABLES AND OUTCOMES

1. We collect perinatal stress perception, Type of birth, Antibiotic use, Diet/exercise/meditation adherence.
2. We analyze milk samples in relation to redcap surveys results.
3. We study mum and babies microbiome, inside postpartum assessment.

VARIABLES

Maternal:

Intention to Breastfeed
Initiation of Breastfeeding
Perception of Support During Breastfeeding
Supplementation and Breastfeeding
Problems or Difficulties During Breastfeeding
Time Between Birth and Start of Infant Feeding
Use of Antibiotics
Obstetric outcomes

Offspring:

Neonatal outcomes at birth
Infant Feeding
Skin-to-Skin Contact
Infant's Weight
Infant's Weight at Discharge
Postpartum Neonatal Pathology

Biomarkers:

Data from weareables

Personal and obstetric:

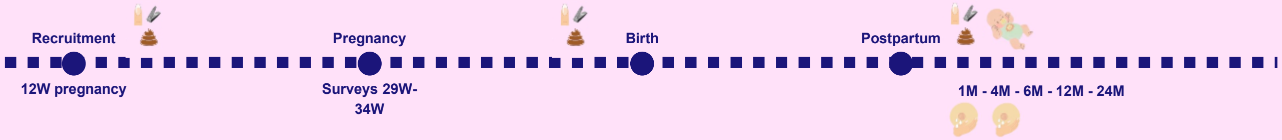
Maternal and neonatal health data

Self reported questionnaires and evaluations

Microbiome:

PROGRESS

Collecting milk and stool samples from mums and babies
Storing samples at -80 degrees in a special freezer
Checking the results of the questionaries in Redcap
Completing birth and postpartum data
During next months we will start milk and stool microbiome study.



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17. Autism Spectrum Disorder in Uruguay: Associations Between Gut Microbiota, Gastrointestinal Symptoms and Clinical Profile in a Sibling-Matched Cohort Cohort

Florencia Peñalba[1], Andrés Parada[1], Nadia Riera[1], Andreina Guisande[2], Lucía Lamberti[3], Camila Rusiñol[4], Maite Irastorza[1], Florencia Konik[5], Claudio Iglesias[3], Paula Mendive[5], Gabriela Garrido[2]

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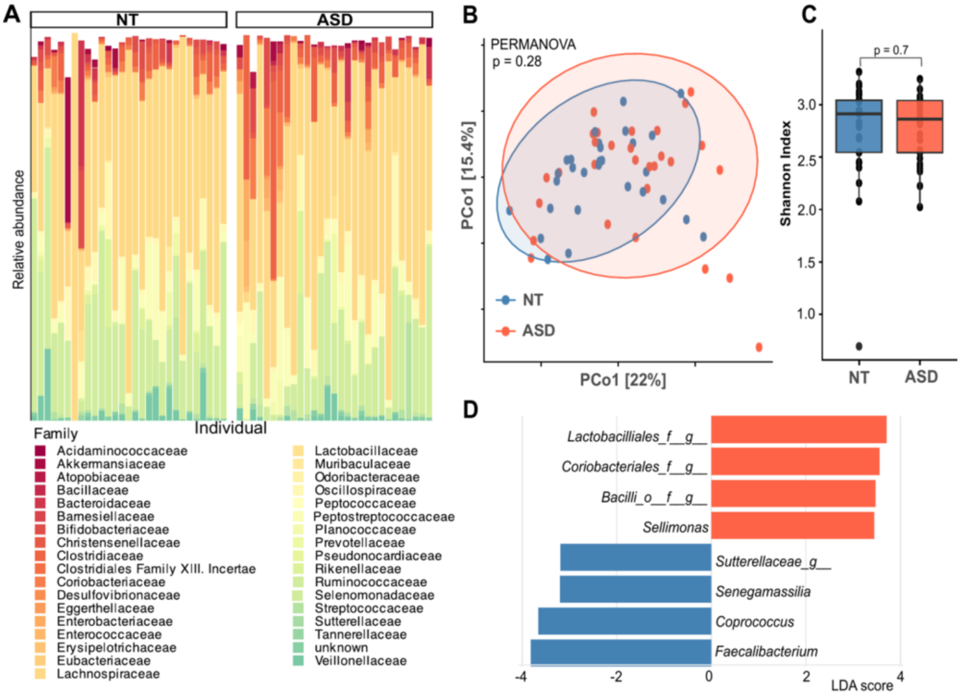
Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by difficulties in social communication and interaction. Given the frequent gastrointestinal symptoms in children with ASD, research is increasingly focusing on the gut microbiota and the gut–brain axis.
We conducted a cross-sectional study in Uruguay involving 55 sibling pairs (aged 4–10 years). Comprehensive clinical, psychiatric, and gastrointestinal data were systematically collected through structured questionnaires and interviews. We selected a subset of 29 sibling pairs for microbial analysis. High-resolution microbiome profiling was achieved through full-length 16S rRNA gene sequencing (V1–V9 regions) using the Oxford Nanopore Technologies.
The ASD group exhibited a high burden of early-life and clinical complications, including pregnancy complications (60%) and universal sensory particularities. Notably, GI symptoms were nearly omnipresent in the ASD group (94.3%). We observed a 65% rate of autistic regression and a concerning 20-month diagnostic. Microbiome analysis revealed distinct taxonomic signatures: the ASD group was significantly enriched in Sellimonas, while NT siblings showed higher abundances of beneficial butyrate-producers, specifically Faecalibacterium and Coprococcus. Pharmacological interventions were strongly associated with microbiome shifts. Antipsychotic use correlated with a reduction in Akkermansia abundance, whereas treatments involving melatonin and methylphenidate were linked to an enrichment of Negativibacillus.
Our findings highlight a significant burden of GI symptoms and its association with microbial communities among Uruguayan children with ASD. Using high-resolution long-read sequencing, we demonstrate how GI symptoms and medication profiles are associated with the gut microbial landscape. This project marks a pioneering effort in the region and establishes a critical foundation for context-specific, multidisciplinary ASD care in Uruguay.



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18. Microbiota Patterns Along the Anal Fistula Tract: Insights from a Pilot Study

Maria Piñana[1], Pablo Pillado[1], Jose Troya[1], Elisa Martró[1], Verónica Saludes[1], David Parés[1], Pere Joan Cardona[1], Poppy Hesketh-Best[1], Alexia Paris de León[1], Antoni Bordoy[1], Edward Sánchez-Haro[1], Ona Torrejón-Llorens[1], Sara González-Gómez[1], Aniol Català[1], Ingrid Tapiolas[1]

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Background. Anal fistulas are abnormal tracts connecting the anal canal to the perianal skin, typically developing after an anorectal abscess. These fistulas can become recurrent, requiring multiple surgical interventions and significantly affecting patients' quality of life. This study aimed to characterize the microbiota across different regions of the anal fistula to explore whether its origin lies in the intersphincteric space, as classically proposed, or whether it may have an external origin.

Methods. Patients with cryptoglandular anal fistulas following a clinically significant anal abscess, and with no evidence of inflammatory bowel disease, underwent total fistulectomy. Each fistula was sectioned into three regions (external opening, central-intersphincteric space, and internal opening). Total DNA was extracted using the QIAamp DNA Mini Kit. The full-length 16S rRNA gene was amplified, and libraries were indexed using the 16S Barcoding Kit v14 (Oxford Nanopore Technologies, ONT). Sequencing was performed using R10.4.1 flow cells on a MinION Mk1C (ONT). Bacterial reads were taxonomically classified using the EPI2ME 16S workflow (ONT), and downstream microbiota analyses were conducted using in-house R scripts.

Results. Five patients were prospectively enrolled, yielding three samples per patient. PCA revealed inter-patient differentiation but no distinct clustering by fistula region. Hierarchical clustering similarly showed grouping by patient rather than by fistula region. The most prevalent species, such as *Campylobacter hominis* or *Fenollaria massiliensis*, were typical gastrointestinal tract bacteria, and were present along the fistula tract.

Conclusions. Microbiota compositions were more similar within the three samples from the same patient than across the different regions of the fistula from different patients, with most detected species originating from the gastrointestinal microbiota. Although this is a pilot study with a limited sample size, these preliminary findings indicate that further investigation is required to determine how the microbiota aligns with current hypotheses on fistula origin and its potential therapeutic implications.

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Microbiota patterns along the anal fistula tract: insights from a pilot study

FANAL study

Maria Piñana[1], Poppy J. Hesketh-Best[1], Alexia Paris de León[1], Antoni E. Bordoy[1,2], Edward Sanchez-Haro[3], Ona Torrejón-Llorens[1], Sara González-Gómez[1], Aniol Català[1], Ingrid Tapiolas[3], Pablo Pillado[1], Jose Troya[3], Elisa Martró[1,2], Verónica Saludes[1,2], David Parés[3], Pere Joan Cardona[1,4]

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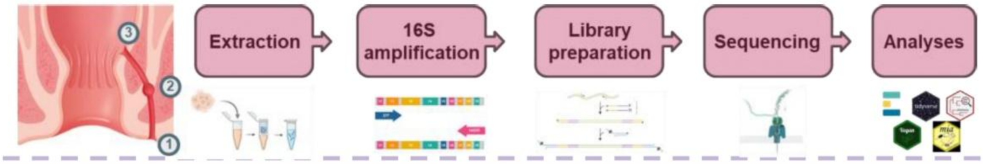
Background

Anal fistulas are abnormal tracts connecting the anal canal to the perianal skin. These fistulas can become recurrent, requiring multiple surgical interventions and significantly affecting patients' quality of life.

This study aimed to characterize the microbiota across different regions of the anal fistula to explore whether its origin lies in the intersphincteric space, as classically proposed, or whether it may have an external origin.

Materials and methods

Patients with cryptoglandular anal fistulas with no evidence of inflammatory bowel disease underwent total fistulectomy.



Extraction → 16S amplification → Library preparation → Sequencing → Analyses

Results

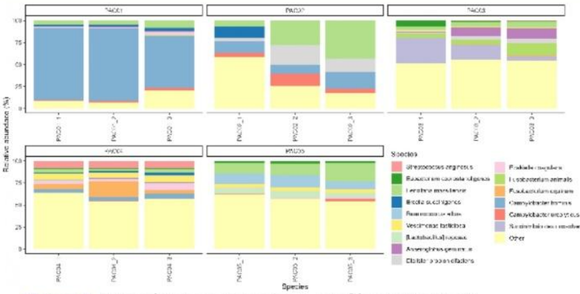


Figure 1. Microbiota composition profiles by patient

Microbiota composition was primarily driven by patient identity rather than fistula region (Fig. 1). Alpha and beta diversity analyses showed significant differences between patients but not among the three fistula regions (Fig. 2 & 3), a pattern also supported by hierarchical clustering (Fig. 4). The most prevalent species belonged to typically-associated gastrointestinal microbiota, including *Campylobacter hominis*, *Fenollaria massiliensis* or *Ruminococcus albus.*

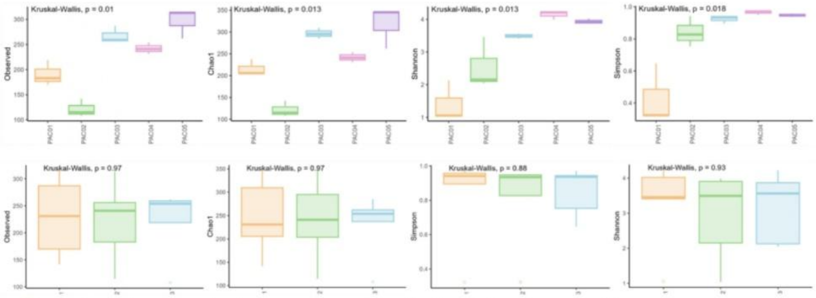


Figure 2. Alpha diversity indexes per patient (up) and fistula region (down).

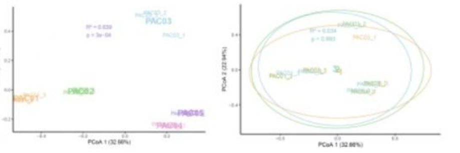


Figure 3. Beta diversity per patient and fistula region.

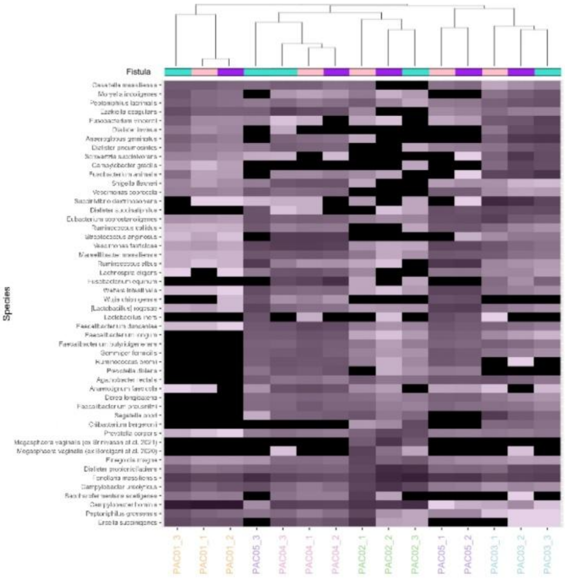


Figure 4. Heatmap of the 50 most prevalent species with hierarchical clustering of samples. Darker color indicate higher relative abundance

Conclusions

Microbiota composition was more similar across different regions of the **same fistula** than across corresponding regions from different patients. In addition, most identified species were characteristic members of the **gastrointestinal microbiota**.

Although limited by the small sample size, this **pilot study** provides preliminary evidence that may contribute to our understanding of anal fistula pathogenesis and highlights the need for larger studies to evaluate the **potential role of microbiota in disease development and treatment**.

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19. Gut Microbiota Oralisation Links Proton Pump Inhibitors Intake with Reduced Response to Immunotherapy in Patients with Hepatocellular Carcinoma

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Background: Gut microbiota composition influences response to immune checkpoint inhibitors (ICIs) across cancers, including hepatocellular carcinoma (HCC). Proton pump inhibitors (PPIs) can disrupt gut microbial communities, influencing immunotherapy efficacy. This study evaluated the impact of PPI intake on baseline gut microbiome and its association with outcomes in ICI-treated HCC patients.
Methods: Fresh faecal samples were collected from 40 patients enrolled in a Spanish multicentre phase I/IIa trial (NCT04170556) of nivolumab plus regorafenib.
PPI exposure was defined as use within 12 weeks prior to immunotherapy. Baseline microbiome composition was analysed by shotgun metagenomics (Illumina), and short-chain fatty acids (SCFAs) were profiled by GC–MS. Overall survival (OS) was assessed using Cox models with inverse probability of treatment weighting and multivariable adjustment. Machine-learning models and SHAP values were used to estimate microbiome feature contributions.
Results: PPI exposure was associated with significant differences in alpha and beta diversity (Shannon $p<0.005$; Bray–Curtis $p<0.05$ across taxonomic levels). Differential abundance of multiple taxa was observed (Figure 1). Higher levels of branched-chain SCFAs and oral-origin taxa (including Actinomyces dentalis, Rothia mucilaginosa, Streptococcus anginosus, S. gordonii and S. vestibularis) were associated with increased mortality risk (Figure 2). PPI exposure was independently associated with poorer OS (HR 4.4; 95% CI 1.6–12.1). Functional pathway differences were observed but not statistically significant (Figure 3).
Conclusions: PPI exposure was associated with reduced microbial diversity and gut microbiome oralisation. These changes, along with altered SCFA profiles, were linked to worse clinical outcomes, suggesting that PPI-induced microbiome shifts may impair immunotherapy response in HCC.

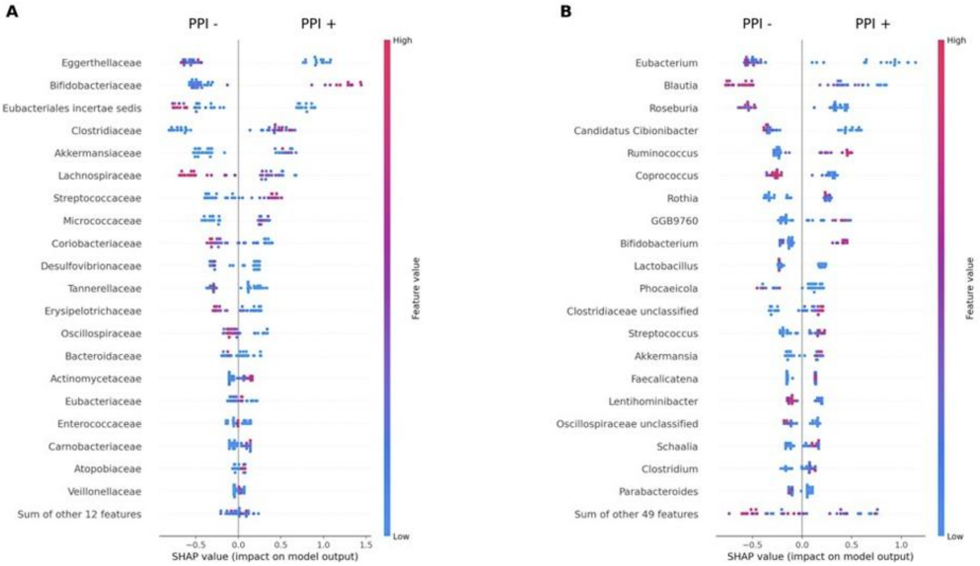

Figure 1. SHAP (Shaplrey Additive exPlanations) values for microbial taxonomic features associated with use of PPIs (panel A for family and pnael B for genus). Each bar represents the Shapley value of a respective taxa, quantifying its impact on the model's predicition. Polarizing features, ie. Those that partitioned the SHAP values, indicate those taxa that are high (blue) or low (red) in each group (with or without PPI).

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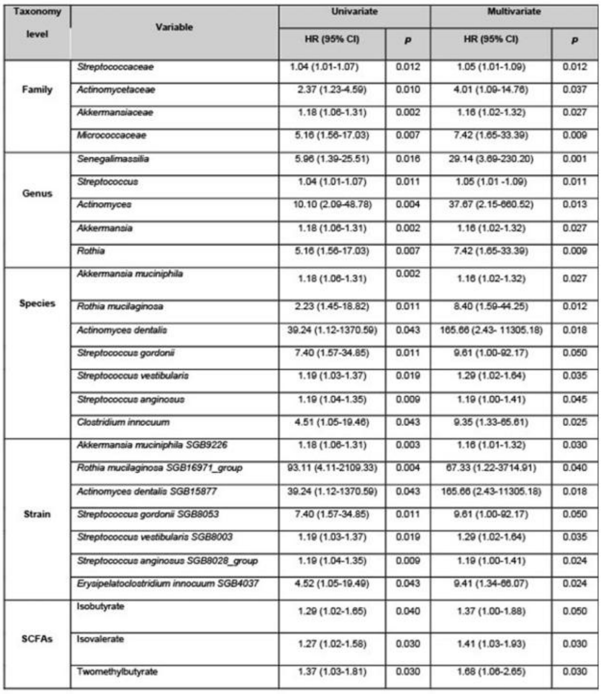

Taxonomy level	Variable	Univariate		Multivariate	
		HR (95% CI)	*p*	HR (95% CI)	*p*
Family	*Streptococcaceae*	1.04 (1.01-1.07)	0.012	1.05 (1.01-1.09)	0.012
	Actinomycetaceae	2.37 (1.23-4.59)	0.010	4.01 (1.09-14.76)	0.037
	Akkermansiaceae	1.18 (1.06-1.31)	0.002	1.16 (1.02-1.32)	0.027
	Micrococcaceae	5.16 (1.56-17.03)	0.007	7.42 (1.65-33.39)	0.009
Genus	*Senegalimassilia*	5.96 (1.39-25.51)	0.016	29.14 (3.69-230.20)	0.001
	Streptococcus	1.04 (1.01-1.07)	0.011	1.05 (1.01 -1.09)	0.011
	Actinomyces	10.10 (2.09-48.78)	0.004	37.67 (2.15-660.52)	0.013
	Akkermansia	1.18 (1.06-1.31)	0.002	1.16 (1.02-1.32)	0.027
	Rothia	5.16 (1.56-17.03)	0.007	7.42 (1.65-33.39)	0.009
Species	*Akkermansia muciniphila*	1.18 (1.06-1.31)	0.002	1.16 (1.02-1.32)	0.027
	Rothia mucilaginosa	2.23 (1.45-18.82)	0.011	8.40 (1.59-44.25)	0.012
	Actinomyces dentalis	39.24 (1.12-1370.59)	0.043	165.66 (2.43- 11305.18)	0.018
	Streptococcus gordonii	7.40 (1.57-34.85)	0.011	9.61 (1.00-92.17)	0.050
	Streptococcus vestibularis	1.19 (1.03-1.37)	0.019	1.29 (1.02-1.64)	0.035
	Streptococcus anginosus	1.19 (1.04-1.35)	0.009	1.19 (1.00-1.41)	0.045
	Clostridium innocuum	4.51 (1.05-19.46)	0.043	9.35 (1.33-65.61)	0.025
Strain	*Akkermansia muciniphila SGB9226*	1.18 (1.06-1.31)	0.003	1.16 (1.01-1.32)	0.030
	Rothia mucilaginosa SGB16971_group	93.11 (4.11-2109.33)	0.004	67.33 (1.22-3714.91)	0.040
	Actinomyces dentalis SGB15877	39.24 (1.12-1370.59)	0.043	165.66 (2.43-11305.18)	0.018
	Streptococcus gordonii SGB8053	7.40 (1.57-34.85)	0.011	9.61 (1.00-92.17)	0.050
	Streptococcus vestibularis SGB8003	1.19 (1.03-1.37)	0.019	1.29 (1.02-1.64)	0.035
	Streptococcus anginosus SGB8028_group	1.19 (1.04-1.35)	0.009	1.19 (1.00-1.41)	0.024
	Erysipelatoclostridium innocuum SGB4037	4.52 (1.05-19.49)	0.043	9.41 (1.34-66.07)	0.024
SCFAs	Isobutyrate	1.29 (1.02-1.65)	0.040	1.37 (1.00-1.88)	0.050
	Isovalerate	1.27 (1.02-1.58)	0.030	1.41 (1.03-1.93)	0.030
	Twomethylbutyrate	1.37 (1.03-1.81)	0.030	1.68 (1.06-2.65)	0.030

Figure 2. Univariable and IPTW plus adjusted multivariable Cox analysis for microbiome variables and death.

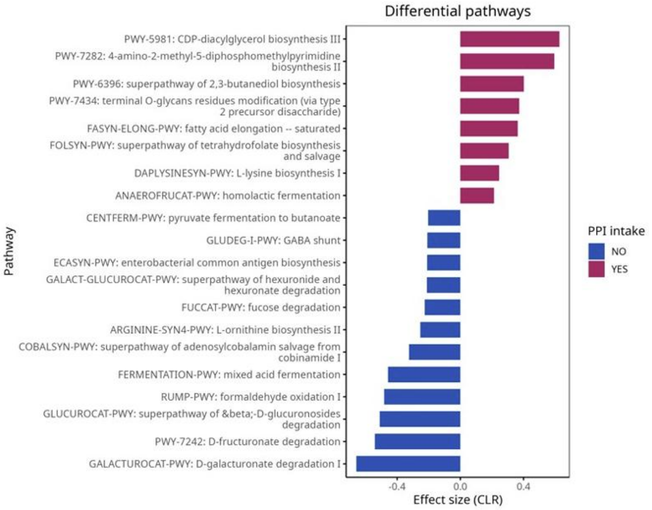

Figure 3. Differential abundance of microbial metabolic pathways by PPI use. Barlplot showing relative enrichment of micrbial metabolic pathways in patients with and without proton pump inhibitor (PPI) use, based in ALDEx2 with centred log-ratio (CLR) transofromation.

Back

Gut microbiota oralisation links proton pump inhibitors intake with reduced response to immunotherapy in patients with hepatocellular carcinoma

M. Montero-Vale[1], M. Sanduzzi-Zamparelli[1], A. González Amezcua[2], A. Sayol[1], E. Rubio[1], J.L. Villanueva[1],
G. Casals[1], J. Argemi[3,4], M. Mari[4,5], F. Torres[2], J. Bruix[5], M. Reig[1,5], C. Casals-Pascual.

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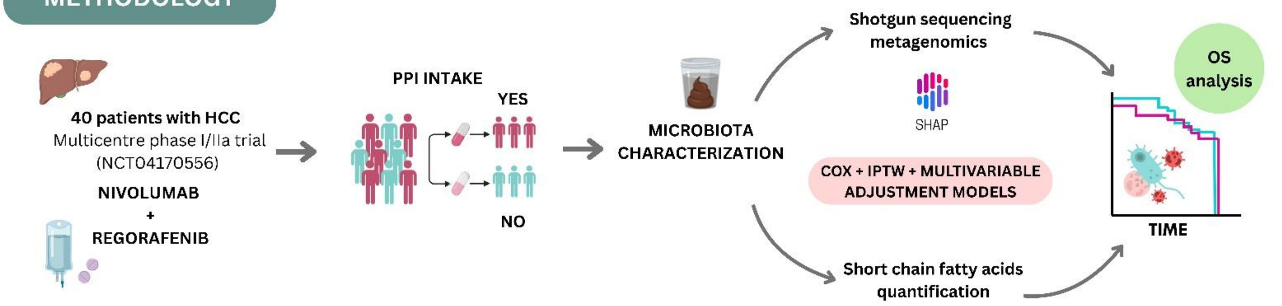
INTRODUCTION

Gut microbiota composition influences the response to immune checkpoint inhibitors (ICIs) across several cancers, including hepatocellular carcinoma (HCC). Proton pump inhibitors (PPIs) are widely prescribed in patients with HCC and are known to alter gut microbial communities, potentially interfering with host immune responses. These alterations may promote dysbiosis and alter microbiota composition, which has been associated with impaired immunotherapy efficacy in different tumour types. Therefore, this study aims to **investigate the effect of PPI exposure on the baseline gut microbiota composition and its association with clinical outcomes in ICI-treated patients with HCC such as overall survival (OS)**. Additionally, it seeks to explore whether PPI-related microbial alterations correlate with differences in treatment response and survival outcomes, providing further insight into the interplay between PPI intake, the microbiome, and immunotherapy efficacy.

METHODOLOGY



40 patients with HCC
Multicentre phase I/IIa trial
(NCT04170556)

NIVOLUMAB
+
REGORAFENIB

PPI INTAKE
YES
NO

MICROBIOTA CHARACTERIZATION

Shotgun sequencing metagenomics

SHAP

COX + IPTW + MULTIVARIABLE ADJUSTMENT MODELS

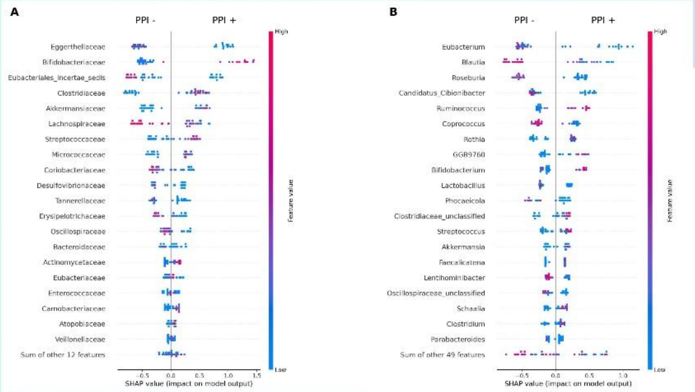
Short chain fatty acids quantification

OS analysis

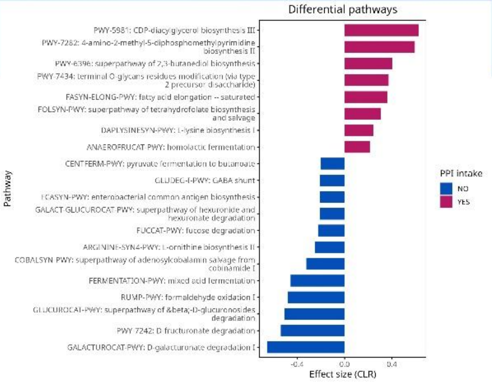
TIME

RESULTS

① **SHAP (SHapley Additive exPlanations) values for microbial taxonomic features associated with use of PPIs (panel A for family and panel B for genus).**



② **Differential abundance of microbial metabolic pathways by PPI use. based on ALDEx2 with centred log-ratio (CLR) transformation.**



③ **Univariable and IPTW plus adjusted multivariable Cox analysis for microbiome variables and death. Only statistically significant results are reported for the multivariate analysis for both family and species levels and short chain fatty acids (SCFAs) quantification.**

Family level

Taxon	HR (95% CI)	p
Streptococcaceae	1.05 (1.01-1.09)	0.012
Actinomycetaceae	4.01 (1.09-14.76)	0.037
Akkermansiaceae	1.16 (1.02-1.32)	0.027
Micrococcaceae	7.42 (1.65-33.39)	0.009

Species level

Taxon	HR (95% CI)	p	Taxon	HR (95% CI)	p
Akkermansia muciniphila	1.16 (1.02-1.32)	0.027	*Streptococcus gordonii*	9.61 (1.00-92.17)	0.05
Rothia mucilaginosa	8.40 (1.59-44.25)	0.012	*Streptococcus vestibularis*	1.29 (1.02-1.64)	0.035
Actinomyces dentalis	165.66 (2.43- 11305.18)	0.018	*Streptococcus anginosus*	1.19 (1.00-1.41)	0.045
Clostridium innocuum	9.35 (1.33-65.61)	0.025			

SCFAs

Type	HR (95% CI)	p
Isobutyrate	1.37 (1.00-1.88)	0.05
Isovalerate	1.41 (1.03-1.93)	0.03
Twomethylbutyrate	1.68 (1.06-2.65)	0.03

CONCLUSIONS

- **Gut microbiome composition was associated with overall survival in patients with HCC treated with ICIs.**
- **PPI exposure was strongly associated with oralization of the gut microbiome, characterized by enrichment of oral-associated taxa from *Streptococcaceae* and *Actinomycetaceae* families.**
- **Higher levels of branched-chain SCFAs (isobutyrate, isovalerate, and 2-methylbutyrate) were associated with worse clinical outcomes.**
- **These findings support a potential role of the gut microbiome as a biomarker of response in HCC patients receiving immunotherapy.**

Clínic Barcelona | Centre de Diagnòstic Biomèdic

RETO

Funding: **Asociación Española Contra el Cáncer (AECC)**

Contact information: mmmontero@clinic.cat

20. STI Burden and Vaginal Microbiome in Late Pregnancy in Sierra Leone

Elisa Rubio García[1], Climent Casals[1], Clara Menéndez[2], Berta Isabel Fidalgo[1], Maria Mercedes Montero[1], Maureen Chileshe[3], Kwabena Owusu-Kyei[3], Estefania Mangas[1], Natàlia Arnalda[1], Alba Morató[1], Andreu Bofill[1]

[1] Hospital Clínic de Barcelona / ISGlobal, Barcelona, Spain; [2] ISGlobal, Barcelona, Spain; [3] Collaborating institutions in Sierra Leone

Background: Neonatal mortality is high in sub-Saharan Africa, where preterm birth (PTB) is a major contributor. Maternal vaginal dysbiosis and sexually transmitted infections (STIs) have been associated with increased risk of PTB. We aimed to characterize STI burden and describe vaginal microbiome (VMB) composition in third-trimester pregnant women attending antenatal care at Makeni Hospital in Sierra Leone

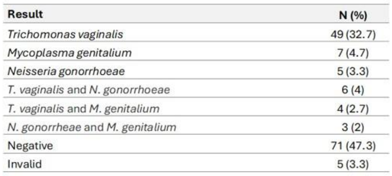
Methods: We analysed 150 vaginal swabs. STIs were screened using the Allplex™ CT/NG/MG/TV PCR assay (Seegene®), detecting C. trachomatis, N. gonorrhoeae, M. genitalium, and T. vaginalis. VMB composition was assessed through full-length 16S rRNA sequencing on the PacBio Sequel II. Sequence processing and analysis were performed using QIIME2 and R. After low-yield exclusions, 111 samples remained for microbiome analysis.

Results: More than half participants tested positive for at least one STI, with T. vaginalis being the most prevalent; C. trachomatis was not detected (Table 1). Alpha diversity was significantly higher among STI-positive women (Faith index, p=0.033), with diversity increasing in correlation with the number of concurrent STIs (Spearman, R=0.22, p=0.02). Microbiome profiling matched STI results, detecting N. gonorrhoeae and M. genitalium only in samples with the lowest Ct values.

Figure 1 shows Community State Type (CST) distribution, notably, L. jensenii, L. paragasseri and Gardnerella spp. were present at low relative abundances (max 12%, 1.6%, 0.11%). No associations were found between VMB diversity, STI results and clinical data. CST I samples had a higher proportion of T. vaginalis–positive cases, and no prematurity was observed in this CST (Table 2).

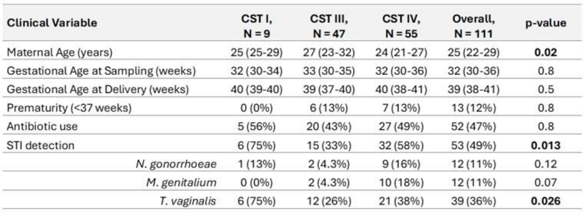
Conclusions: STI prevalence was high, but C. trachomatis was absent, indicating low circulation of this pathogen. STI-positive women showed higher VMB diversity, consistent with dysbiosis. The VMB was dominated by L. iners and non-Lactobacillus CSTs, a pattern typical in sub-Saharan Africa.

Table 1. Detection rates of Sexually Transmitted Infections in the study cohort



Result	N (%)
Trichomonas vaginalis	49 (32.7)
Mycoplasma genitalium	7 (4.7)
Neisseria gonorrhoeae	5 (3.3)
T. vaginalis and *N. gonorrhoeae*	6 (4)
T. vaginalis and *M. genitalium*	4 (2.7)
N. gonorrheae and *M. genitalium*	3 (2)
Negative	71 (47.3)
Invalid	5 (3.3)

Table 2. Clinical characteristics of the study cohort and Sexually Transmitted Infections (STI) results according to Community State Type (CST) analysis.



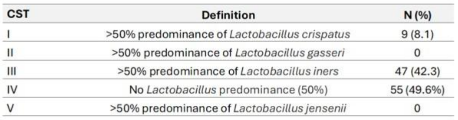
Clinical Variable	CST I, N = 9	CST III, N = 47	CST IV, N = 55	Overall, N = 111	p-value
Maternal Age (years)	25 (25-29)	27 (23-32)	24 (21-27)	25 (22-29)	**0.02**
Gestational Age at Sampling (weeks)	32 (30-34)	33 (30-35)	32 (30-36)	32 (30-36)	0.8
Gestational Age at Delivery (weeks)	40 (39-40)	39 (37-40)	40 (38-41)	39 (38-41)	0.5
Prematurity (<37 weeks)	0 (0%)	6 (13%)	7 (13%)	13 (12%)	0.8
Antibiotic use	5 (56%)	20 (43%)	27 (49%)	52 (47%)	0.8
STI detection	6 (75%)	15 (33%)	32 (58%)	53 (49%)	**0.013**
N. gonorrhoeae	1 (13%)	2 (4.3%)	9 (16%)	12 (11%)	0.12
M. genitalium	0 (0%)	2 (4.3%)	10 (18%)	12 (11%)	0.07
T. vaginalis	6 (75%)	12 (26%)	21 (38%)	39 (36%)	**0.026**

[1] Median (25%-75%); n (%)

[2] Kruskal-Wallis rank sum test; Fisher's exact test

Figure 1. (A) Relative abundances of the 20 most abundant species by Community State Type (CST) classification. (B) CST distribution and definitions among the 111 samples retained for vaginal microbome analysis.

B)



CST	Definition	N (%)
I	>50% predominance of *Lactobacillus crispatus*	9 (8.1)
II	>50% predominance of *Lactobacillus gasseri*	0
III	>50% predominance of *Lactobacillus iners*	47 (42.3)
IV	No *Lactobacillus* predominance (50%)	55 (49.6%)
V	>50% predominance of *Lactobacillus jensenii*	0

Back

Sexually Transmitted Infections Burden and Vaginal Microbiome in Late Pregnancy in Sierra Leone

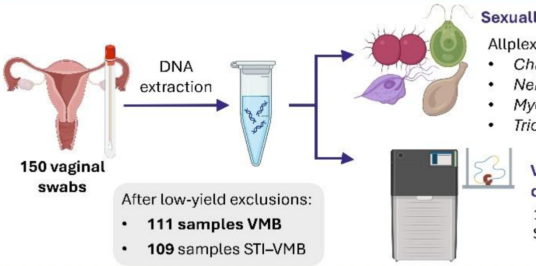
Elisa Rubio[1-3], Berta I Fidalgo[1], Maria Mercedes Montero[1,2], Maureen Chileshe[3], Kwabena Owusu-Kyei[3], Estefania Mangas[1], Natàlia Arnalda[1], Alba Morató[3], Andreu Bofill[3], Climent Casals-Pascual[1-3], Clara Menendez[3]

1. Department of Microbiology, CDB, Hospital Clinic, University of Barcelona, Barcelona, Spain **2.** Molecular CORE Facility, CDB, Hospital Clinic of Barcelona **3.** ISGlobal Barcelona Institute for Global Health, Barcelona, Spain

Background

Neonatal mortality is high in sub-Saharan Africa, where preterm birth (PTB) is a major contributor. Maternal vaginal dysbiosis and sexually transmitted infections (STIs) have been associated with increased risk of PTB, yet data from Sierra Leone is limited. We aimed to characterize STI burden and describe vaginal microbiome (VMB) composition in **third-trimester pregnant women** (>28 weeks) attending antenatal care at **Makeni Regional Hospital** in Bombali, Sierra Leone

Methods



150 vaginal swabs

DNA extraction

After low-yield exclusions:
- **111 samples VMB**
- **109** samples STI–VMB

Sexually transmitted infections (STI)

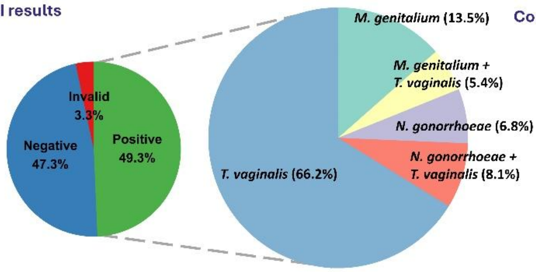
Allplex Assay (Seegene®):
- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- *Mycoplasma genitalium*
- *Trichomonas vaginalis*

Vaginal microbiome composition (VMB)

16S rRNA V1-V9 (1465 pb)
Sequel II PacBio system

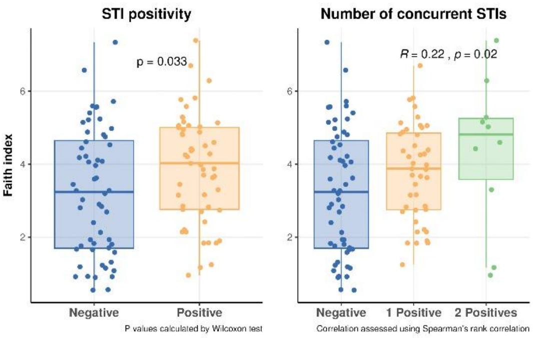
Results

STI results



- More than half participants tested positive for at least one STI, with *T. vaginalis* being the most prevalent; *C. trachomatis* was not detected

STI – VMB alpha diversity

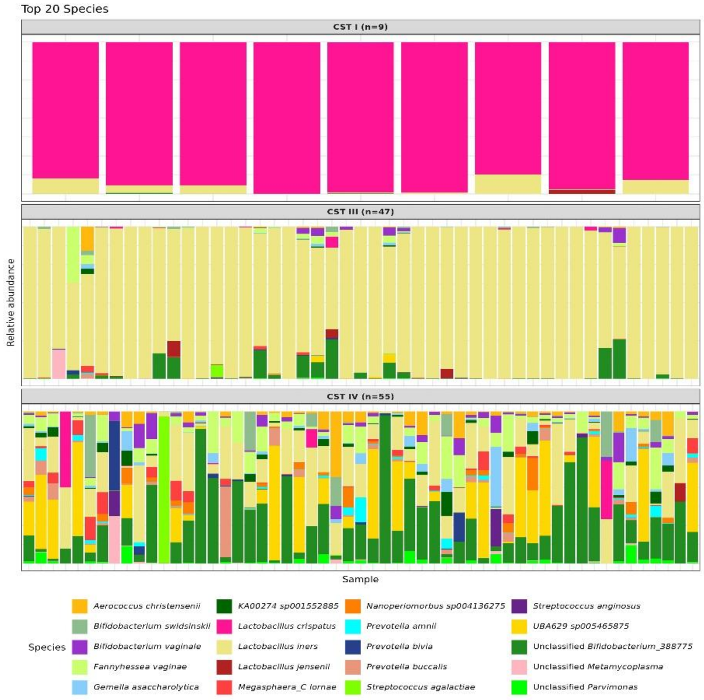


P values calculated by Wilcoxon test

Correlation assessed using Spearman's rank correlation

- Alpha diversity was significantly higher among STI-positive women with diversity increasing in correlation with the number of concurrent STIs.
- Microbiome profiling matched STI results, detecting *N. gonorrhoeae* and *M. genitalium* only in samples with the lowest Ct values

Community State Type (CST) distribution



- *L. jensenii*, *L. paragasseri* and *Gardnerella* spp. were present at low relative abundances (max 12%, 1.6%, 0.11%).
- No clear associations were found between VMB diversity, STI results and clinical data.
- CST I samples had a higher proportion of *T. vaginalis*–positive cases, and no prematurity was observed among women in this CST.

Clinical Variable[1]	CST I, N = 9	CST III, N = 47	CST IV, N = 55	Overall, N = 111	p-value[2]
Maternal Age (years)	25 (25-29)	27 (23-32)	24 (21-27)	25 (22-29)	0.02
Gestational Age at Sampling (weeks)	32 (30-34)	33 (30-35)	32 (30-36)	32 (30-36)	0.8
Gestational Age at Delivery (weeks)	40 (39-40)	39 (37-40)	40 (38-41)	39 (38-41)	0.5
Prematurity (<37 weeks)	0 (0%)	6 (13%)	7 (13%)	13 (12%)	0.8
Antibiotic use	5 (56%)	20 (43%)	27 (49%)	52 (47%)	0.8
STI detection	6 (75%)	15 (33%)	32 (58%)	53 (49%)	0.013
N. gonorrhoeae	1 (13%)	2 (4.3%)	9 (16%)	12 (11%)	0.12
M. genitalium	0 (0%)	2 (4.3%)	10 (18%)	12 (11%)	0.07
T. vaginalis	6 (75%)	12 (26%)	21 (38%)	39 (36%)	0.026

[1] Median (25%-75%); n (%)

[2] Kruskal-Wallis rank sum test; Fisher's exact test

CST	Definition	n (%)
I	>50% predominance of *Lactobacillus crispatus*	9 (8.1)
II	>50% predominance of *Lactobacillus gasseri*	0
III	>50% predominance of *Lactobacillus iners*	47 (42.3)
IV	No *Lactobacillus* predominance (50%)	55 (49.6%)
V	>50% predominance of *Lactobacillus jensenii*	0

Conclusions

In this cohort, STI prevalence was high, but *C. trachomatis* was absent, indicating low circulation of this pathogen. STI-positive women showed higher VMB diversity, consistent with dysbiosis and increased infection risk. The VMB was dominated by *L. iners* and non-Lactobacillus CSTs, a pattern typical in sub-Saharan Africa. *L. crispatus*–dominated communities were rare but may be protective against PTB.

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21. A Blood Metabolomic Signature of the Dietary Index of Gut Microbiota and its Association with Cognitive Function in a Population-Based Cohort of Older Persons

Salomé Darblade[1], Emmanuelle Orsini[1], Blandine Gendre[1], Catherine Helmer[1], Cécilia Samieri[1]

[1] Univ Bordeaux, INSERM, Bordeaux Population Health Research Center, France

Background: Dementia and Alzheimer's disease are public health issues. The Diet-Gut-Brain axis is a promising prevention target. The Dietary Index of Gut Microbiota (DI-GM) reflects diet quality for microbiota health. We aimed to identify a DI-GM blood metabolomic signature and test its link with cognition.

Methods: We analyzed 1,128 participants aged 55-80 years from the B cube study, with up to three 24h dietary recalls, a comprehensive cognitive battery, and plasma metabolomics (Metabolon). The DI-GM summarizes 13 dietary components reflecting microbiota-healthy and unhealthy foods. Among 1,307 detected blood metabolites, 430 previously linked to gut microbiota composition (GUTSY atlas) were selected. Cognitive performance was summarized into latent composite scores for global cognition, memory, and executive function. A bootstrap-enhanced LASSO adjusted for age, sex, BMI, and recent antibiotic use identified a blood metabolomic signature of the DI-GM. The resulting metabolomic score and individual metabolites were tested for associations with cognition.

Results: The DI-GM metabolomic signature included 67 metabolites. In multivariable models, higher DI-GM scores and higher metabolomic signature scores were associated with better memory ($\beta=0.03$ [0.01–0.04] per DI-GM point; $\beta=0.06$ [0.01–0.10] per SD metabolomic score). The metabolomic score was also associated with better global cognition ($\beta=0.07$ [0.02–0.11]), while the DI-GM showed a weaker, borderline significant association. No associations were observed with executive function. Among the 67 metabolites, 18 were significantly associated with cognition, including ergothioneine and hydroxy-CMPF, both positively related to memory and global cognition.

Conclusion: We identified a blood metabolomic signature of a gut microbiota-supportive diet that was associated with better global cognition and memory in older adults. Replication and prospective studies are needed to confirm its relevance for cognitive aging.

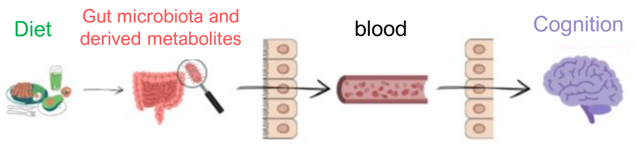
Back

A blood metabolomic signature of the Dietary Index of Gut Microbiota and its association with cognitive function in a population-based cohort of older persons

Salomé Darblade[a], Emmanuelle Orsini[a], Blandine Gendre[a], Catherine Helmer[a], Cécilia Samieri[a]

[a]Univ Bordeaux, INSERM, Bordeaux Population Health Research Center, UMR 1219, Bordeaux, France

Introduction



Diet-Gut-Brain axis

- **Prevention of Alzheimer's disease and related dementias** is a major challenge in aging populations, with **environmental factors** (the exposome) contributing to disease risk (1).
- Diet may influence cognition through the **Diet-Gut-Brain** axis. (2)
- **Gut-derived metabolites** are potential mediators linking diet and brain health, and these remain poorly characterized.
- The **Dietary Index for Gut Microbiota** (DI-GM) reflects adherence to microbiota-supportive diet (3).

Objective : Identify a blood metabolomic signature of the DI-GM and investigate secondarily its association with cognitive function in a large population-based cohort of older persons from late midlife through older age

Methods

DATA

Population-based

B cube

Biobank and Brain health in Bordeaux

between 55 and 80 yrs
in 2022-2025
in Bordeaux Métropole

DI-GM

Based on repeated **24h dietary recalls**. 0 or 1 assigned to each item using sex-specific population medians (1 indicating favorable intake). Beneficial to gut microbiota : fiber, whole grains, fermented dairy, coffee, green tea, avocado, broccoli, chickpeas, soy. Unfavorable to gut microbiota : processed and red meat, red, refined grains, and energy from fat ≥ 40%.

Metabolites

Metabolon® untargeted LC-MS profiling (P=1270 detected across 3 batches)

Metabolites with **≥ 30% of data** and **associated with ≥ 30% microbial species according to GUTSY Atlas** (4) were retained (P=399)

Cognition

Cognitive performances assessed using a battery of neuropsychological tests. Three cognitive composite scores derived from a latent process model: **global cognition, memory, executive function**

(1) IDENTIFICATION OF A METABOLOMIC SIGNATURE OF THE DI-GM

LASSO penalized regression with 1000 bootstrap resampling
adjusted for age, sex, antibiotic use in the past 3 months and batch

Metabolites retained in at least 60% of bootstraps

Derivation of a **single metabolomic score**

$$\sum_{p=1}^{P} \beta_p X_p$$

(2) ASSOCIATION BETWEEN THE METABOLOMIC SIGNATURE AND COGNITION

$$\mathrm{COGNITION}_i = \beta_0 + \beta_1\,\mathrm{SCORE}_i + \beta_{\mathrm{AdjCov}}\,\mathrm{AdjCov}_i + \varepsilon_i$$

$$\mathrm{COGNITION}_i = \beta_0 + \beta_1\,\mathrm{METABOLITE}_i + \beta_{\mathrm{AdjCov}}\,\mathrm{AdjCov}_i + \varepsilon_i$$

Adjusted for age, sex, education, number of medication used, alcohol consumption, smoking history, batch, BMI, hypertension, type 2 diabetes and hypercholesterolemia

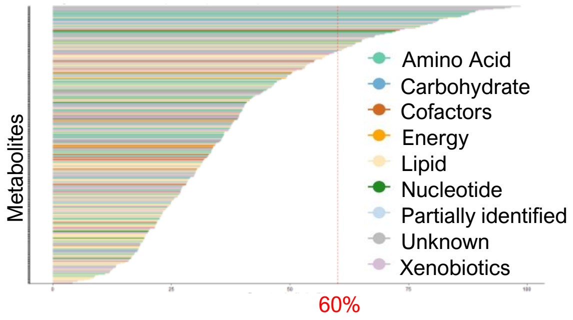
Results

N=1623
With at least one 24h recall and metabolomic data

65% female – 71% > high school level - 36% hypertension - 6% type 2 diabetes – 26% hypercholesterolemia
Mean (±sd): age 66.6 yrs (±6) BMI 25.3kg/m² (±4) medications 1.9 (±2)

(1) METABOLOMIC SIGNATURE OF THE DI-GM

Rate of selection across bootstraps (≥60% P=66)



Among the 66 metabolites selected:
→**Lipids** including hydroxy-CMPF, 14:0 dicarboxylic acid…
→**Amino Acids** including indoleacetate, indoleproprionate…
→**Xenobiotics** including ergothioneine, xanthine …

(2) ASSOCIATION BETWEEN THE METABOLOMIC SIGNATURE AND COGNITION

Metabolomic score

	β	CI95%
Global cognition	0.05	[0.02 ; 0.09]
Memory	0.05	[0.02 ; 0.09]
Execut. function	0.01	[-0.03 ; 0.05]

Metabolites
FDR-adjusted significance
(FDR <0.05**)**

DI-GM		Cognition
+	**Pyruvate** →energy metabolism	+
-	**2-naphtol sulfate** →environmental exposure to pollution and smoke	-
+	**X-11315**	+

Conclusion

- **The blood metabolomic signature reflecting a gut microbiota-beneficial diet was associated with better memory and global cognitive performance.**
- **These findings require validation in an independent cohort**
- **Results consistent with the literature, with expected directions of association**

Pyruvate : higher circulating levels associated with slower cognitive decline and reduced brain atrophy (5)
2-naphtol sulfate : lower circulating level associated with better general cognition (6)
X-11315 : unidentified, but associated with apple consumption (7)

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(6) Ahmad, et al. medRxiv. 2024
(7) Low, et al. Nutrients. 2025

This work is supported by a grant overseen by the French National Research Agency (ANR) as part of the "France 2030" Program ANR-23-IAHU-00001, ANR-17-EURE-0027, and ANR-20-SFRI-0001.

22. Potential Gut Microbiota-Associated Biomarkers in Wilson Disease

Carmen Espinós[1], Edna Ripollés[1], Ariadna Bono[1], Anna Miralpeix[2], Isabel Sastre-Bataller[3], Zoe Mariño[4], Marina Berenguer[2], Alfonso Benítez-Sáez[5]

[1] Universitat de València / INCLIVA / CIBERER, Spain; [2] Hospital La Fe, Valencia, Spain; [3] Hospital Clínic, Barcelona, Spain; [4] Hospital Clínic / Universitat de Barcelona, Spain; [5] IATA-CSIC, Spain

Background: The microbiota-liver axis and the development of hepatic fibrosis are closely linked. This interconnection is regulated by an intricate metabolic, endocrine, and immune host-microbe crosstalk. We aimed to characterize the gut microbiota in patients with Wilson's disease (WD) to identify potential bacterial biomarkers that could facilitate the development of novel treatment strategies.
Methods: Clinical series: faecal samples from two independent cohorts (discovery & validation) comprising 24 in-depth phenotyped patients, and age and sex-matched cohorts of healthy individuals. Molecular assays: first approach by 16S RNA gene sequencing whose results are under validation using quantitative PCR (qPCR). Bacterial cultures: Akkermansia species growth rate determined in presence/absence of D-penicillamine, D-penicillamine + CuSO4 or zinc acetate.
Results: We find significant changes in the GM community structure and species abundance in WD patients, who exhibit lower levels of Akkermansia muciniphila, a species associated with disruptions in gut barrier function, imbalances in metabolism, and hepatic status. We focus on the drug-microbe interaction following its growth rate when cultured anaerobically with D-penicillamine and zinc acetate mimicking treatment dosage. A. muciniphila cannot survive in the presence of zinc acetate, and its growth rate is meaningfully decreased in presence of D-penicillamine and restored with CuSO4 supplementation.
Conclusions: GM profile of WD patients differs from that of healthy individuals. The attenuated abundance of A. muciniphila from WD patients is likely derived from pharmacological treatment. Our findings pave the way for designing novel probiotic-based strategies to improve WD patients' health, given the pivotal role of A. muciniphila in controlling host metabolism at different levels.
Funding: Instituto de Salud Carlos III (PI21/00103 & PI25/00046), cofunded by the European Union, and Foundation Per Amor a l'Art.

Back

Potential gut microbiota-associated biomarkers in Wilson disease

ESPINÓS, Carmen[1,2], RIPOLLÉS, Edna[3], BONO, Ariadna[4], MIRALPEIX, Anna[5], SASTRE-BATALLER, Isabel[4], MARIÑO, Zoe[5,6,7], BERENGUER, Marina[4,6,8], BENÍTEZ-PÁEZ, Alfonso[9]

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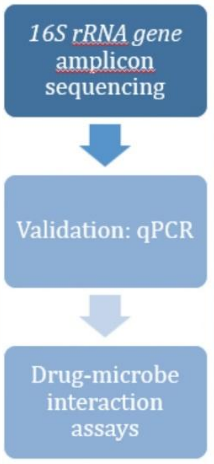
BACKGROUND. The gut microbiota makes an axis with various extraintestinal organs, including liver. This interconnection is regulated by an intricate metabolic, endocrine, and immune host-microbe crosstalk. Intestinal dysbiosis and increased intestinal permeability lead to translocation of microorganisms and microbial products, which trigger and maintain inflammatory cascades that ultimately lead to liver damage. To date, there is a lack of data on gut microbiota in Wilson's disease (WD) patients undergoing chronic copper chelation treatment.

AIM. To identify bacterial biomarkers that could provide insight into the development of microbiome-based approaches to enhance the management of the disease.

METHODS

Fecal samples from two independent cohorts (discovery & validation) comprising 24 and 10 in-depth phenotyped patients, and age and sex-matched cohorts of healthy individuals.

Bacterial growth in presence/absence of Cupripen ®, Cupripen ® + $CuSO_4$ or Wilzin ®. RNAseq.

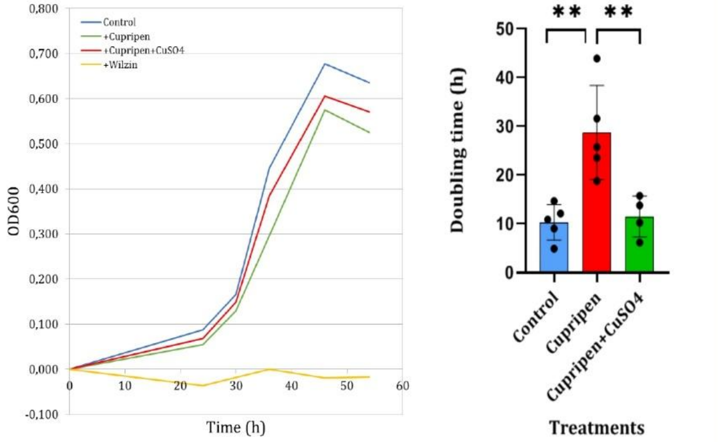


16S rRNA gene amplicon sequencing

Validation: qPCR

Drug-microbe interaction assays

RESULT. Figure 2. The growth rate of *A. muciniphila* influenced by WD drugs



A. muciniphila cannot survive in the presence of Wilzin®, and its growth rate is meaningfully decreased with Cupripen® but restored with $CuSO_4$ supplementation.

FUNDING

FUNDACIÓ PER AMOR A L'ART

reMIDAS Microbiota humana Aplicada a la Salud
RED2022-134934-T

Co-funded by the European Union

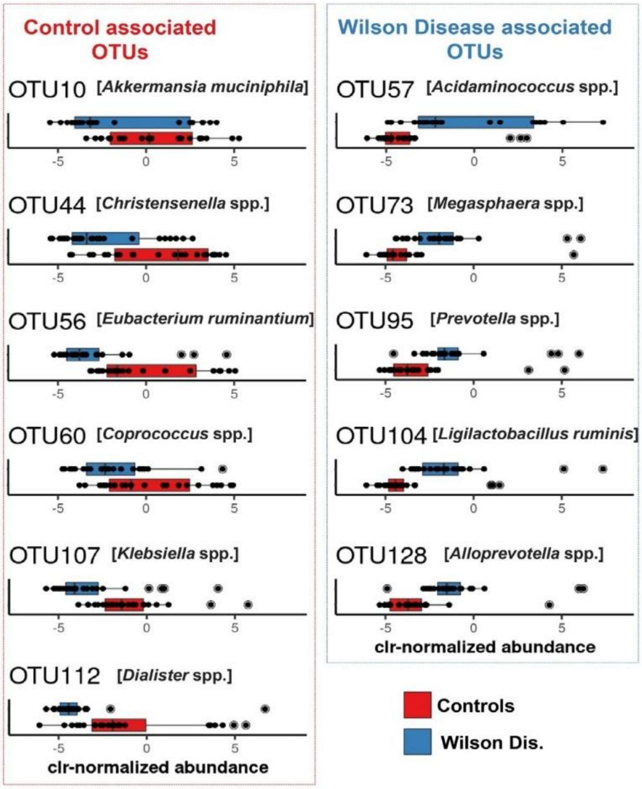
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES
AGENCIA ESTATAL DE INVESTIGACIÓN

Instituto de Salud Carlos III

PI21/00103
PI25/00046

GENERALITAT VALENCIANA
Conselleria d'Educació, Cultura i Universitats
CIACIF/2021/252

RESULT. Figure 1. There exists significant changes in the gut microbiota community structure.



We have not identified significant differences in α or β diversity between controls and WD patients. However, specific species were identified as either overrepresented or underrepresented in patients

CONCLUSIONS

- The gut microbiota profile of WD patients undergoing pharmacological treatment differs from that of healthy individuals.
- The attenuated abundance of *A. muciniphila* from WD patients is likely derived from treatment.
- Our findings pave the way for designing novel probiotic-based strategies to improve WD patients' health, given the pivotal role of *A. muciniphila* in controlling host metabolism.

BEST ABSTRACT

23.Gut Microbiome–Cardiorenal Crosstalk as a Determinant of Future Disease

Kanta Chechi[1,2,3]#, Rima Chakaroun[4,5,6]*, Antonis Myridakis[1]*, Sofia K. Forslund Startceva[7,8,9,10,11,12]*, Sebastien Fromentin[13]*, Trine Nielsen[14,15,16,17], Judith Aron-Wisneswky[18,19], Eugeni Belda[18,20], Edi Prifti[18,20], Pierre Bel Lassen[18,19], Gwen Falony[21,22,23,24], Sara Vieira Silva[21,22,23,25,26], Julien Chilloux[1], Kazuhiro Sonomura[27], Lesley Hoyles[1,28], Laura Martinez-Gili[1], Francesco Pallotti[1,29], Petros Andrikopoulos[1], Francesc Puig-Castellví[30], Romina Pacheco Tapia[30], Inés Castro-Dionicio[30], Hugo Roume[13], Nicolas Pons[13], Emmanuelle Le Chatelier[13], Benoit Quinquis[13], Nathalie Galleron[13], Magali Berland[13], Michael T. Olanipekun[1], Manyi Jia[1], Angelos Manolias[1], Bridget Holmes[31,32], Solia Adriouch[18], Matthias Blüher[4,5], Luis Pedro Coelho[8], Kévin Da Silva[13], Pilar Galan[33], Boyang Ji[34,35], Ana Luisa Neves[1,36], Christine Rouault[18], Joe-Elie Salem[37], Valentina Tremaroli[6], Tue H. Hansen[14,15,16], Nadja B. Søndertoft[14], Christian Lewinter[14], Helle K. Pedersen[14], The MetaCardis Consortium§, Peter D. Mark[38], Jens P. Goetze[39], Lars Køber[39,40], Henrik Vestergaard[14,17], Torben Hansen[14,41], Jean-Daniel Zucker[18,20], Taka-Aki Sato[27], Serge Hercberg[33], Fredrik Bäckhed[6,42], Ivica Letunic[43,44], Jean-Michel Oppert[19], Jens Nielsen[34], Jeroen Raes[21,22], Ioanna Tzoulaki[3,45,46], Abbas Dehghan[3,46], Verena Zuber[3,46,47], Emmanuelle Bouzigon[48], Mark Lathrop[49], Parminder Raina[50,51], Philippe Froguel[30,52], Fumihiko Matsuda[53], Florence Demenais[48], Dominique Gauguier[49,53,54], Michael Stumvoll[4,5], Peer Bork[8,9,44], Oluf Pedersen[14,40,55]#, S. Dusko Ehrlich[13,56]#, Karine Clément[18,19]#, Marc-Emmanuel Dumas[1,2,30,49]#*

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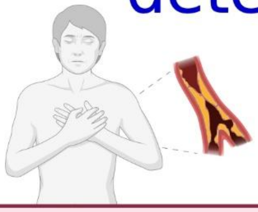
Cardiovascular diseases (CVD) remain a major global health challenge. Early markers of disease initiation/progression are urgently needed. We previously reported major gut microbiome alterations in association with early stages of CVD. Here, we report associations among gut microbial metabolism of aromatic amino acids, phenylalanine-tyrosine, and circulating pro-atrial natriuretic peptide and estimated glomerular filtration rate as markers of heart and kidney function, respectively, in metabolically healthy individuals from the European MetaCardis study (n = 275). Using mediation and Mendelian Randomization approaches, we next find microbiome-related metabolites that serve as the mediators of the gut microbiome-kidney axis on one hand and engage in a potentially causal crosstalk with the kidneys on the other. Notably, baseline levels of key microbiome-related metabolites also associate with CVD incidence during a 3-year follow-up in the Canadian Longitudinal Study on Aging (n = 8,669). Altogether, our work suggests that the gut microbiome participates in an interorgan crosstalk affecting host physiology and disease aetiology. As our discovery population was free of obesity, type 2 diabetes, metabolic syndrome or heart disease, these data further suggest that the gut microbiome can capture metabolic disturbances associated with CVD initiation, thereby serving as a potential target for prevention and early intervention.

Back

Gut microbiome–cardiorenal crosstalk as a determinant of future disease

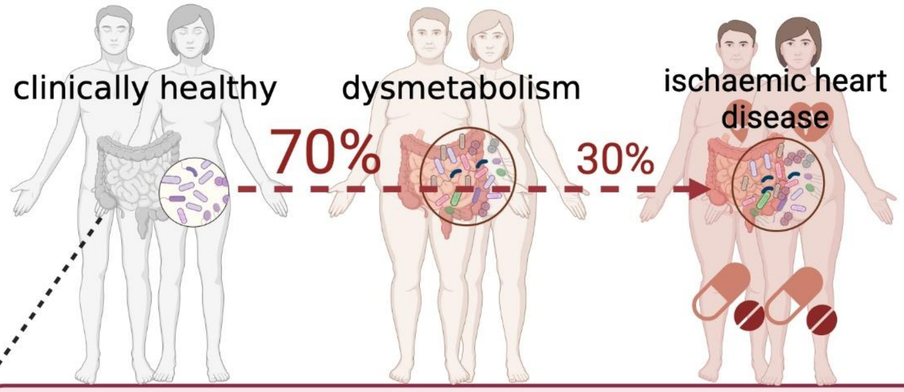


Cardiovascular disease (CVD) remain the **leading** cause of deaths globally

Mechanistic understanding and early detection especially of the underlying silent pathogenesis is much needed

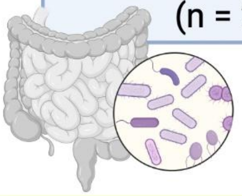
MetaCardis

European population
(Germany, Denmark, France)
n ~ 2,350



Microbiome and metabolomic markers shifted primarily in association with dysmetabolism relative to CVD
Fromentin*, Forslund*, **Chechi*** et al *Nat Med* 2022
*co-first authorship

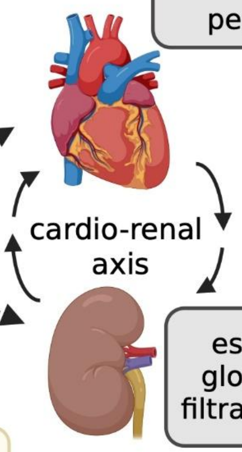
individuals free of metabolic syndrome, obesity, type 2 diabetes and heart disease
(n = 275)



Markers related to phenylalanine and tyrosine metabolism

gut microbial metabolic modules

circulating metabolites



pro-atrial natriuretic peptide

estimated glomerular filtration rate

Baseline levels of key metabolites associated with CVD incidence during 3-year follow up in the Canadian Longitudinal Study of Aging (n = 8,669)

Genetic and observational evidence link gut microbial aromatic amino acid metabolism with subclinical variations in heart and kidney function, revealing an inter-organ axis, which is predictive of future cardiovascular disease (Chechi K et al, *Nat Comms, 17,* 2026)

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UKRI Medical Research Council

KANTA CHECHI
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Diagnostics

24. Microbiome Taxa as Biomarkers of Irritable Bowel Syndrome Symptomatology in Patients with Ulcerative Colitis in Remission

Natalia Balaguer-Sanjuán[1], Ruben Salas Reixach[1], Xavier Aldeguer[1], Robert Carreras-Torres[1]

[1] Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI), Girona, Spain

Patients with ulcerative colitis (UC) in remission could still present symptomatology compatible with irritable bowel syndrome (IBS). Alterations in the gut microbiome could underlie this symptomatology. This study pretends to identify taxonomic biomarkers for this phenotype through the analysis of 16S rRNA sequencing data.
Stool samples from 77 patients from 3 clinical groups were collected. They comprise 31 patients with UC in remission (reference group), 27 patients with UC in remission with IBS symptomatology and 19 patients diagnosed with IBS, as study groups. Sequences from the V3-V4 region from the 16S rRNA gene were generated.
Data was processed using the DADA2 and phyloseq pipeline, while the SILVA's v138.2 reference database was used for taxonomic assignment. Predicted microbiome functional abundances were estimated with PICRUSt2. Finally, differential abundances between the reference group and the study groups were assessed with ALDEX2.
The analysis of diversity indices (Shannon and Simpson) did not present significant differences between groups (P> 0.73). Differential abundance analyses identified the genus Flavonifractor as depleted in patients with IBS symptomatology (P= 0.003; FDR P= 0.37). This may reflect reduced butyrate production, a metabolite with anti-inflammatory effects. Consistently, functional analysis highlighted a decreased abundance of the "biosynthesis of unsaturated fatty acids" pathway (ko01040) (P= 6.1x10-4; FDR P= 0.07).
In conclusion, the metabolic activity of specific taxa from the gut microbiome could explain the IBS symptomatology in UC patients in remission. These findings could be relevant as possible diagnostic biomarkers or for future therapeutic options.

Back

Microbiome as biomarkers for Inflammatory Bowel Syndrome (IBS)-like symptoms in Ulcerative Colitis (UC) patients in remission

Natalia Balaguer Sanjuan*[1], Xavier Aldeguer[1], Robert Carreras-Torres[1]

[1]*Institut d'Investigació Biomèdica de Girona Dr Josep Trueta (IDIBGI), Digestive Diseases and Microbiota Group, Girona, Spain*

INTRODUCTION

A significant subset of patients with Ulcerative Colitis (UC) in clinical remission continue to experience persistent irritable bowel syndrome (IBS)-like symptoms. The underlying biological mechanism remains unclear, but subclinical alterations in the gut microbiota are hypothesized to drive this symptomatic phenotype (UC&IBS).

This study aimed to **identify taxonomic and functional microbiome biomarkers** associated with IBS symptomatology in UC patients in remission.

METHODS

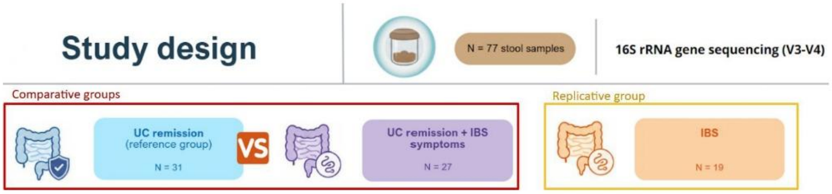

Fig.1 Overview of study groups and sequencing strategy. Schematic representation of the 77 clinical stool samples analyzed to identify specific taxonomic and functional biomarkers differentiating the persistent UC&IBS phenotype from silent UC remission and standard IBS controls.

Worflow

Bioinformatic analysis pipeline

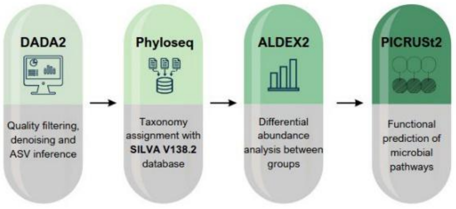

Fig.2 Sequential stages of the 16S rRNA bioinformatic processing. Schematic flow illustrating raw data denoising (DADA2) , community taxonomic assignment (Phyloseq/SILVA) , targeted biomass abundance variations (ALDEx2) , and functional potential modeling (PICRUSt2)

RESULTS

No statistically significant differences were observed in Shannon or Simpson alpha diversity indices across clinical groups (P > 0.73). Demonstrating that persistent **symptoms are not driven by major ecosystem collapses.**

However, high-resolution taxonomic profiling identified a significant and targeted **depletion** of the genus **Flavonifractor** in the symptomatic UC&IBS cohort (P = 0.003; FDR P = 0.37). This finding is particularly interesting because Flavonifractor species are involved in the metabolism of complex carbohydrates and have been linked to short-chain fatty acid production, particularly **butyrate**, which plays an important **anti-inflammatory role** in the gut.

Consistent with this specific taxonomic loss, functional pathway prediction revealed a significant **downregulation of the Biosynthesis of unsaturated fatty acids pathway (ko01040)** in the UC&IBS group (P = 6.1 x 10^4; FDR P = 0.07), further supporting the existence of **metabolic alterations associated with the symptomatic phenotype.**

Overall diversity

Shannon and Simpson analysis

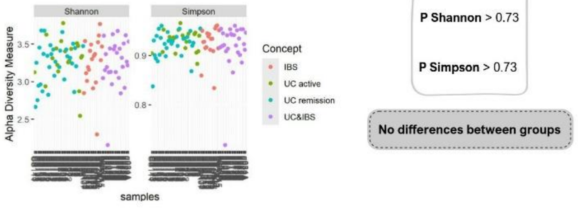

Fig.3 Comparison of gut microbiota alpha diversity. Statistical profiling using Shannon and Simpson metrics reveals no significant differences in global diversity between symptomatic UC&IBS patients and reference cohorts (P > 0.73)

Microbial biomarkers

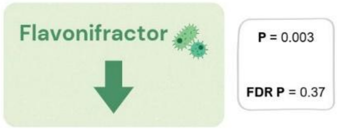

Fig.4 Microbial biomarker profiling. The genus Flavonifractor is significantly depleted in the active UC&IBS phenotype (P = 0.003; FDR P = 0.37), serving as a key candidate diagnostic and therapeutic biomarker.

Functional prediction

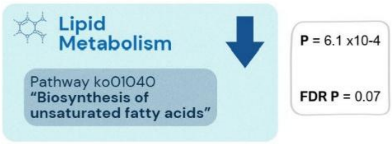

Fig.5 Predicted functional alterations. A significant decrease in the lipid metabolism pathway ko01040 (P = 6.1x10-4; FDR P = 0.07) associated with the symptomatic phenotype.

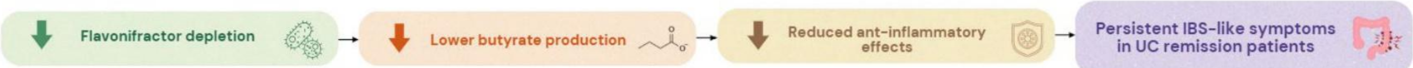

Fig.6 Proposed biological mechanism of the UC&IBS phenotype. Schematic overview of the downstream effects triggered by taxonomic dysbiosis, connecting with the initial objective: identify taxonomic and functional microbiome biomarkers associated with IBS symptomatology in UC patients in remission.

CONCLUSIONS

"IBS-like symptoms in UC remission are linked to specific taxonomic and metabolic alterations rather than global diversity changes, highlighting Flavonifractor depletion as a key potential biomarker and therapeutic target."

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GRANT REFERENCE:
This study was funded by the Ministry of Economy, Industry and Competitiveness (MINECO) RETOS program (RTC-2017-6467-2). The Instituto de Salud Carlos III supported RCT through CP21/00058.

25. MICK Clinical: Automated Qualitative Identification of Gastrointestinal Pathogens Via Ivd-certified Ngs-Based Metagenomic Analysis

Andreu Paytuví-Gallart[1], Roberto Malinverni[1], Erik Andrés Reig[1], Guillermo Chumaceiro[1], Daniel Julian[1], Riccardo Aiese Cigliano[1]

[1] Sequentia Biotech S.L., Barcelona, Spain

Background: Early identification of gastrointestinal pathogens is essential for effective symptom management. Traditional diagnostics lack the comprehensive scope of whole-genome analysis. Here, we present MICK Clinical, an in vitro diagnostic (IVD) software solution qualified as a medical device under IVDR (EU) 2017/746, enabling automated qualitative detection of genetic markers associated with specific gastrointestinal microorganisms.

Methods: MICK Clinical is a novel IVD software for bioinformatic analysis of Next-Generation Sequencing (NGS) data from adult fecal samples. The software processes Illumina paired-end FASTQ files through four automated modules: read trimming and quality control, taxonomic profiling, and finally, gastrointestinal pathogen identification via genomic bin coverage and virulence gene assessment. The IVD certification involved comprehensive validation: analytical validation on 464 simulated samples established the Limit of Detection (LOD) and performance; clinical validation on 76 hospitalized patient samples evaluated diagnostic efficacy against reference methods (qPCR, cell cultures).

Results: MICK Clinical generated structured reports including quality metrics and the qualitative presence of predefined pathogens. The software's QC module verifies sample reliability and biological integrity by assessing fragmentation, read percentages, and core gut microbiota against thresholds. Analytical validation demonstrated a total sensitivity of 98.5% and a specificity of 100% across all tested pathogens. In clinical validation, the software achieved an overall sensitivity of 91.7% and specificity of 99.57%, with a Positive Likelihood Ratio of 216.33.

Conclusions: MICK Clinical is a robust, automated, IVD-certified platform for the qualitative screening of gastrointestinal pathogens from whole-genome sequencing data, enhancing clinical workflows with standardized reports and integrated quality control.

Back

MICK Clinical

Automated qualitative identification of gastrointestinal pathogens via IVD-certified NGS-based metagenomic analysis

Powered by SEQUENTIA
DECODE · DISCOVER · DISRUPT

Roberto Malinverni

INTRODUCTION

Diagnostic Challenges

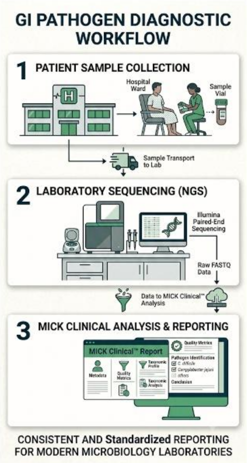
Early identification of traditional gastrointestinal (GI) pathogens is essential for effective management of symptoms such as diarrhoea and abdominal pain.

Change of Paradigma

As clinical microbiology shifts from traditional culture to Metagenomics, the primary bottleneck has moved from "**data generation**" to "**data interpretation**".

Our solution

Here, we present **MICK Clinical™** , an in vitro diagnostic (IVD) software qualified as medical device under **European Union Regulation (EU) 2017/746 (IVDR).** By analyzing **shotgun metagenomics** data, it enables the automated qualitative detection of genetic markers associated with specific gastrointestinal microorganisms."



PATHOGEN PANEL

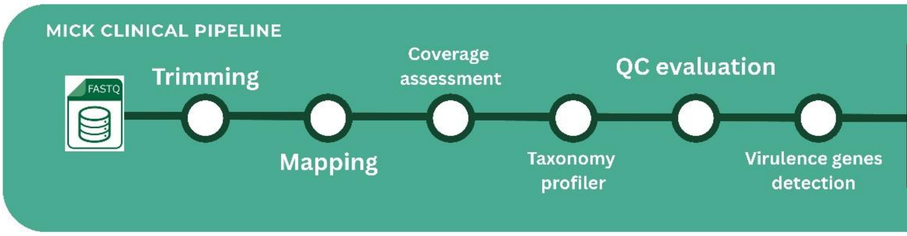
Campylobacter jejuni: The leading bacterial cause of human gastroenteritis worldwide; driven heavily by poultry reservoirs.[1]

Clostridioides difficile: The primary cause of hospital-acquired (nosocomial) diarrhea; poses a massive healthcare and economic burden (€300M+/year in the EU).[2]

Aeromonas spp.: A rapidly emerging enteropathogen; recent molecular data shows it is the 2nd most common bacterial enteric pathogen in some hospital cohorts (up to 20.6% of positive isolates).[3]

Yersinia enterocolitica: The 4th most common zoonotic GI infection in the EU; psychrotrophic nature and specialized culture needs make it a major diagnostic blind spot.[4]

MICK CLINICAL PIPELINE



Trimming

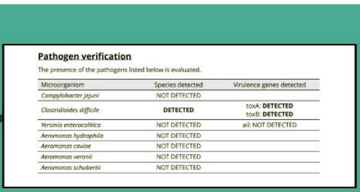
Mapping

Coverage assessment

Taxonomy profiler

QC evaluation

Virulence genes detection



Pathogen verification

The presence of the pathogens listed below is evaluated.

Microorganism	Species detected	Virulence genes detected
Campylobacter jejuni	NOT DETECTED	
Clostridioides difficile	**DETECTED**	toxA: **DETECTED** toxB: **DETECTED**
Yersinia enterocolitica	NOT DETECTED	ail: NOT DETECTED
Aeromonas hydrophila	NOT DETECTED	
Aeromonas caviae	NOT DETECTED	
Aeromonas veronii	NOT DETECTED	
Aeromonas schubertii	NOT DETECTED	

standardize pdf report

VALIDATION METHODS (ANALYTICAL & CLINICAL)

Comprehensive validation studies were conducted to establish the software's limits of detection and confirm its clinical diagnostic efficacy.

1. **Analytical Validation: 464** simulated samples across various sequencing depths to establish Limit of Detection (**LODs**).
2. **Clinical Validation: 76** samples from hospitalized adult patients from Vall d'Hebron Hospital (18–64 years).
3. **Reference Methods:** Diagnostic efficacy evaluated against qPCR and standard cell cultures.

Sensitivity (Overall)
91.7%

Specificity (Overall)
99.5%

PPV
96.5%

NPV
98.9%

Vall d'Hebron
Hospital

CE 0123

International Organization for Standardization
ISO
13485
Quality Management System

CONCLUSIONS

MICK Clinical™ is a robust, **IVDR-certified (UE 2017/746)** platform that successfully automates the bioinformatic analysis of adult fecal samples. By integrating comprehensive quality control metrics and generating standardized PDF reports, it enables clinical laboratories to safely and efficiently adopt direct metagenomics for routine gastrointestinal screening.

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MICK Clinical
by Sequentia

mailto:info@sequentiabiotech.com

26. Identification of Microbial Markers Associated with Treatment Response in IBD

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There is growing evidence that the intestinal microbiome plays a key role in how patients respond to biologic treatments for IBD. However, given the variety of current therapies and inter-individual differences, predicting who will respond to each drug remains a major challenge.

We retrieved shotgun sequencing data from fecal samples of 114 patients (Lee et al., 2021) to re-analyze them using a k-mer-based strategy, including not only bacteria but also viruses and archaea. Our goal was to understand how the microbiome influences patient outcomes based on the biologic received and why some achieve remission while others do not, using ALDEx2.

In Crohn's disease, a potential link was observed between the increased abundance of Streptococcus dysgalactiae and Streptococcus oriscaviae and non-response to anti-TNF therapy. In contrast, for vedolizumab, several species of the genus Clostridium appear to be associated with this lack of improvement.

Regarding ulcerative colitis, higher levels of Methylocystis rosea and Veillonella parvula correlate with a poorer response to anti-TNF, while the virus Kehishuvirus primarius seems to be linked to a favorable outcome. Furthermore, clinical improvement with vedolizumab seems to be accompanied by the presence of Gracilibacillus caseinilyticus.

In conclusion, these findings suggest that the microbiome could serve as a key guide to understanding which therapy best fits each patient, bringing us closer to the implementation of truly personalized medicine.

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ABSTRACTS BY TOPIC

Therapeutics

27. Enhancing Anti-pd1 Immunotherapy with the Microbiome-based Drug MVT-201 in Melanoma

David Ríos-Covian[1], Pilar Manrique Ronquillo[1], Ignacio Montero Ordóñez[1], Ugo Verzi Borgese[1], Carlos Moncada Martins[1], Carmela Mitre Rodríguez[1], Alejandro Ojeda Suárez[1], Noelia Martínez Álvarez[1], Claudio Hidalgo Cantabrana[1]

[1] Microviable Therapeutics, Gijón, Spain

Background: Melanoma incidence continues to rise globally, while the rate of success of immunotherapy remains nearly 50%. As consequence, there is a current need for new strategies to enhance the efficacy of immune checkpoint inhibitors (ICI). The immune system plays a crucial role both in the efficacy of immunotherapeutic agents and in mediating host-microbiome interactions. Fecal microbiota transplantation (FMT) is currently being evaluated as an adjuvant for immunotherapy showing promising results in clinical trials. This work aimed to study the adjuvant effect of MVT-201, an orally delivered, ultra purified microbiome drug that consists on the bacterial fraction. The effect of MVT-201 was compared with traditional FMT in a murine model of melanoma treated with anti-PD1 immunotherapy.

Methods: To evaluate the adjuvant effect of MVT-201, immunocompetent mice were injected with a melanoma cell line resistant to anti-PD1 (B16F10) and treated with anti-PD1 alone and in combination with classical FMT or MVT-201. Gut microbiome composition was sequenced by shotgun and analyzed for composition, functionality and correlation with efficacy. Several immune-related proteins, including ICI-associated markers and inflammatory cytokines, were quantified in several tissues using multiplex ELISA. Selected findings were validated by RT-qPCR.

Results:. MVT-201 enhanced anti-PD1 activity, achieving tumor control in almost all treated animals, whereas anti-PD1 alone and anti-PD1 in combination with FMT had significantly lower success rates (40-50%). Anti-PD1 response correlated positively with the abundance of bacterial taxa such as Alistipes and Eubacteriaciae. Moreover, some pro-inflammatory cytokines including MCP-1 and IL-1β were associated with the modulation of tumor progression alongside proteins implicated in the mechanisms of action of anti-PD1 such as PD-1 and PD-L1.

Conclusions: MVT-201 demonstrated better efficacy as adjuvant for anti-PD1 than FMT.

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Enhancing anti-PD1 immunotherapy with the microbiome-based drug MVT-201 in melanoma

David Rios-Covian; Ignacio Montero; Pilar Manrique ; Ugo Verzi Borgese; Carlos Moncada Martins; Carmela Mitre Rodríguez, Alejandro Ojeda Suárez; Noelia Martínez-Álvarez; Claudio Hidalgo-Cantabrana.
Microviable Therapeutics SL. Gijón, Asturias, Spain. http://www.microviable.com. david.rios@microviable.com

Background

- Melanoma incidence continues to rise globally.
- Immunotherapy, particularly immune checkpoint inhibitors (ICI), success remains 40-60%
- Fecal microbiota transplantation (FMT) has shown promising results as an adjuvant increasing the efficacy by 20%
- MVT-201 is presented as a more concentrated, hygienic, safer alternative to FMT to be used adjuvant for anti-PD1 immunotherapy in solid tumors

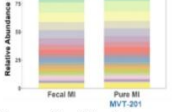

Figure 1. Microbiome analysis analysis of the fecal sample and the highly purified microbiome-based medicinal product MVT-201

MVT-201

- MVT-201: Highly purified microbiome-based medicine that only contains the microbial fraction.
- Proprietary manufacturing process (WO 2025/141228) that eliminates >90% of eucaryotic virus and >97% of toxic metabolites
- Maintains microbial community structure from donor (Figure 1)

Methods

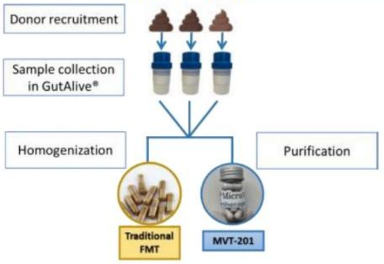

Figure 2. Fecal samples from different donors were processed for both, traditional FMT and MVT-201 at the same time, for a direct comparison.

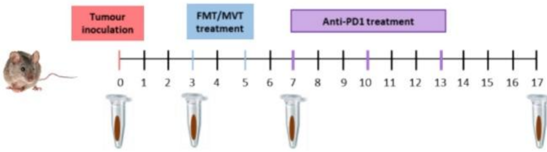

Figure 3. Experimental design timeline. At days 3 and 5, each experimental group received vehicle, FMT or MVT-201 in a high or low dose.

The adjuvant effect of MVT-201 is stronger than traditional FMT

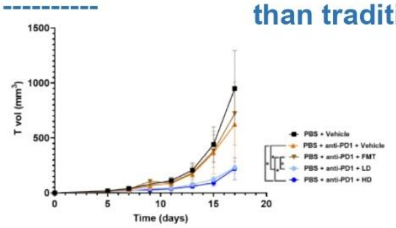

Figure 4. Tumour progression (mm^3) over time in all experimental groups. The groups colored in blue received MVT-201 in low dose (LD) or high dose (HD).

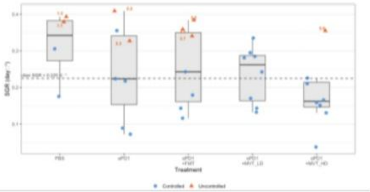

Figure 5. Tumor size specific growth rate (SGR). Tumors were catalogued into controlled or uncontrolled growth depending on whether they reached exponential phase or not. Blue dots: controlled growth. Orange dots: uncontrolled growth.

→ MVT-201 significantly delayed tumor progression compared with anti-PD-1 alone and anti-PD-1 combined with traditional FMT, exhibiting a dose-independent effect. In addition, it avoided exponential growth in almost all tumors.

Stability of the Gut Microbiome Throughout the Experiment

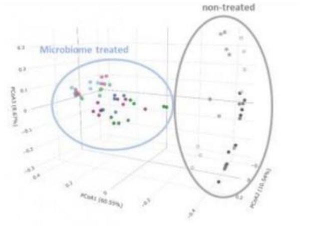

Figure 6. PCoA based on Bray-Curtis dissimilarity. Grey circle: vehicle groups. Blue circle: groups treated with microbiota (FMT or MVT-201)

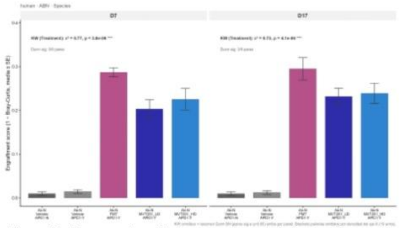

Figure 7. Human microbiota engraftment index in the murine model. Dark grey: Vehicle. Light grey: anti-PD1 + vehicle. Pink: anti-PD1 + MFT. Dark blue: MVT-201 low dose. Light blue: MVT-201 high dose.

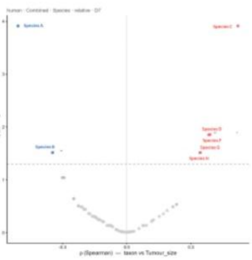

Figure 8. Volcano plot of bacterial species correlated with tumour volume. Blue dots: negative correlation. Red dots: positive correlation

- At composition level, FMT and MVT-201 microbiome engrafted similarly in the murine model. However, **total engraftment in MVT-201 was lower than FMT and dose-independent.**
- **Several bacterial species were negatively correlated with tumour progression and will be isolated to perform further analysis.**

MVT-201 might act through immune system modulation

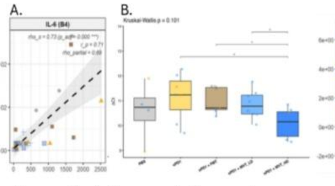

Figure 9. IL-6 concentration and gene expression in spleen. A. Correlation between cytokine concentration (pg/mg tissue, Y axis) and tumor size (X axis). B. Gene expression of IL-6 by treatment group.

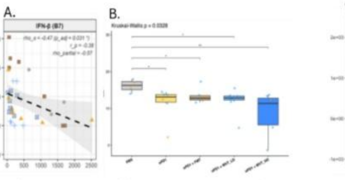

Figure 10. IFN-β concentration and gene expression in spleen. A. Correlation between cytokine concentration (pg/mg tissue, Y axis) and tumor size (X axis). B. Gene expression of IFN-β by treatment group.

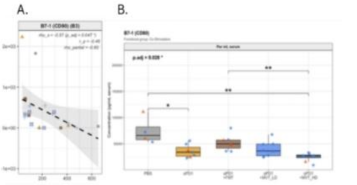

Figure 11. CD80 concentration and gene expression in tumor. A. Correlation between cytokine concentration (pg/mg tissue, Y axis) and tumor size (X axis). B. Gene expression of CD80 by treatment group.

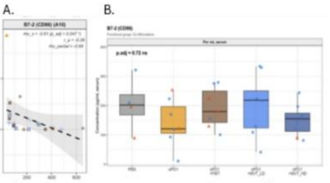

Figure 12. CD86 concentration and gene expression in tumor. A. Correlation between cytokine concentration (pg/mg tissue, Y axis) and tumor size (X axis). B. Gene expression of CD86 by treatment group.

- IL-6 was positively correlated with tumor size in spleen (Figure 8) and serum (data not shown) and its splenic gene expression was significantly lower in mice treated with a high dose of MVT-201. Whereas IFN-β was negatively correlated, but its splenic gene expression was significantly higher in the control group compared to the rest of the groups**. The mechanism of action of MVT-201 could be partially exerted through the immune system modulation**.
- Tumoral CD80 and CD86 concentrations were negatively correlated with tumor size, but only CD80 gene expression was significantly lower in all anti-PD1 treated groups. **MVT-201 does not seem to interact with the ICP regulating pathways**, but more experiments should be conducted.

Acknowledgements

This work has been partially funded by Centro de Desarrollo Tecnológico e Innovación (CDTI) under the PID call (ref. EXP00169553/IDI-20250023).

28. Animal By-Product Protein Hydrolysate Shapes Gut Microbiota and Improves Metabolic Outcomes in Diet-induced Obese Rats

Diego Alavarsa-Cascales[1], Fernando Aniento-Marcote[1], Júlia Adrada-Schnell[1], Francisca I. Bravo[1], Cristina Torres-Fuentes[1], Josep M. del Bas[1], Enrique Calvo[1]

[1] *Universitat Rovira i Virgili, IRB CatSud and TecnATox, Tarragona, Spain*

Background: Obesity is linked to gut microbiota dysbiosis and cardiometabolic dysfunction. Protein hydrolysates can modulate microbial composition and metabolic pathways, acting as bioactive compounds that influence host metabolism. The animal protein hydrolysate (APH) studied here is derived from an agricultural by-product, representing a sustainable source of bioactive peptides. **Methods:** This study assessed the effects of an APH on gut microbiota composition and systolic blood pressure (SBP). Forty-eight Wistar rats were fed either a standard (STD) or cafeteria (CAF) diet for 12 weeks.
During the last 4 weeks, animals received either vehicle or APH (55 mg/kg/day).
Results: Hydrolysate supplementation lowered SBP in both sexes to levels comparable to the STD, accompanied by remodeling of microbiota–SBP relationships. In CAF animals, SBP correlated with specific bacterial genera. Negative associations were observed with Suilimivivens, Fimenecus, and Sporofaciens, suggesting a potential protective role, whereas positive correlations were identified with Coprococcus, and Borkfalkia. Although not significant after multiple testing correction, these associations indicate a microbiota–SBP interaction. Hydrolysate treatment altered this pattern, weakening and in some cases reversing associations observed in CAF animals. In particular, the positive Coprococcus association in CAF animals shifted to a negative association after APH supplementation, while new associations emerged with Leuconostoc, Schaedlerella, and Pseudobutyricicoccus. Multivariate analysis (MaAsLin2) identified compositional shifts associated with APH supplementation, including increased Acetatifactor, Porcipelethomonas, Eisenbergiella, and Brotaphodocola, and decreased abundance of Enterocloster, Longicatena, Eubacterium, Faecousia, and Fimenecus.
Conclusions: APH lowers SBP and remodels gut microbiota, partly uncoupling microbiota–SBP relationships and suggesting a shift in vascular regulation.

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Animal by-product protein hydrolysate shapes gut microbiota and improves metabolic outcomes in diet-induced obese rats

Diego Alavarsa-Cascales[1,2,3]*, Fernando Aniento-Marcote[1,2,3], Julia Adrada-Schnell[1], Francisca Isabel Bravo[1,2,3], Cristina Torres-Fuentes[1,2,3], Josep M. del Bas[1,2,3], and Enrique Calvo[1,2,3]

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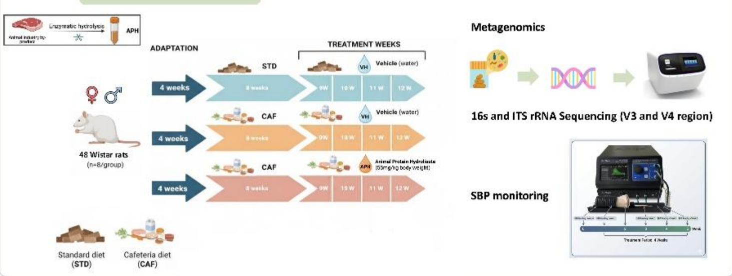
Introduction

Obesity is critically linked to gut microbiota dysbiosis and associated cardiometabolic dysfunction. To address these systemic challenges, protein hydrolysates offer a promising therapeutic avenue by serving as potent bioactive compounds capable of favorably modulating microbial composition and host metabolic pathways. The animal protein hydrolysate (APH) evaluated in this study is upcycled from an agricultural by-product, establishing a highly sustainable and functional source of bioactive peptides.

Objective

The To evaluate the therapeutic efficacy of a sustainably sourced animal protein hydrolysate (APH) on systolic blood pressure (SBP) and gut microbiota composition in a rat model of diet-induced obesity.

Methodology



Results

APH significantly decreased systolic blood pressure (SBP) in CAF-fed rats in both sexes

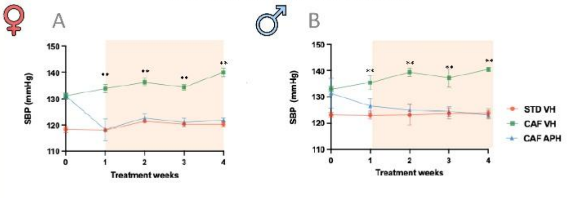


Figure 1. Systolic Blood Pressure (SBO) showing millimeter of mercury (mmHg) levels during the treatment week in females (A) and males (B). Graphs represent the means (n=8 per group) ± SEM. One-way ANOVA followed by Tukey's post hoc test. ** indicates significant differences between CAF VH and CAF APH ($p < 0.01$)

APH reduces alpha diversity in females while driving sex-specific β-diversity restructuring

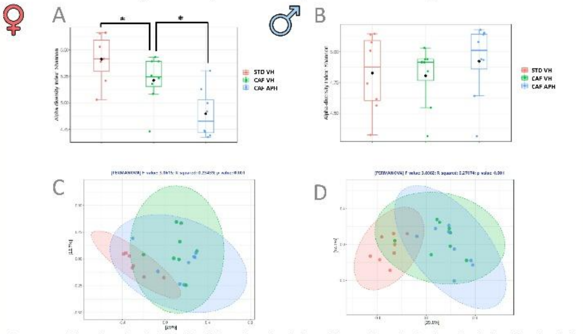


Figure 2. Alpha-diversity in females (A) and males (B), and beta diversity in females (C) and males (D). Graphs represent the means (n=8 per group) ± SEM. Shannon alpha diversity was calculated at the feature level from non filtered data to evaluate microbial diversity across treatment groups. Differences among groups (STD VH, CAF VH, and CAF APH) were assessed using Welch's t-test/ANOVA with Benjamini–Hochberg FDR correction for pairwise comparisons. Beta diversity of microbial communities was assessed using Principal Coordinates Analysis (PCoA) based on the Bray–Curtis dissimilarity index at the feature level. Samples are colored according to the experimental factor (Tx), and ellipses represent group clustering. Community composition differences were tested using PERMANOVA.

APH shows no sex differences in alpha diversity but causes clear sex-dependent changes in beta diversity

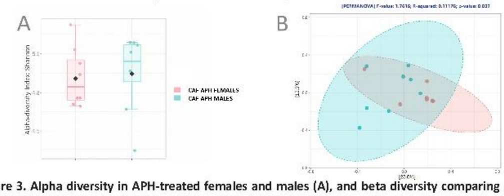


Figure 3. Alpha diversity in APH-treated females and males (A), and beta diversity comparing APH-treated females and males (B). Graphs represent the means (n=8 per group) ± SEM. Shannon alpha diversity was calculated at the feature level from non filtered data to evaluate microbial diversity across treatment groups. Differences between sexes were evaluated using Welch's t-test/ANOVA with Benjamini–Hochberg FDR correction for pairwise comparisons. Beta diversity of microbial communities was assessed using Principal Coordinates Analysis (PCoA) based on the Bray–Curtis dissimilarity index at the feature level. Samples are colored according to the experimental factor (Tx), and ellipses represent group clustering. Community composition differences were tested using PERMANOVA.

APH supplementation alters the relative abundance of key gut bacterial phylum and genera in a sex-dependent manner CAF-fed rats

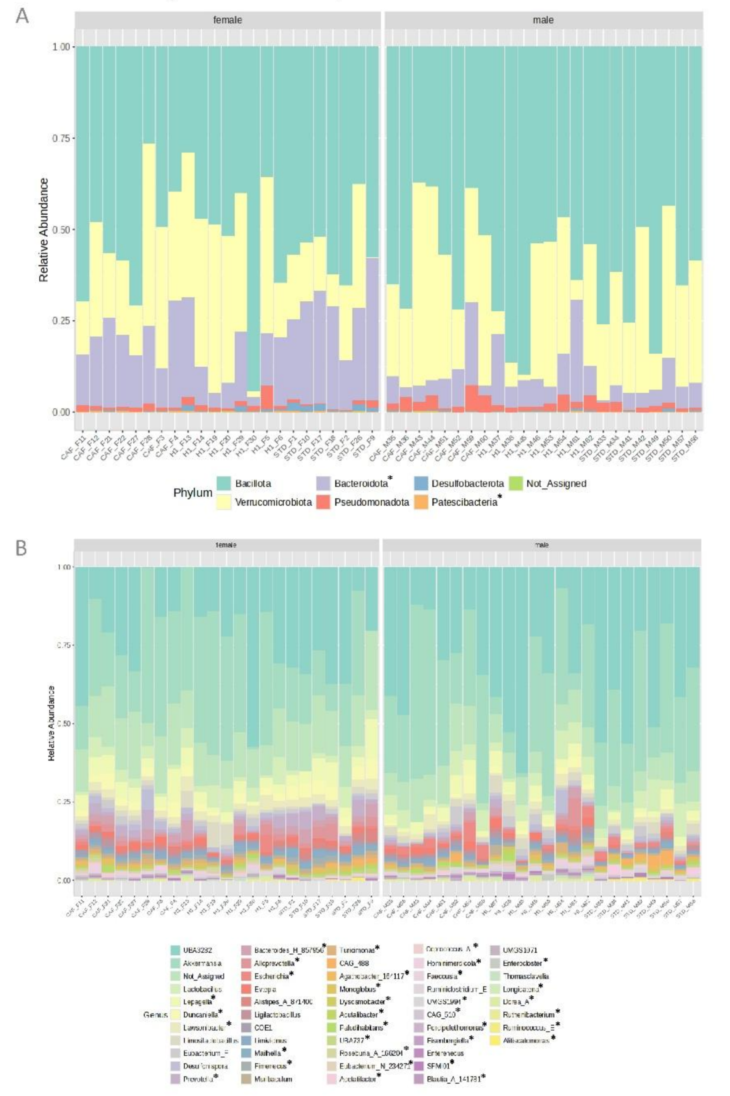


Figure 4. Relative abundance of microbial taxa by sex at the Phylum (A) and Genus (B) levels. Associations between microbial Phyla and Species with sex were analyzed using MaAsLin2 employing a negative binomial regression model (NEGBIN), with sex as the primary variable and treatment included as a covariate. * indicates significant differences between groups ($p < 0.05$, FDR-adjusted).

Conclusion

APH lowers blood pressure and remodels gut microbiota, partly uncoupling microbiota–SBP relationships and suggesting a shift in vascular regulation, supporting its potential as a sustainable modulator of host–microbiome interactions in cardiometabolic disease.

Acknowledgements: This was funded by project PID2023-151563OB-I00 from MCIN/AEI/10.13039/501100011033. D.A.C. was supported by the Universitat Rovira i Virgili through the Martí i Franquès program (2024PMF-PIPF-03). F.A.M. is the recipient of the FPI predoctoral fellowship associated with this project (PRE2023-151563OB-I00).

29. Sex-dependent Effects of a Plant By-product-derived Protein Hydrolysate on Gut Microbiota and Metabolic Health in Obese Rats

Cristina Torres[1], Fernando Aniento[1], Diego Alavarsa[1], Francisca Isabel Bravo[1], Nerea Clavero-Murciego[1]

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Background: Obesity is associated with gut microbiota dysbiosis, contributing to metabolic dysfunction. Increasing evidence identifies biological sex as a key determinant of microbiota composition and metabolic responses, potentially influencing the efficacy of nutritional interventions. Plant-derived protein hydrolysates have emerged as promising modulators of gut microbiota and metabolic health. However, whether their effects differ between sexes in obesity remains unclear.

Methods: Thirty-two 8-week-old Wistar rats (16 males, 16 females) were fed a cafeteria (CAF) diet for 12 weeks. During the final 4 weeks, animals were allocated to two groups (n = 8/sex/group): CAF + vehicle or CAF supplemented with a plant protein hydrolysate (PPH; 55 mg/kg/day), administered daily by pipette. Body weight was monitored weekly. Metabolic responses were evaluated using an oral lipid tolerance test (OLTT) and an oral glucose tolerance test (OGTT) after 2 and 3 weeks of treatment, respectively. In addition, leptin, adiposity and gut microbiota composition were analyzed at the end of the intervention.

Results: PPH reduced adiposity and plasma leptin levels and showed a trend towards lower postprandial triglycerides in females, whereas it improved glucose tolerance in males. These sex-specific effects were accompanied by differences in gut microbiota composition, including a higher relative abundance of the genus *Escherichia* in PPH-treated males compared to females. Notably, *Escherichia* abundance positively correlated with plasma leptin levels and OLTT (AUC).

Conclusions: PPH induced sex-dependent effects in different metabolic parameters as well as in gut microbiota. Changes in *Escherichia* and its association with metabolic parameters suggest a potential involvement of this genus in host metabolic regulation, although causal relationships require further investigation. These findings highlight the importance of considering biological sex in microbiota-targeted nutritional strategies.

Back

Sex-dependent effects of a plant by-product-derived protein hydrolysate on gut microbiota and metabolic health in obese rats

Fernando Aniento-Marcote, Diego Alavarsa-Cascales, Nerea Clavero-Murciego, Francisca I. Bravo, Cristina Torres-Fuentes*
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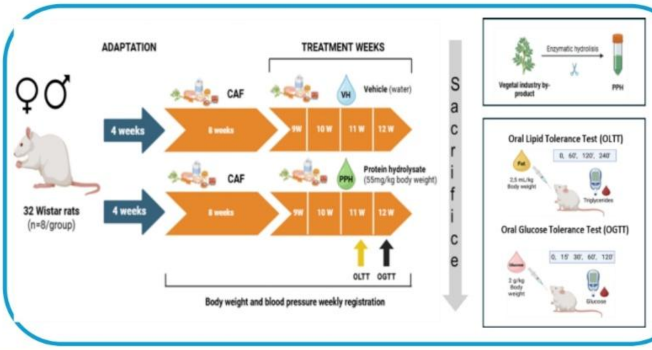
INTRODUCTION

- Obesity is associated with gut microbiota dysbiosis and metabolic dysfunction.
- Biological sex may influence responses to microbiota-targeted nutritional interventions.
- Plant-derived protein hydrolysates have emerged as promising modulators of metabolic health.

AIM

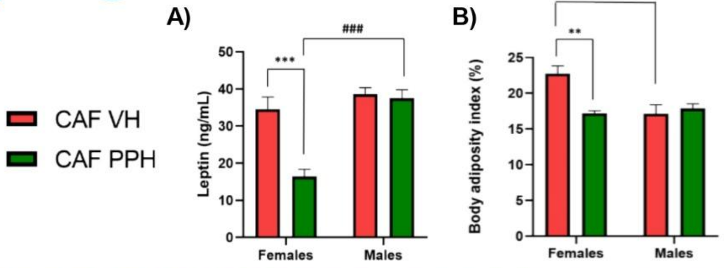
To determine whether the metabolic and gut microbiota effects of a plant-derived protein hydrolysate are influenced by biological sex in a diet-induced obesity model.

METHODOLOGY



RESULTS

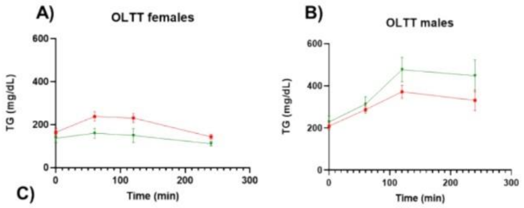
1 PPH improved adiposity-related parameters in females



PPH reduced adiposity and circulating leptin levels in female CAF-fed rats.

Figure 1. Plasma leptin levels **(A)** and body adiposity index (%) **(B)**. Graphs represent the mean ± SEM. Statistical analysis performed by two-way ANOVA followed by Tukey's post hoc test. *: treatment effect; #: sex effect (*p<0.05, **p < 0.01, ***p<0.001) .

2 Sex-dependent effects of PPH on metabolic responses



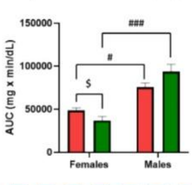
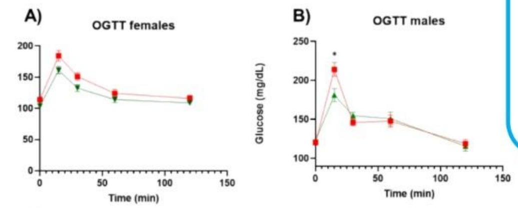
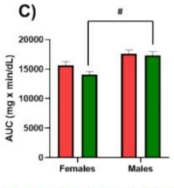


Figure 2. Oral lipid tolerance test (OLTT) in (**A**) females and (**B**) males. (**C**) Area Under the Curve (AUC) for females and males. Graphs represent the mean ± SEM. Statistical analysis was assessed by two-way ANOVA for repeated measures and for by two-way ANOVA followed by Tukey's post hoc test for AUC. #: sex effect. # $p < 0.05$; ### $p<0.001$; $ indicates a nonsignificant trend ($0.05 < p < 0.1$).

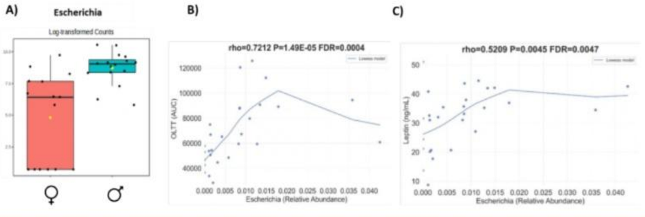




PPH improved glucose tolerance in males and showed a trend towards lower postprandial triglycerides in females.

Figure 3. Oral glucose tolerance test (OGTT) in (**A**) females and (**B**) males. (**C**) Area Under the Curve (AUC) for females and males. Graphs represent the mean ± SEM. Statistical analysis was assessed by two-way ANOVA from repeated measures and by was two-way ANOVA followed by Tukey's post hoc test for AUC. *: treatment effect. #: sex effect. # $p < 0.05$.

3 Gut microbiota may contribute to the metabolic effects of PPH



Sex-specific changes in *Escherichia* abundance were linked to adiposity and lipid metabolism

Figure 4. Differential abundance and correlation analysis of *Escherichia* in PPH-treated CAF-fed female and male rats. **(A)** Differential abundance based on CLR-transformed data assessed using Wilcoxon test with FDR correction. Correlation between *Escherichia* abundance and OLTT **(B)** (AUC) and between *Escherichia* abundance and plasma leptin **(C)**. Correlations were assessed using Spearman correlation analysis.

CONCLUSION

PPH induced sex-dependent effects in different metabolic parameters as well as in gut microbiota. Changes in *Escherichia* and its association with metabolic parameters suggest a potential involvement of this genus in host metabolic regulation, although causal relationships require further investigation. These findings highlight the importance of considering biological sex in microbiota-targeted nutritional strategies.

Funding: PID2023-151563OB-I00 (MCIN/AEI/10.13039/501100011033) and predoctoral FPI fellowship PRE2023-151563OB-I00.

30. Targeting a Microbiome Pathobiont: Antigen Prioritization and Preclinical Evaluation of Protective Efficacy and Immunogenicity Against Circulating Klebsiella Pneumoniae Strains

Marta Vicente-Pazos[1], Sandra Fernández-Piñán[1], Michael J. McConnell[2], Mireia López-Siles[1], Antoni Benito[1], Jessica Castro[1], Gal·la Ustrell Sallent[1], Pau Riera Garcia[1], Laura Quílez Agraz[1], Joana Velasco Castillo[1], Clàudia Navarro Sánchez[1], Laura Martín Ortega[1]

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Background: Klebsiella pneumoniae, a frequent colonizer of the human gut, is a major cause of healthcare-associated infections and a driver of antimicrobial resistance worldwide. Carriage of this opportunistic pathobiont represents an important risk factor for invasive infections, highlighting the need to develop preventive strategies as vaccination.

This study systematically evaluated the potential of K. pneumoniae virulence factors as antigens using a DNA vaccine platform, and assessed their protective efficacy and immunogenicity against clinical strains.

Methods: To identify antigens, an in silico comparative approach was used to analyse immunogenic potential of 34 K. pneumoniae virulence factors. Codon-optimized coding sequences were cloned independently into pVAX1. Expression was confirmed in HEK-293 cells by RT-qPCR. After a three-dose intramuscular immunization of C57BL/6 mice, the immune response was characterized by antigen-specific antibody quantification and cytokine profiling. A panel of clinically circulating K. pneumoniae strains was used to test the breadth of antibody recognition. Challenge with K. pneumoniae ATCC 43816 was used to evaluate efficacy of protection against sepsis.

Results: Eight antigens -four porins, three siderophore receptors, and one fimbrial subunit- were shortlisted, successfully cloned and expressed in HEK-293 human cells. Sera from immunized mice showed cross-reactivity against multiple circulating clinical isolates, regardless of the vaccine candidate. Fimbrial subunit and porin vaccine candidates induced the highest levels of total IgG and distinct cytokine signatures. A porin-encoding vaccine was the best candidate to protect against K. pneumoniae ($p<0.0378$).

Conclusion: These findings establish the basis for antigen prioritization in the development of vaccines against K. pneumoniae and support the feasibility of targeting microbiome-associated pathobionts to prevent infections caused by antimicrobial-resistant bacteria.

Back

Targeting a microbiome pathobiont: antigen prioritization and preclinical evaluation of protective efficacy and immunogenicity against circulating *Klebsiella pneumoniae* strains

M. Vicente-Pazos [1,2], A. Benito [3], J. Castro [3], G. Ustrell-Sallent [1], P. Riera-Garcia [1], L. Quílez Agraz [1], J. Velasco Castillo [1],
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Background

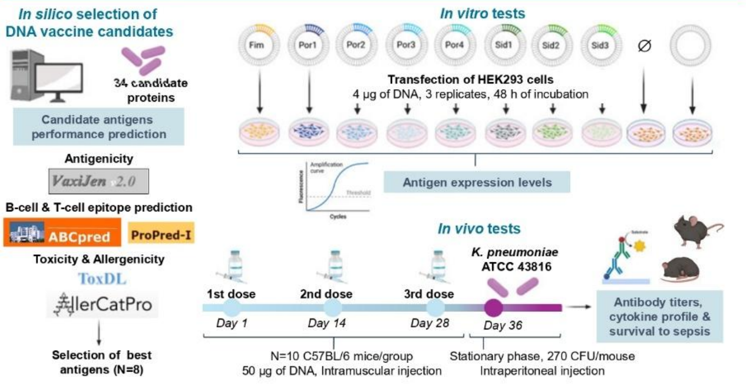
Klebsiella pneumoniae, a frequent colonizer of the human gut, is a pathogen that represents an important clinical problem in the community and hospital settings due to the increase of infections caused by hypervirulent and multidrug-resistant strains, respectively [1]. Carriage of this opportunistic pathobiont represents an important risk factor for invasive infections.

The development of vaccines against this species may help to prevent infections and reduce the associated mortality and morbidity [2]. A promising approach could be the use of DNA vaccines given their stability, their ability to be easily modified to target multiple antigens or pathogens, and their capacity to induce both humoral and cellular immune responses.

Aim

To systematically evaluate the potential of *K. pneumoniae* virulence factors as antigens using a DNA vaccine platform, and assess their immunogenicity against clinical strains and protective efficacy against sepsis.

Methodology



***In silico* selection of DNA vaccine candidates**

34 candidate proteins

Candidate antigens performance prediction

Antigenicity

VaxiJen v2.0

B-cell & T-cell epitope prediction

ABCpred ProPred-I

Toxicity & Allergenicity

ToxDL

AllerCatPro

Selection of best antigens (N=8)

***In vitro* tests**

Transfection of HEK293 cells
4 µg of DNA, 3 replicates, 48 h of incubation

Antigen expression levels

***In vivo* tests**

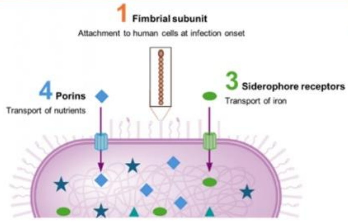
1st dose – Day 1
2nd dose – Day 14
3rd dose – Day 28
K. pneumoniae ATCC 43816 – Day 36

N=10 C57BL/6 mice/group
50 µg of DNA, Intramuscular injection

Stationary phase, 270 CFU/mouse
Intraperitoneal injection

Antibody titers, cytokine profile & survival to sepsis

Results

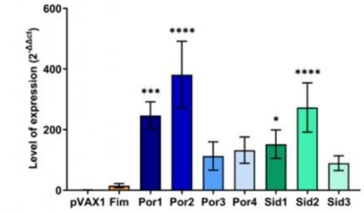


Candidate antigens performance prediction

Characteristic	Analysis tool	Fim	Por 1	Por 2	Por 3	Por 4	Sid1	Sid2	Sid 3
Antigenicity	VaxIJen v2.0 (score)	0.76	0.77	0.68	0.73	0.75	0.78	0.60	0.70
B-cell epitopes	ABCpred (nº epitopes)	34	48	22	23	40	79	81	73
T-cell epitopes	ProPred-I (sum of best epitope probability for all HLA alleles)	1772.02	1767.20	1750.27	1838.38	1591.27	1919.90	2032.05	1932.24
Toxicity	ToxDL (toxicity score)	0.0012	6.609×10^{-5}	7.069×10^{-6}	1.072×10^{-5}	3.456×10^{-5}	3.528×10^{-5}	0.0001	0.0001
Allergenicity	AllerCatPro v2.0	No	No	Weak evidence	No	No	No	No	No

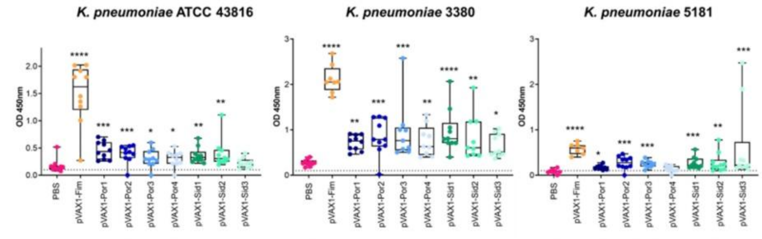
- ▸ All proteins are predicted to be immunogenic, able to induce humoral immune response and have a high number of predicted MHC Class-I binding sequences. No toxicity or high evidence of allergenicity are predicted.

Antigen expression levels



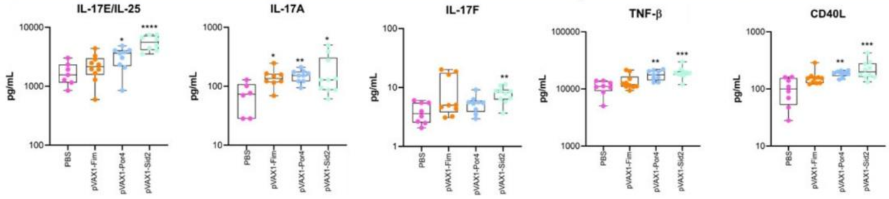
- ▸ Expression of all antigenic sequences is detected.
- ▸ The quantity of transcripts detected is between 15- to 380-times higher compared to control (pVAX1).

IgG levels at day 35



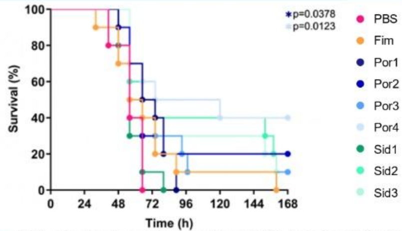
- ▸ Significant IgG levels are generated by all vaccine constructs and recognise the three strains tested.
- ▸ The highest antigen-specific antibodies are detected for pVAX1-Fim, and *K. pneumoniae* 3380 strain.

Cytokine profile at day 35



- ▸ IL-25/IL-17 suggest Th2 cells activation whereas detection of IL-17A and IL-17F indicate activated Th17 cells.
- ▸ TNFβ may boost adaptive immunity and CD40L could drive IgA isotype switching.
- ▸ The cytokine profile can represent an innate intestinal immune response.

Survival curves



- ▸ 40% of mice immunized with pVAX1-Por4 survive to sepsis by an hypervirulent and hyperencapsulated *K. pneumoniae* strain.

Conclusions

- **A collection of 8 DNA vaccine candidates encoding different *K. pneumoniae* virulence factors has been constructed and transcription of the genes encoding the antigens has been confirmed in HEK293 cells.**
- **Despite pVAX1-Fim elicits the best humoral response, a better survival has been observed with pVAX1-Por4. This suggests involvement of cellular response which merits further investigation.**
- **Cytokine profile can represent the induction of an innate intestinal immune response.**
- **These findings establish the basis for antigen prioritization in the development of vaccines against *Klebsiella pneumoniae* and support the feasibility of targeting microbiome-associated pathobionts to prevent infections caused by antimicrobial-resistant bacteria.**

Abbreviations

ELISA: enzyme-linked immunosorbent assay
RT-qPCR: Quantitative reverse transcription polymerase chain reaction
CFU: colony-forming unit

References

[1] PMID: 36183727
[2] PMID: 33289833

Symbols

∅: not transfected, no antigen
*: p-value<0.05
**: p-value<0.01
***: p-value<0.001
****: p-value<0.0001

Funding

MPY 227/23, MPY 516/19, MPY-380/18, CP22CIII-00007, F19III/00006

2023 PROD 00034

LCF/ TR/CI23/56000019

31. Engineering the Skin Commensal Cutibacterium Acnes for the Treatment of Skin Diseases

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Cutibacterium acnes is a natural skin commensal with well-established interactions with the human host. It inhabits human hair follicles, making it the most abundant bacteria in the sebaceous sites of the skin (Fourniere et al. 2020). Recent work discovered that C. acnes applied onto the skin are able to engraft and modulate the hosts skin microbiome composition (Paetzold et al. 2019). Additionally, the population of C. acnes in each hair follicle is mainly clonal (Conwill et al 2022). C. acnes colonization changes throughout our life, and its presence is associated to skin homeostasis by producing acidic molecules impede the growth of pathogens.

By leveraging synthetic biology and microbiome engineering, we transformed C. acnes into a living biotherapeutic platform capable of producing and delivering targeted molecules directly to the skin.

We created strains with inducible expression systems and plan to expand this toolbox to only work within the natural skin environment where C. acnes proliferates: the microaerophilic, acidic and lipophilic environment of the pilosebaceous units. We also plan to create sophisticated biocontainment systems that impede C. acnes growth outside of hair follicles, controlling its spread. Thus, making C. acnes a controlled live biotherapeutic for the treatment of different skin conditions such as acne, anti-aging or wound healing.

Back

Host - Microbiome

33. Rectal Microbiome Associations with HIV Vaccine-Induced Binding Antibody Responses in RV306

Nel Marín-Sánchez[1], Sorachai Nitayaphan[2], Punnee Pitisuttithum[3], Alessandra Borgognone[1], Adam Burgener[4], Roger Paredes[1], Alexandra Schuetz[5], Samantha Horne[4], Francesc Catalá-Moll[1], Mariona Parera[1], Maria Casadellà[1], Leighanne R. Main[4], Siriwat Akapirat[5], Carolina Herrera[6]

[1] IrsiCaixa, Badalona, Spain; [2] Royal Thai Army Clinical Research Center, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [3] Vaccine Trial Center, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [4] Center for Global Health and Diseases, Case Western Reserve University School of Medicine, Cleveland, United States; [5] WRAIR - Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [6] CONRAD, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, United States

The rectal mucosa, a major portal for HIV-1 entry, harbors a microbial community that shapes local immune homeostasis and might influence systemic immune responses to vaccines. We investigated the interactions between the rectal microbiome and vaccine-induced binding antibody (bAb) responses in the RV144 ALVAC-HIV/AIDSVAX® B/E prime/boost vaccine regimen of the RV306 trial.

Rectal mucosal samples were collected from male participants at baseline (n=44), two weeks post 1st boost (n=45) and two weeks post 2nd boost (n=41). Participants were classified as high or low responders based on tertiles of all available bAb responses after the 2nd boost (peak immunogenicity). Rectal microbiota was profiled with shotgun metagenomics (MetaPhlAn3) and differential abundance analyses were conducted using MaAsLin2 and Linear Mixed Models. Correlations with complete blood count parameters were also assessed.

A consistent pattern of discriminant core bacteria distinguished high and low responders at peak immunogenicity across four classes of plasma IgG, previously associated to lower acquisition risk. Bacteroides and Blautia spp. were enriched in high responders, while Eubacterium and Dorea spp. were depleted. Ruminococcus gnavus was consistently more abundant in high responders after 1st and 2nd boosts, while R. torques was reduced in high responders after vaccinations. The majority of enriched bacteria in high responders positively correlated with neutrophil percentage at baseline and peak immunogenicity, while depleted bacteria were negatively associated. In contrast, the opposite trend was observed for lymphocyte percentage.

Observed differences in bacterial taxa between plasma IgG high and low responders, and their contrasting associations with neutrophil and lymphocyte frequencies, suggest a dynamic interaction between the rectal mucosal microbiota and systemic host humoral responses to a preventive HIV vaccine, likely through distinct inflammatory signaling pathways.

Back

Rectal Microbiome Associations with HIV Vaccine-Induced Binding Antibody Responses in RV306

Nel Marín-Sánchez[1,2], Alexandra Schuetz[3,4,5], Samantha Horne[6,7], Francesc Català-Moll[1], Mariona Parera[1], Maria Casadellà[1], Leighanne R. Main[6], Siriwat Akapirat[3], Carolina Herrera[8], Sorachai Nitayaphan[9], Punnee Pitisuttithum[10], Alessandra Borgognone[1], Adam Burgener[6,7,11], Roger Paredes[1,6,12] and the RV306 study group

[1]IrsiCaixa. Badalona, Barcelona, Spain; [2]Universitat de Barcelona, Barcelona, Spain; [3]WRAIR - Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [4]US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA; [5]Henry M. Jackson Foundation for the Advancement of Military Medicine Inc., Bethesda, Maryland, USA; [6]Center for Global Health and Diseases, Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA; [7]Department of Obstetrics, Gynecology & Reproductive Sciences, University of Manitoba, Winnipeg, Manitoba, Canada; [8]CONRAD, Macon & Joan Brock Virginia Health Sciences at Old Dominion University, Norfolk, Virginia, USA; [9]Royal Thai Army Clinical Research Center, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [10]Vaccine Trial Center, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; [11]Department of Medicine Solna, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden. [12]Department of Infectious Diseases, Hospital Germans Trias i Pujol, Badalona, Spain

BACKGROUND

The RV306 trial[1] tested late boosts of the RV144 vaccine regimen, the first and only large HIV vaccine clinical trial to show a modest protective effect to HIV-1 acquisition (~31%)[2]. Immune-correlates analyses of RV144 identified plasma IgG antibodies binding to the V1V2 region of HIV-1 envelope as inversely correlated to infection risk[3].

We investigated **interactions between the rectal microbiome and vaccine-induced binding antibody (bAb) responses** in the RV144 vaccine series of the RV306 study design.

METHODS

Rectal swabs were collected from male participants in the RV306 active arm and analyzed using shotgun metagenomic sequencing (Fig. 1).

Participants were classified as high and low responders based on bAb levels at Wk26 (Fig. 2). MaAsLin2 was used for differential abundance (DA) analyses.

***Figure 1.** Study design and rectal sample collection*

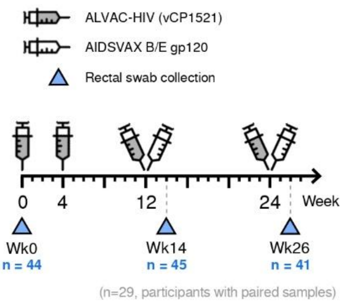
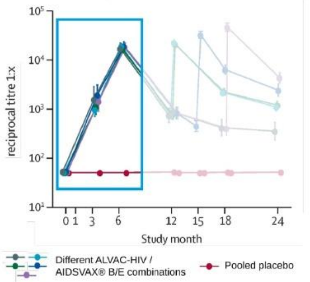


***Figure 2.** Plasma HIV-1 Env-specific IgG bAb levels peak at Wk26 (study month 6)*

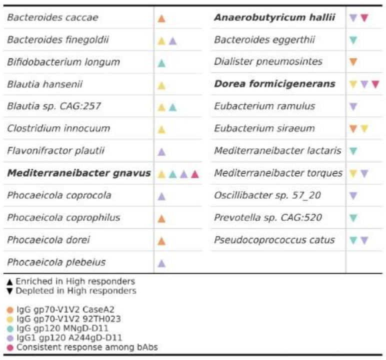


Note: Figure adapted from Pitisuttihum et al., 2020. Only IgG gp120 A244gD- D11 responses are shown. All IgG bAb responses exhibited similar immunogenicity profiles.

RESULTS

- Distinct core bacterial species discriminated high and low responders for each plasma IgG bAb at Wk26 (Fig. 3).
- No significant differences were detected between high and low responders at Wk0.
- *Mediterraneibacter gnavus* (ex *Ruminococcus gnavus*) was enriched in consistently high responders across all four bAbs. *Dorea formicigenerans* and *Anaerobutyricum hallii* (ex *Eubacterium hallii*) were depleted (Fig. 4).

***Figure 3.** Differentially abundant bacterial species at Wk26*

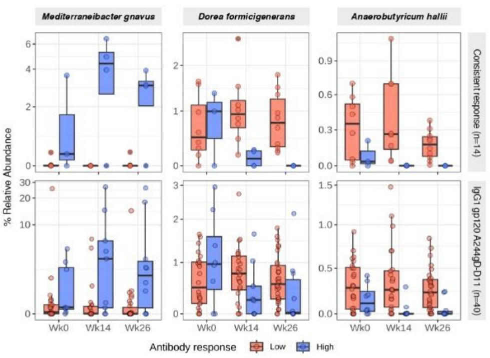


Bacteroides caccae	▲	***Anaerobutyricum hallii***	▼▼
Bacteroides finegoldii	▲▲	*Bacteroides eggerthii*	▼
Bifidobacterium longum	▲	*Dialister pneumosintes*	▼
Blautia hansenii	▲	***Dorea formicigenerans***	▼▼▼
Blautia sp. CAG:257	▲▲	*Eubacterium ramulus*	▼
Clostridium innocuum	▲	*Eubacterium siraeum*	▼▼
Flavonifractor plautii	▲	*Mediterraneibacter lactaris*	▼
Mediterraneibacter gnavus	▲▲▲▲	*Mediterraneibacter torques*	▼▼
Phocaeicola coprocola	▲	*Oscillibacter sp. 57_20*	▼
Phocaeicola coprophilus	▲	*Prevotella sp. CAG:520*	▼
Phocaeicola dorei	▲	*Pseudocoprococcus catus*	▼▼
Phocaeicola plebeius	▲		

▲ Enriched in High responders
▼ Depleted in High responders

● IgG gp70-V1V2 CaseA2
● IgG gp70-V1V2 92TH023
● IgG gp120 MNgD-D11
● IgG1 gp120 A244gD-D11
● Consistent response among bAbs

***Figure 4.** Abundance boxplots of species in the subset with consistent responses among bAbs*



- Bacterial species enriched in high responders tended to positively associate with neutrophil levels and negatively with lymphocyte levels, although with marginal statistical significance (p-value>0.1). In contrast, depleted bacteria showed the opposite correlation trend (Fig. 5).
- At community-level, the gut microbiota of participants classified as high responders for the four bAb classes was positively associated with pathways involved in short-chain fatty acids (SCFAs) and bile acids production. Low responders showed a more general regulatory functions (Fig. 6).

***Figure 5.** Correlations of discriminant bacteria with neutrophil and lymphocyte percentage at Wk0 and Wk26*

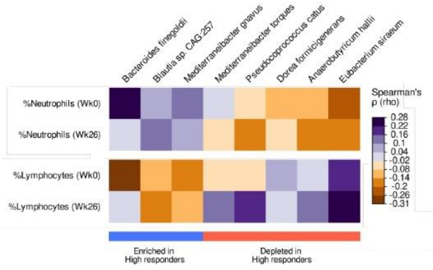
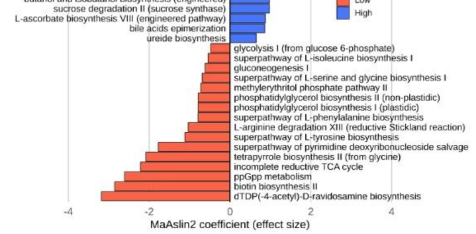


***Figure 6.** Discriminant microbial metabolic pathways at Wk26*



DISCUSSION AND CONCLUSION

M. gnavus has shown the potential to modify bile acids in the gut[5] and to produce pro-inflammatory polysaccharides[6] linked to inflammatory bowel diseases. However, marked strain-level diversity may explain inconsistent results across vaccine studies[7,8].

D. formicigenerans* and *A. hallii, similarly to *M. gnavus*, are members of the *Lachnospiraceae* family known for being SCFA-producing bacteria and potential modulators of vaccine-induced immune responses[9].

Results suggest that a **transient shift from an SCFA-dominated gut environment toward a more immune-stimulatory microbiome** may support higher HIV-specific bAb levels induced by the RV144 prime/ boost vaccine regimen.

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ADDITIONAL KEY INFORMATION

Presenting Author: nmarin@irsicaixa.es

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Disclaimer: Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of War. The investigators have adhered to the policies for protection of human participants as prescribed in AR 70–25.

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AFRIMS

34. Horizontal Gene Flow Among Bacteria in the Gut Microbiome in Non-westernised Human Populations and Their Domesticated Animals

Mark Tefero Kivumbi[1], Giacomo Orsini[2], Amelie Baud[3], Mireia Vallès-Colomer[1]

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Host microbiota associations play an important role in human health and disease. However, the evolutionary dynamics shaping host microbiome relationships remain poorly understood. Horizontal gene transfer (HGT) has been identified as a major driver of diversification and adaptation within microbial communities. Here, we explore bacterial HGT in the gut microbiome of two non-Westernised human populations and their domesticated animals.

550 stool samples were analysed from humans, cows, goats, sheep, and dogs from 100 households in Agogo, Ghana, and Korogwe, Tanzania. Shotgun metagenomics was performed (median 45.7M reads/sample). HGT events were inferred using WAAFLE v1.0 (PMID: 39747694). AMRFinderPlus v3.12.8 (PMID:34135355) was used to identify antibiotic resistance genes and GeNomad v1.8.0 (PMID:37735266) to identify mobile genetic elements.

In total, 362,293 HGT events were identified and normalized by bacteria species richness per sample. Animals showed significantly higher numbers of HGT events than humans, with effect sizes of 0.86 in cows, 1.02 in goats, 0.87 in sheep, and 0.20 in dogs ($q<2.2e-16$). Males had significantly fewer HGT events than females ($r=-0.09$, $p=3.7e-03$), and HGT events increased with age ($r=5e-3$, $p=1.9e-09$). Additionally, fenced animals exhibited significantly more HGT events than non-fenced animals ($r=0.16$, $p=2.4e-02$). HGT contigs were enriched in tetracycline resistance genes, ($r=0.27$, $q=1e-03$). A total of 7518 species were involved in HGT, 89 being significantly overrepresented in HGT. Most involved were Tenericutes SGB6571 ($r=5.3$, $q=6.9e-08$), Oscillospiraceae SGB119519, ($r=4.77$, $q=3.3e-06$), Lysinibacillus odysseyi SGB7477 ($r=4.42$, $q=3.6e-05$), Firmicutes SGB121692 ($r=4$, $q=6e-04$), Lachnospiraceae SGB118254 ($r=3.96$, $q=6.4e-04$).

In conclusion, HGT varies by host species, with animals displaying higher HGT. Man-made animal habitats may drive HGT rates in animals. Physiological differences may play a role in variation of HGT between sexes.

Back

35. Metabolomics Analyses Reveal Lignin Depolymerization by Microbiota in the Human Gut

Christopher Dicksion[1], Elizabeth Bess[1]

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Consumption of dietary fiber imparts numerous nutritional benefits. Lignin is a polyphenolic plant fiber and an important dietary source of polyphenolic metabolites that promote human health via anticancer, anti-inflammatory, and antioxidant mechanisms. Even though consensus in the field is growing, molecular-level structural evidence that microbiota in the human gut depolymerize lignin has heretofore remained elusive. We cultured microbiota derived from human fecal samples with isotopically-labelled lignin to show that microbiota in the human gut depolymerize lignin under anaerobic conditions. Mass spectrometric analyses reveal the first-ever detailed structural changes in lignin, and a metabolomic timecourse analysis shows that this process happens on the scale of hours. This work not only opens new avenues for efficient and sustainable production of boutique polyphenolic compounds via enhanced lignin valorization, but it also provides a new avenue toward a molecular-level understanding of how lignin metabolism by human gut microbiota impacts human health.

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36. Species-resolved Enterococcus Profiling Identifies Associations with Sars-cov-2 Infection and Metastatic Status in Patients with Solid Tumors

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Background: Members of the genus Enterococcus are common intestinal commensals and opportunistic pathogens. Unlike the marked expansion observed in inflammatory states, cancer-associated Enterococcus changes are generally modest and reflect selective enrichment of specialized strains. We characterized Enterococcus spp distribution in cancer patients with and without SARS-CoV-2 infection and assessed its association with disease stage and tumor type.

Methods: Shotgun metagenomic analysis was conducted on 195 stool samples from cancer patients with (n=98) and without (n=97) SARS-CoV-2 infection. Tumor types included gastrointestinal (CRC, n=61; NOCRC, n=11), breast (n=43), genitourinary (GU, n=14), gynecologic (GYN, n=15), head and neck (n=16), thoracic (n=20) and others (n=15). Tumors were localized (n=118) or metastatic (n=77) at sample collection. Abundances were analyzed using adjusted linear models on log10-transformed values; p-values were FDR-corrected.

Results: Enterococcus genus represented ~1.7% of the gut microbiota composition with E. faecium, E. faecalis, E. lactis, E. avium, and E. gilvus being the most represented species.

SARS-CoV-2 infection was associated with increased Enterococcus abundance (ratio=3.01, IC95%:1.45–6.24, p=0.003) with selective enrichment of E. avium (p=0.003), E. faecium (p=0.010), and E. lactis (p=0.04). Patients with metastatic cancer showed higher Enterococcus genus abundance compared with localized disease (ratio=3.12, IC95%:1.46–6.66, p=0.004), with an enrichment of E. faecalis (p=0.004) and E. avium (p=0.018). E. faecium was predominant across most tumors while E. gilvus consistently exhibited the lowest abundance. A non-significant enrichment of E. faecalis in NOCRC and GYN and of E. lactis and E. avium in GU tumors was observed. Conclusions:Variation in Enterococcus composition across SARS-CoV-2 infection and disease stage is driven by few species, supporting a species-specific contribution rather than a genus-wide effect.

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37. Not All Scars Heal: Persistent Microbiota-liver Crosstalk After Dietary Intervention in Obesity

Jordi Rofes Herrera[1], Matthias Schlesner[2], Pablo M. Garcia-Roves[3], Óscar Yanes[4], Joan Miró-Blanch[1], Christian M. Heyer[2], Pau Gama[5], Ignasi Forné[6], Aurélie Balvay[7], Claire Maudet[7], Sylvie Rabot[7], Axel Imhof[6]

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Obesity is associated with impaired metabolic plasticity and weight regain, suggesting that diet-induced molecular alterations persist despite lifestyle intervention. To dissect reversible and persistent components of this remodeling, we performed a multi-omic characterization of the Life-Style Matters (LiMa) mouse model, integrating liver transcriptomics, epigenomics, metabolomics, and metallomics with gut metagenomics and metallomics.

Multi-omics factor analysis (MOFA) resolved two dominant axes of variation. The first captured a broadly reversible response (~72% of altered variables), restored by dietary restriction and exercise, involving coordinated remodeling of hepatic metal homeostasis, epigenetic regulation, and immune and cellular turnover pathways. The second reflected persistent alterations resistant to intervention, primarily driven by microbial functional profiles, notably, functional diversity remained reduced despite partial taxonomic recovery.

To causally link these hepatic alterations to the microbiota, we defined the microbiota-responsive hepatic space using germ-free mice, showing that a significant fraction of persistent liver features falls within it. These functions were enriched in lipid metabolism — PPAR signaling, steroid and cholesterol biosynthesis, peroxisomal activity — and retinol metabolism. Sequential correlation analysis traced these changes to HFD-induced loss of specific low-abundance taxa and their biosynthetic capacities, particularly vitamin synthesis (folate, biotin, cobalamin, pantothenate, thiamine) and cofactor metabolism. Strikingly, cobalt — the metal core of cobalamin — was the only essential element persistently dysregulated, directly linking microbiota-derived vitamin metabolism to sustained hepatic dysfunction.

These findings support a dual regulatory framework governed by reversible host-intrinsic and persistent microbiota-dependent processes, offering a systems-level explanation for obesogenic memory.

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Not all scars heal: persistent microbiota-liver crosstalk after dietary intervention in obesity

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Introduction

The microbiota plays a vital role in the organism by aiding the development of the immune system, producing key metabolites, such as vitamins and amino acids and maintaining gut structure[1]. Alterations of the microbiota have been associated with obesity[2] and its restoration has been effective in restoring the phenotype[3] but the mechanisms are still unclear. The microbiota produces metabolites that can change the epigenome such as short chain fatty acids (SCFA), vitamins, and folate[4,5] and we hypothesized that these metabolites affect gene expression from the host and that changes in the diet and lifestyle can alter these interactions.

To study this, we applied a multi-omic approach to a lifestyle intervention C57BL/6J mice model with three groups (each n = 10): fed a standard diet (CNT), a high fat diet (HFD), and a high fat diet followed by a lifestyle intervention with a healthier diet and exercise (INT) (Figure 1). Then, we acquired liver gene expression (RNA-Sequencing), epigenetics (Whole Genome Bisulphite sequencing and histone proteomics) and metabolites (targeted epigenetically relevant metabolites and untargeted); cecum and colon microbiome data (16S sequencing and shotgun sequencing), metabolites (targeted) and metals; and serum metabolites.

Methods

A multi-omics factor analysis (MOFA2) model was trained with 2 factors using the variables significant in atleast one univariate group comparison. Variables were classified into each factor using the weights from the model. A KEGG partial joint pathway enrichment analysis was performed of the genes and metabolite features using clusterProfiler. Pearson cross-correlations were calculated between omic layers, tested for statistical significance, and filtered by FDR adjusted p-value <0.05 and >0.75 correlation strength.

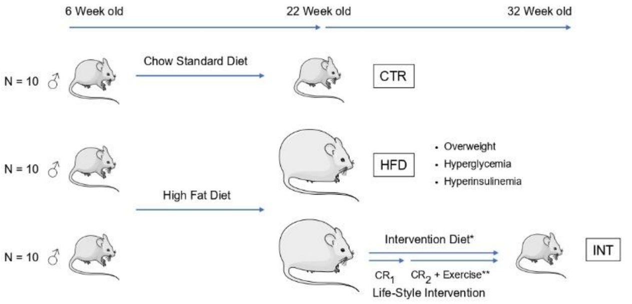

Figure 1. Life-style intervention model overview. From top to bottom: CTR group fed standard diet, HFD group a high fat diet and INT group a high fat diet and a following life-style intervention changes. *Flaxseed and olive oil instead of lard and soybean oil; corn starch instead of sucrose **CR1 80% of caloric intake from CTR mice. CR2 100% of caloric intake from CTR mice. Exercise 1h/day, 5days/week

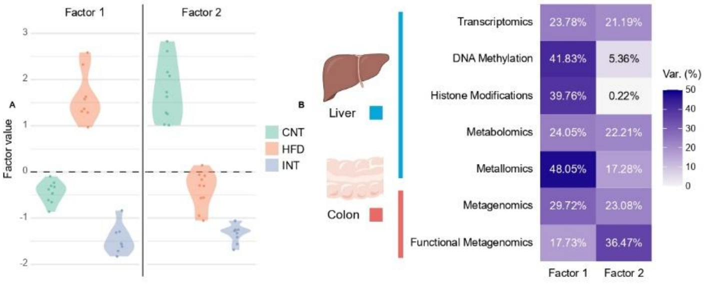

Figure 2. A.Violin plot of the two first factors from the multi omics factor analysis (MOFA) model. B. Percentage of explained variance of each omic layer on the two first MOFA factors.

Results

The MOFA factors capture two main trends of the HFD alterations (Figure 2A):

1) Molecular features that are restored with the intervention (72% of features)
2) Molecular features are resistant to the intervention (28% of features)

Factor 1 explains the variability from the epigenetic layers and liver metallomics, while factor 2 captures microbiota functionalities (Figure 2B).

The factor 1 KEGG join pathway enrichment showed inflammation, immune system and sugar regulation pathways (Figure 3A). while factor 2 reveals lipid, bile acid, branched chain amino-acids, and circadian rhythm metabolisms (Figure 3B).

Sequential correlations between factor 2 microbiota genus, cluster of orthologous genes (COG) microbiota functionalities and KEGG pathways of the second factor revealed correlated persistent reduction of key genera (except lactococcus) with loss of diverse vitamin and cofactor functionalities associated with up and down-regulated impaired liver pathways (Figure 3C).

Conclusions

The results show that the life-style intervention can restore most liver epigenetic, expression and metabolic changes caused by the high fat diet. However, many molecular features remain altered and they strongly correlate with the microbiota and their functionalities.

KEGG enrichment of factor variables show that liver inflammation and sugar metabolisms can be reversed by the intervention but there remain persistent changes to the circadian rhythm and fatty acid metabolism.

These persistent changes can be associated with reduction of key genera and the consequent loss of many vitamin production and other diverse COG microbiota functionalities.

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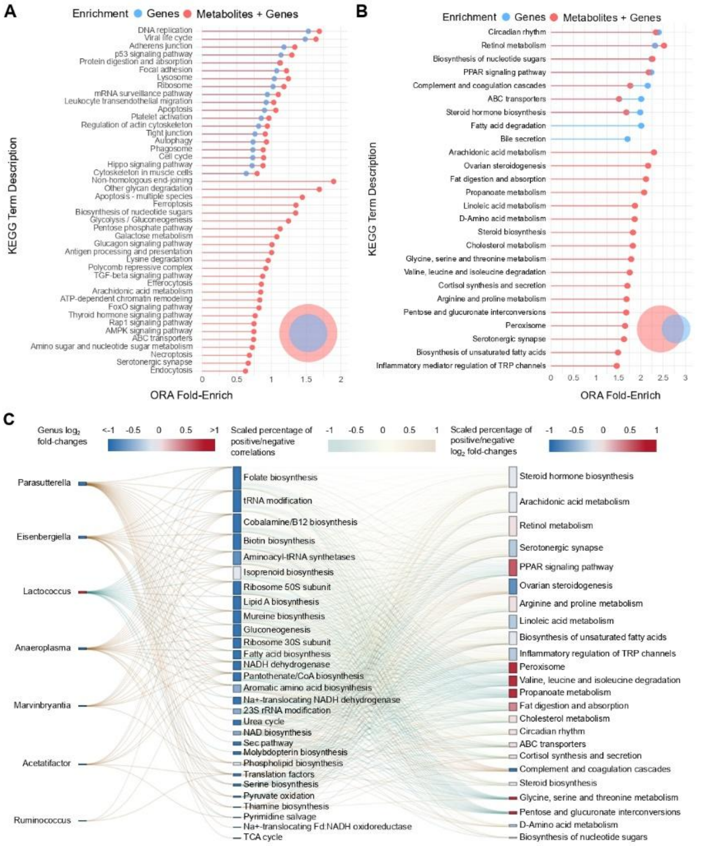

Figure 3. Lollypop plot of the joint KEGG pathway enriched KEGG terms comparing genes+metabolite features (red) with only genes (blue) enrichments using only genes (adj. p-value <0.05). Venn Diagrams indicate the overlap between enriched terms. A. Factor 1 enrichments B. Factor 2 enrichments. C. Sequential correlations between microbiota genus, COG functionalities and factor 2 liver KEGG enrichment. Node colors indicate the HFD-CNT log2 fold-change. Line colors indicate the number of positive/negative correlations between variables

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38. Decoding the Gut-Brain Axis in Parkinson’s Disease: Unraveling the Impact of Obesity Using Organ-on-a-Chip Platforms

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Parkinson’s disease (PD) is a multifactorial neurodegenerative disorder characterized by dopaminergic neuronal loss and α-synuclein aggregation, with early non-motor symptoms such as gastrointestinal dysfunction. Increasing evidence highlights the gut–microbiota–brain axis (GMBA) as a key player in PD pathogenesis. In parallel, obesity is associated with gut microbiota dysbiosis and systemic inflammation, yet its role in PD remains unclear.

In previous work, we investigated the association between PD and gut microbiota composition, identifying dysregulation in specific bacterial populations through gene expression analysis using PCR-based approaches. These findings support the involvement of microbiota alterations in PD and highlight the need to further explore underlying mechanisms.

Building on this, the present project aims to investigate the interplay between gut microbiota, obesity, and PD progression. We are developing advanced 3D microfluidic organ-on-a-chip platforms, including brain-on-a-chip and gut-on-a-chip systems using human-derived cell lines. These models will be integrated to recreate a physiologically relevant gut–brain axis and incorporate microbiota components and adipocyte-derived secretome to mimic obesity-related conditions.

This is an ongoing project. We expect to demonstrate that obesity-associated gut dysbiosis exacerbates PD-related neurodegenerative processes, including increased α-synuclein aggregation, neuroinflammation, and dopaminergic dysfunction.

Overall, this work will provide a novel human-relevant platform to study the gut-brain axis in PD and may contribute to the identification of new biomarkers and therapeutic targets.

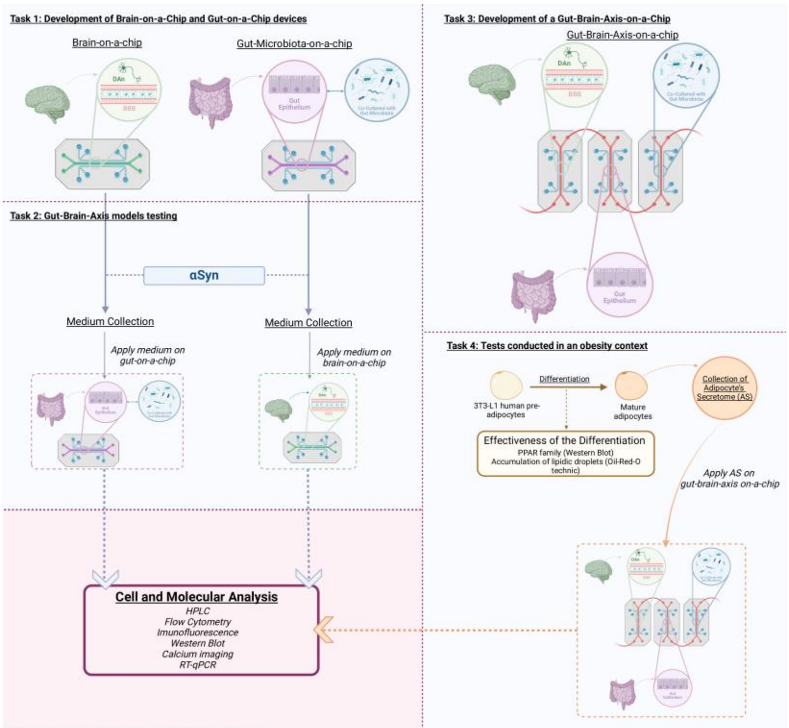

Figure 3: Schematic representation of the PhD project.
Note: microfluidic devices herein represented are just an example, not corresponding to the prototypes that will be developed.

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39. Elucidating the Impact of Ultra-processed Foods on the Gut Microbiome in Animal Models

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Ultra-processed foods (UPF) are ubiquitous in industrialized societies. Increasing evidence shows association between UPF diets and gastrointestinal diseases, including inflammatory bowel disease, as well as mental health.

We evaluated whether the introduction timing of UPF diet affects microbiome functional and taxonomic profiles in 102 cecal samples of Rattus norvegicus. Animals were exposed to 3 dietary conditions: 1) chow diet, 2) UPF introduced from weaning onward, and 3) lifelong UPF exposure. Conditions were assessed independently in adolescent (7weeks) and adult (18weeks) animals (N=43 and 59 respectively). Shotgun metagenomic sequencing was performed with an average depth of 85M reads/sample. Taxonomic composition assessed with MetaPhlAn4.2.2 (vJan25) and functional profile with HUMAnN3.9 (vJun23).

In adolescents, timing of UPF introduction presented a significant effect on beta diversity (30% variation explained, $r=0.54$, $p<1e-5$). In adults, UPF influenced microbiome composition (13%, $r=0.38$, $p<1e-5$), but introduction at weaning vs lifelong could not be distinguished. UPF was associated with reduced taxonomic alpha diversity in both cases, with timing of introduction showing differential effect: adolescents exposed from birth exhibited lower alpha diversity than those introduced at weaning ($r=0.39$, $p<1e-3$), while all adults exposed to UPF displayed a similar reduction compared to chow diet ($r=0.65$, $p<0.01$). The only sex-specific effect was observed in adults, with decreased taxonomic alpha diversity. In addition, Parabacteroides SGB1941 was significantly increased in UPF diets ($r=0.49$, $p<1e-3$), consistent with previous studies. In contrast, functional diversity was higher in UPF ($r=0.18$, $p<0.05$), and adults showed increased carbohydrate/lipid degradation ($r=0.58$, $p<1e-3$), consistent with fat content (UPF:47.1%-Chow:9.2%).

These results suggest timing and duration of UPF exposure, along with age and sex, can shape gut microbiome taxonomy and function.

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40. Contribution of the Intestinal Microbiota to the Progression of Kidney Damage in Patients with Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC)

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Background: Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC) is an ultra-rare autosomal recessive tubulopathy caused by mutations in CLDN16 or CLDN19.

It is characterized by massive renal loss of Mg^{2+} and Ca^{2+}, progressive nephrocalcinosis, and early onset of chronic kidney disease. Patients homozygous for the Spanish founder mutation p.G20D in CLDN19 exhibit variable progression to renal failure, suggesting additional molecular or environmental factors modulate disease course. This study aimed to investigate the intestinal microbiota composition and functional potential in FHHNC patients to understand mechanisms underlying phenotypic variability.

Methods: Patients were classified based on estimated glomerular filtration rate (eGFR) decline into fast (≥10 mL/min/1.73 m²/year), moderate (<10 mL/min/1.73 m²/year), or slow (stable) progression groups. A marker-based approach targeting the 16S ribosomal RNA (16S rRNA) gene was performed in 37 fecal samples (12 patients and 25 healthy controls). Analyses included microbial α- and β-diversity, taxonomic profiling, and functional prediction.

Results: No significant differences in α-diversity were observed between patients and controls or between progression groups. However, β-diversity differed significantly between patients and controls, with a similar but non-significant trend between moderate and slow progression groups. Specific bacterial genera contributed to these differences. Functional inference revealed enrichment of short-chain fatty acid and D-amino acid metabolism in patients. Moreover, distinct functional profiles were observed between progression groups.

Conclusions: FHHNC patients exhibit a dysbiotic gut microbiota with altered metabolic potential. These microbial changes may contribute to variability in clinical outcomes, even among patients carrying the same CLDN19 p.G20D mutation, highlighting a potential role of gut microbiota in modulating disease progression and kidney damage.

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Contribution of the intestinal microbiota to the progression of kidney damage in patients with Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC)

[1]Julieta Torchia Pruzzo, [1]Anna Meseguer, [1,2,3]Gema Ariceta, [1]Cristina Martinez.

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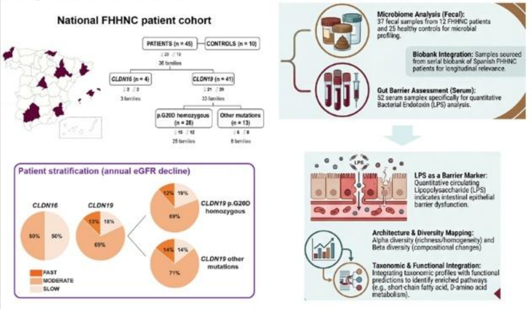
BACKGROUND

FHHNC is an ultra-rare inherited tubulopathy caused by mutations in *CLDN16* or *CLDN19*, which encode tight junction proteins essential for paracellular magnesium and calcium reabsorption in the thick ascending limb of Henle's loop. The disease is characterized by severe renal wasting of Mg^{2+} and Ca^{2+}, nephrocalcinosis, and progressive chronic kidney disease, leading to kidney failure at a young age. Despite sharing the same causative mutation, patients homozygous for the Spanish founder variant p.G20D in *CLDN19* exhibit marked variability in disease progression, suggesting the existence of additional modifiers influencing disease severity. Emerging evidence highlights the gut microbiota as an important regulator of host metabolism, inflammation, and kidney function. Therefore, we investigated the composition and functional potential of the intestinal microbiota in FHHNC patients to explore its contribution to phenotypic variability and disease progression.

OBJECTIVE

To characterize the gut microbiota composition and predicted functional profile of patients with FHHNC carrying the homozygous CLDN19 p.G20D mutation, and to identify microbial features associated with renal disease progression and phenotypic variability.

METHODS



National FHHNC patient cohort

Patient stratification (annual eGFR decline)

Microbiome Analysis (Fecal): 37 fecal samples from 12 FHHNC patients and 25 healthy controls for microbial profiling.

Biobank Integration: Samples sourced from serial biobank of Spanish FHHNC patients for longitudinal relevance.

Gut Barrier Assessment (Serum): 52 serum samplex specifically for quantitative Bacterial Endotoxin (LPS) analysis.

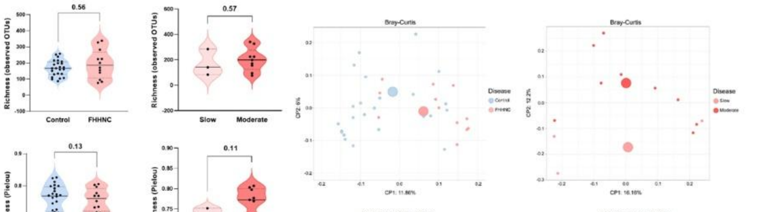
LPS as a Barrier Marker: Quantitative circulating Lipopolysaccharide (LPS) indicates intestinal epithelial barrier dysfunction.

Architecture & Diversity Mapping: Alpha diversity (richness/homogeneity) and Beta diversity (compositional changes)

Taxonomic & Functional Integration: Integrating taxonomic profiles with functional predictions to identify enriched pathways (e.g., short-chain fatty acid, D-amino acid metabolism).

RESULTS

1. FHHNC-Driven Gut Dysbiosis



A. Alpha diversity analysis

B. Beta diversity analysis

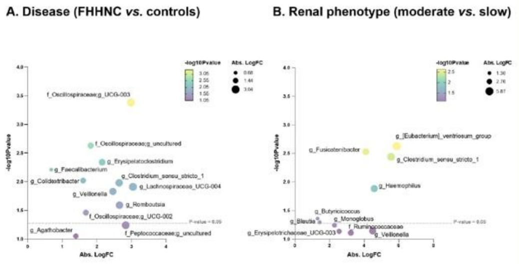
ANOSIM ANALYSIS

Variable Name	ANOSIM statistic R	Significance
Disease	0.3337	0.001

ANOSIM ANALYSIS

Variable Name	ANOSIM statistic R	Significance
Renal_phenotype	0.37132	0.076

Fig. 1. Gut microbial community structure is altered in FHHNC patients despite preserved microbial richness and diversity. A. Measures of α-diversity showed comparable microbial richness and evenness across study groups. **B.** In contrast, β-diversity analysis identified significant differences in microbial community composition between FHHNC patients and healthy controls. Patients with moderate and slow progression also exhibited partial separation of microbial communities, suggesting progression-associated microbiome differences, although statistical significance was not reached.

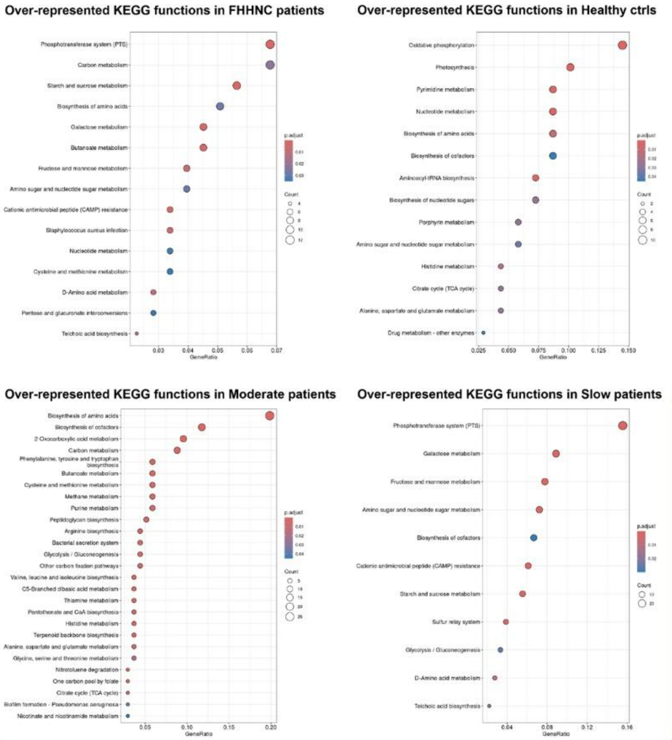


A. Disease (FHHNC *vs.* controls)

B. Renal phenotype (moderate *vs.* slow)

Fig. 2. FHHNC is associated with distinct taxonomic signatures that partially overlap with renal progression–associated microbial changes. A. Differential abundance analysis identified multiple bacterial taxa significantly altered in FHHNC patients compared with healthy controls, indicating disease-associated gut microbiota dysbiosis. **B.** Comparison of patients with moderate and slow renal disease progression revealed a smaller set of differentially abundant taxa, suggesting that specific microbial populations may contribute to phenotypic variability and disease progression. Dot size represents relative abundance, and color intensity reflects statistical significance.

2. Metabolic Markers of Renal Progression



Over-represented KEGG functions in FHHNC patients

Over-represented KEGG functions in Healthy ctrls

Over-represented KEGG functions in Moderate patients

Over-represented KEGG functions in Slow patients

Fig. 3. Functional prediction analysis revealed divergent metabolic signatures between FHHNC patients and healthy controls, as well as specific functional shifts associated with the rate of renal progression. Dot plots illustrate the significantly over-represented metabolic functions inferred from 16S rRNA sequencing data across the study groups. The panels display enriched pathways for (top-left) FHHNC patients, (top-right) healthy controls, (bottom-left) patients with moderate renal progression, and (bottom-right) patients with slow (stable) renal progression. The x-axis represents the GeneRatio (the proportion of genes in the sample associated with a specific pathway), while dot size corresponds to the sequence count and dot color indicates the statistical significance (adjusted p-value).

Functional prediction analysis revealed significant differences in the metabolic potential of the gut microbiota between FHHNC patients and healthy controls, as well as between renal progression groups. Compared with controls, FHHNC patients showed enrichment of pathways involved in carbohydrate uptake and metabolism, including the phosphotransferase system (PTS), carbon metabolism, and starch and sucrose metabolism, together with pathways related to D-amino acid metabolism and teichoic acid biosynthesis. In contrast, healthy controls were enriched in oxidative phosphorylation and nucleotide metabolism pathways, consistent with a more energy-efficient metabolic profile. Stratification by renal progression rate identified distinct functional signatures: moderate progressors exhibited marked enrichment of amino acid biosynthesis, cofactor biosynthesis, and 2-oxocarboxylic acid metabolism, whereas slow progressors were characterized by enrichment of PTS, galactose and fructose/mannose metabolism, and CAMP resistance pathways. These findings suggest that FHHNC is associated with a core functional shift toward carbohydrate metabolism, while amino acid biosynthesis and specific sugar-processing pathways may distinguish different rates of renal disease progression.

3. Stable Systemic Translocation

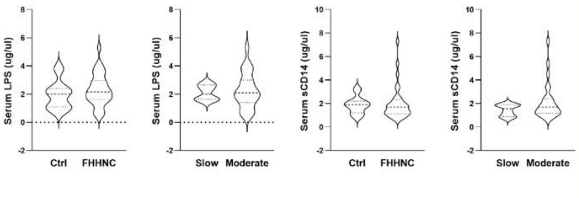


Fig. 4. Systemic markers of microbial translocation are not altered in FHHNC or associated with renal disease progression. Violin plots showing plasma levels of LPS and sCD14 in FHHNC patients and healthy controls, and according to renal progression rate. No significant differences were detected between groups. These findings indicate that, despite the taxonomic and functional alterations observed in the gut microbiota of FHHNC patients, systemic markers of microbial translocation and monocyte activation remain largely unchanged.

CONCLUSIONS

FHHNC patients exhibit a dysbiotic gut microbiota with altered metabolic potential. These microbial changes may contribute to variability in clinical outcomes, even among patients carrying the same CLDN19 p.G20D mutation, highlighting a potential role of gut microbiota in modulating disease progression and kidney damage.

hipofam — Asociación para la información y la investigación de la hipomagnesemia familiar; AIRG España; Vall d'Hebron Barcelona Campus Hospitalari; Vall d'Hebron Institut de Recerca; Hub de Recerca Pediàtrica; Gobierno de España, Ministerio de Ciencia e Innovación; Instituto de Salud Carlos III; Cofinanciado por la Unión Europea; AGAUR Agència de Gestió d'Ajuts Universitaris i de Recerca; UAB Universitat Autònoma de Barcelona

41. Skin Microbiome Engineering Against Chronic Inflammatory Diseases

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disorder characterized by cycles of flare-ups and remission, driven by skin barrier impairment and microbial dysbiosis. A key factor in AD progression is the loss of taxonomic diversity and the colonization of Staphylococcus aureus, which triggers a pro-inflammatory cascade. Conventional treatments, such as corticosteroids and antibiotics, often fail to resolve this underlying dysbiosis and may further disrupt commensal microbiota.

Methods: We developed a synthetic biology framework utilizing the dominant skin commensal Cutibacterium acnes as a biological chassis for autonomous, in situ therapeutic delivery. We engineered genetic circuits designed to sense diverse AD-relevant biomarkers—including metal ions, amino acids, vitamins, and peptide signals—by harnessing transmembrane receptors and intracellular transcription factors.

Results: The engineered sensing modules successfully detected an array of target ligands, including tryptophan and its derivatives, alongside various vitamins and small peptides representative of the AD inflammatory milieu. Rigorous testing confirmed the sensitivity and specificity of these sensors, with significant fold-induction observed across physiologically relevant concentrations.

Conclusions: This work represents a significant advancement in precision microbial sensing, transforming a common skin commensal into a responsive participant in skin homeostasis. By establishing a platform capable of monitoring signals linked to dysbiosis and inflammation, this synthetic biology toolkit offers a sophisticated foundation for future autonomous, "sense-and-respond" interventions for chronic inflammatory skin conditions.

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42. Intranasal Inoculation of Commensal Bacteria Drives Pig Nasal Microbiota Composition and Improves Immunity and Vaccine Efficacy

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The microbiota plays a pivotal role in host physiology and health. Strategies for modulating these commensal communities, such as probiotics, are gaining attention. Here, we evaluate an early-life intranasal inoculation of piglets with a cocktail of five commensal bacteria in the presence/absence of maternal antibodies against a protein fragment (F4) from virulent Glaesserella parasuis. Piglets belonged to 4 groups: colonized with commensals or not (C/NC piglets) and born to sows vaccinated or not with F4 (V/NV piglets). All groups were challenged with virulent G. parasuis at 3 weeks of age. ▤-F4 antibodies together with several cytokines were monitored before challenge, and lung SP-D, weight gain and clinical signs were monitored until study end. The nasal microbiota was profiled at day 15 using 16S rRNA sequencing. When controlling for sow vaccination status, we found that colonization increased the ▤-F4 antibody levels in V piglets and TGF-β (although not consistent across litters) at day 15, while no significant differences were detected for IL-8 or IL-10. Colonization strongly shaped the nasal microbiota composition, with marked differences between C and NC piglets (either V/NV). The microbiota of C piglets was dominated by a few taxa commonly found in the porcine nasal cavity, including two of the inoculated taxa, whose abundance positively correlated with TGF-β levels and weight gain. In contrast, NC piglets were dominated by gut-associated bacteria, which are present but not abundant in the nasal cavity, along with low-abundant environmental taxa. After challenge, colonization improved weight gain in V piglets, indicating a dependence on maternal vaccination. In addition, clinical signs after challenge tended to be fewer in C piglets. No differences were found for lung SP-D. In conclusion, early-life colonization with selected commensals shapes nasal microbiota composition, supports immune development, and may enhance performance and resilience to infection.

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Intranasal inoculation of commensal bacteria drives pig nasal microbiota composition and improves immunity and vaccine efficacy

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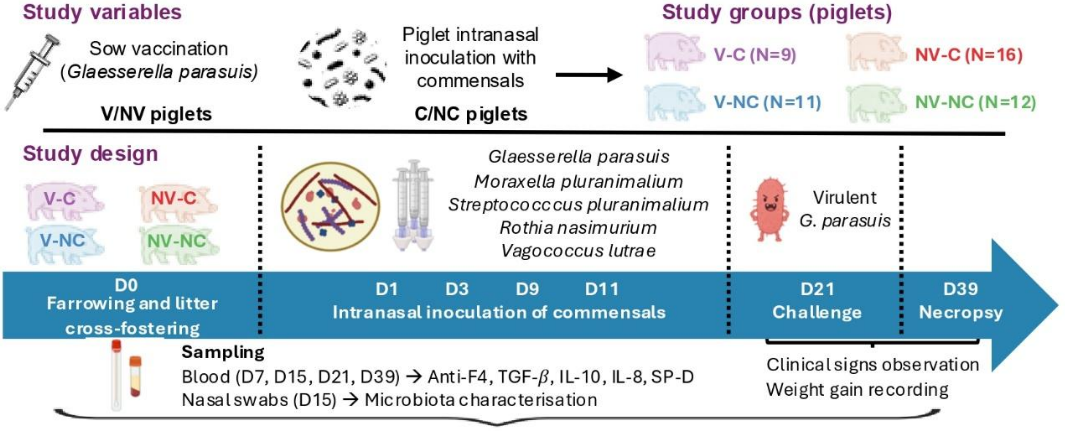
BACKGROUND

The nasal microbiota contributes to host health through metabolic functions, pathogen exclusion, and immune system development. Strategies aimed at deliberately modulating this microbiota to enhance its beneficial functions may represent a promising approach to help prevent and control infectious diseases. Here, we evaluate the impact of intranasal administration of five commensal strains on the nasal microbiota composition, immune response and productive performance under controlled challenge conditions, comparing animals from vaccinated and non-vaccinated sows.

MATERIALS AND METHODS



Study variables

Sow vaccination (*Glaesserella parasuis*)
V/NV piglets

Piglet intranasal inoculation with commensals
C/NC piglets

Study groups (piglets)

V-C (N=9)
NV-C (N=16)
V-NC (N=11)
NV-NC (N=12)

Study design

V-C
NV-C
V-NC
NV-NC

Glaesserella parasuis
Moraxella pluranimalium
Streptococccus pluranimalium
Rothia nasimurium
Vagococcus lutrae

Virulent
G. parasuis

D0
Farrowing and litter cross-fostering

D1 D3 D9 D11
Intranasal inoculation of commensals

D21
Challenge

D39
Necropsy

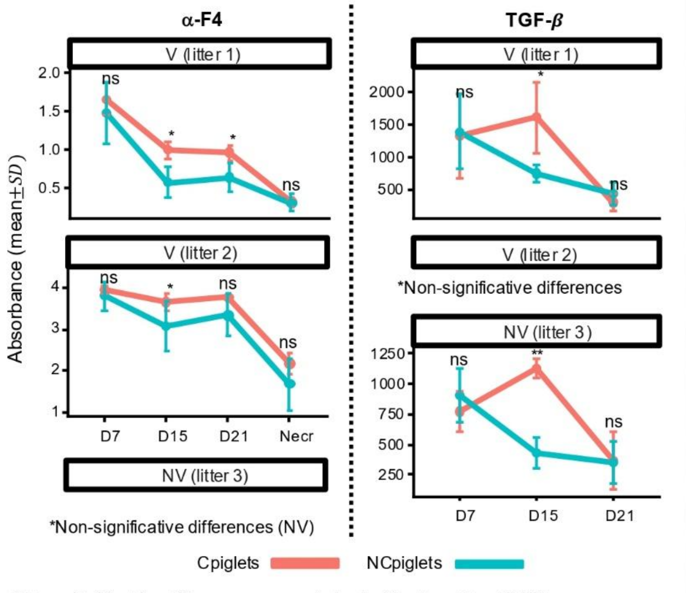
Sampling
Blood (D7, D15, D21, D39) → Anti-F4, TGF-β, IL-10, IL-8, SP-D
Nasal swabs (D15) → Microbiota characterisation

Clinical signs observation
Weight gain recording

Immunity, growth and clinical signs → Generalized Linear Mixed Models / Microbiota characterisation → v3v4 16S rRNA

RESULTS

Commensals inoculation increased the α-F4 levels in V piglets and TGF-β (in V and NV) at day 15 (evaluated within litters).

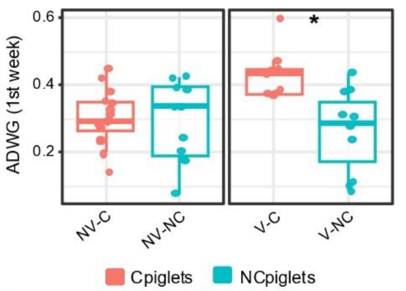


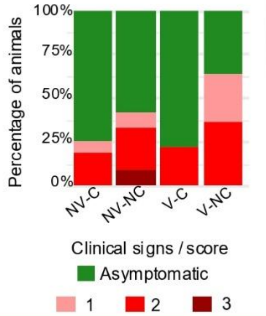
*Non-significative differences (NV)

*Non-significative differences

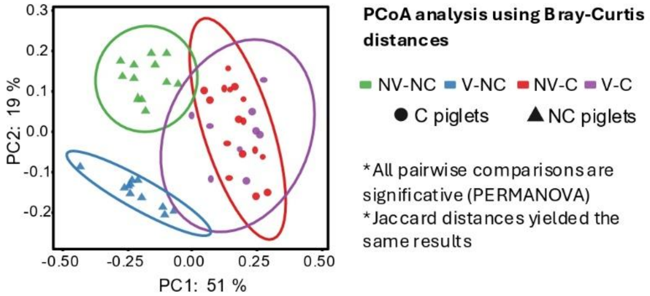
*Non-significative differences were detected for the other NV litter.
** Non-significative differences were detected for IL-8/IL-10 and SP-D levels.

After challenge, colonization significantly improved the average daily weight gain (ADWG) in **V** piglets. Colonized piglets showed less clinical signs after challenge.



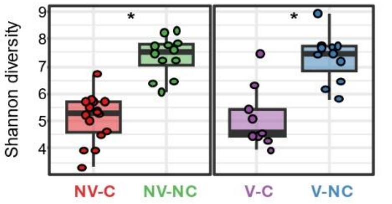


Inoculation strongly shaped the nasal microbiota composition (either V/NV). C Piglets had lower alpha diversity and a different community composition.



PCoA analysis using Bray-Curtis distances

*All pairwise comparisons are significative (PERMANOVA)
*Jaccard distances yielded the same results

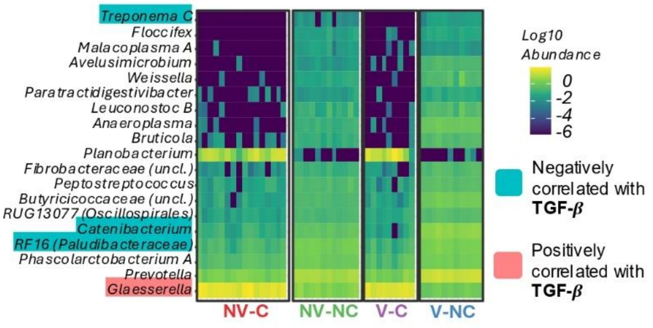


Alpha diversity estimated with Shannon index

*The same results were obtained using Chao1 (richness) and pielou_e (evenness) indexes.

C piglets: dominated by a few taxa commonly found in the porcine nasal cavity, such as *Glaesserella* and *Moraxella*.
NC piglets: enriched in gut-associated bacteria (normally present but not abundant in the nasal cavity), along with low-abundant environmental taxa.

Genera contributing to piglet inoculation prediction (Random Forest)



In conclusion, early-life inoculation with selected commensals shapes the nasal microbiota composition, supports immune development, and may enhance performance and resilience to infection.

43. Gut Microbiome-Derived Metabolites and LPS Contribute to Epithelial Barrier Dysfunction in Pediatric Idiopathic Nephrotic Syndrome

Shuyue He[1], Víctor López-Baez[2], Neus Roca[3], Carmen Larramendi[3], Álvaro Madrid[3], Anna Meseguer[1], Gema Ariceta[2], Cristina Martínez[1]

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Introduction: Idiopathic nephrotic syndrome (INS) is one of the most common kidney disorders in children and is characterized by disruption of the glomerular filtration barrier, with an incompletely understood pathogenesis. Recently, the gut microbiome has emerged as a potential contributor to INS pathogenesis, as microbial components and metabolites may translocate into the circulation and affect epithelial barrier integrity and kidney function.

Methods: Stool and blood samples were collected from children with INS and healthy controls. Gut microbiome composition was analyzed by 16S rRNA sequencing, and fecal metabolomics was performed. Serum lipopolysaccharide (LPS) and soluble CD14 (sCD14) were quantified. Functional assays used Caco-2 cells treated with fecal supernatants (FSN) from INS patients or controls. Barrier integrity was evaluated by transepithelial electrical resistance (TEER) and ZO-1 expression. Cytokines were measured using a multiplex array.

Results: No differences in alpha or beta diversity were found between INS patients and controls or across disease states. In contrast, a reduction in total short-chain fatty acids (SCFAs) was observed in INS, mainly driven by lower butyrate levels. Serum LPS was elevated in active INS and negatively correlated with glomerular function (eGFR) (Spearman $r = -0.65$, $p = 0.001$), while sCD14 showed no differences. Stimulation with FSN from active INS patients and LPS reduced TEER and ZO-1 expression and increased IL-17A production in Caco-2 cells after 48h, whereas control FSN had no effect.

Conclusion: Microbiome-derived metabolites and microbial products contribute to epithelial barrier dysfunction in INS. Reduced SCFAs, together with increased LPS and IL-17A induction, support a role for gut–derived inflammatory mediators in disease pathogenesis, highlighting functional microbiome alterations as potential therapeutic targets.

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Gut microbiome-derived metabolites and LPS contribute to epithelial barrier dysfunction in pediatric idiopathic nephrotic syndrome

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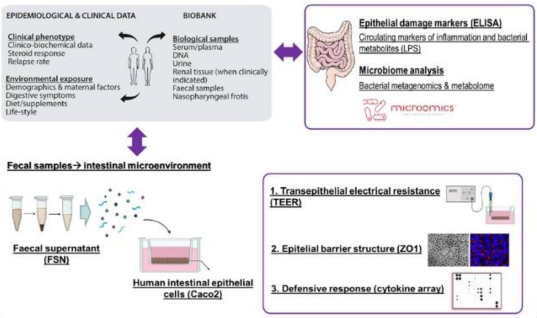
BACKGROUND

Idiopathic nephrotic syndrome (INS) is one of the most frequent glomerular diseases in children and is characterized by disruption of the glomerular filtration barrier. Although its pathogenesis remains incompletely understood, increasing evidence suggests that the gut–kidney axis may contribute to disease development. Alterations in gut microbiota-derived metabolites and microbial products can impair epithelial barrier integrity, promote systemic inflammation, and potentially affect renal function. In particular, short-chain fatty acids (SCFAs) and lipopolysaccharide (LPS) have emerged as key mediators linking intestinal dysbiosis with immune and epithelial dysfunction.

OBJECTIVE

To investigate the role of gut microbiome-derived metabolites and microbial products in INS by analyzing fecal microbiome composition, metabolomic profiles, circulating inflammatory markers, and their effects on epithelial barrier integrity *in vitro*.

METHODS



EPIDEMIOLOGICAL & CLINICAL DATA

Clinical phenotype
Clinico-biochemical data
Steroid response
Relapse rate

Environmental exposure
Demographics & maternal factors
Digestive symptoms
Diet/supplements
Life-style

BIOBANK

Biological samples
Serum/plasma
DNA
Urine
Renal tissue (when clinically indicated)
Faecal samples
Nasopharyngeal frotis

Epithelial damage markers (ELISA)
Circulating markers of inflammation and bacterial metabolites (LPS)

Microbiome analysis
Bacterial metagenomics & metabolome

microomics

Fecal samples→ intestinal microenvironment

Faecal supernatant (FSN)

Human intestinal epithelial cells (Caco2)

1. Transepithelial electrical resistance (TEER)
2. Epitelial barrier structure (ZO1)
3. Defensive response (cytokine array)

RESULTS

1. Patient cohort

Demographic data	Cases n= 26	SSNS n=11	SSNS-FR n=8	SRNS n=7	HC n=20
Age (at inclusion)	9 [3-17]	6 [3-9]	9 [3-13]	11 [9-17]	8.5 [4-16]
Age (at the onset)	3.5 [1-10]	4 [1-8]	2 [2-4]	6 [2-10]	NA
Sex					
Female	4 (15%)	0	4 (50%)	0	11 (55%)
Male	22 (85%)	11 (100%)	4 (50%)	7 (100%)	9 (45%)
Ethnicity					
Hispanic	22 (85%)	10 (90%)	5 (62.5%)	7 (100%)	16 (80%)
Caucasian	2 (7.5%)	1 (10%)	1 (12.5%)	0	4 (20%)
North Africa	0	0	0	0	0
Black	0	0	0	0	0
White	2 (7.5%)	0	2 (25%)	0	0
Asiatic	0	0	0	0	0
Type of diagnosis					
Debut	7 (27.5%)	6 (55%)	0	1 (14%)	NA
Active	2 (7.5%)	0	1 (12.5)	1 (15%)	NA
Remission	17 (65%)	5 (45%)	7 (87.5%)	5 (72%)	NA

Table 1. Clinical and demographic characteristics of the study cohort. The study included 26 paediatric patients diagnosed with idiopathic nephrotic syndrome (INS) and 20 healthy controls (HC). INS patients were classified as steroid-sensitive nephrotic syndrome (SSNS, n=11), frequently relapsing steroid-sensitive nephrotic syndrome (SSNS-FR, n=8), and steroid-resistant nephrotic syndrome (SRNS, n=7). Samples were collected at disease debut, during active disease, or in remission. Median age at inclusion ranged from 6 to 11 years across patient subgroups, with a predominance of male patients in the INS cohort. Demographic and clinical characteristics of all study groups are summarized in the table.

2. Microbiome composition is preserved in INS

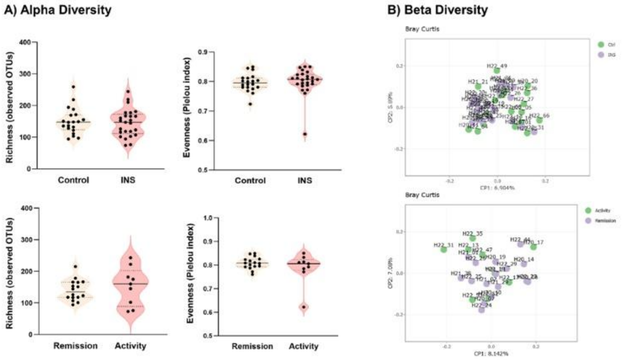


Fig. 1. Gut microbiome composition is preserved in idiopathic nephrotic syndrome (INS). Alpha diversity analysis (A), including richness and evenness indices, showed no significant differences between INS patients and healthy controls, nor between patients in active disease and remission. Similarly, beta diversity analysis (B) based on Bray–Curtis dissimilarity and principal coordinate analysis (PCoA) revealed no distinct clustering among groups, indicating that overall gut microbiome composition remains largely preserved in INS..

3. INS is associated with increased circulating LPS and reduced SCFAs

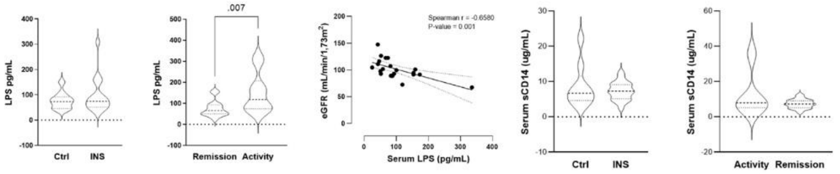


Fig. 2. Active INS is associated with increased circulating LPS levels. Violin plots showing serum levels of lipopolysaccharide (LPS) and soluble CD14 (sCD14) in healthy controls and INS patients, including patients in remission and active disease. Active INS patients displayed significantly increased circulating LPS levels compared with controls and remission patients, whereas no significant differences were observed for sCD14 levels. Scatter plot analysis revealed a negative correlation between serum LPS levels and glomerular filtration rate (eGFR)

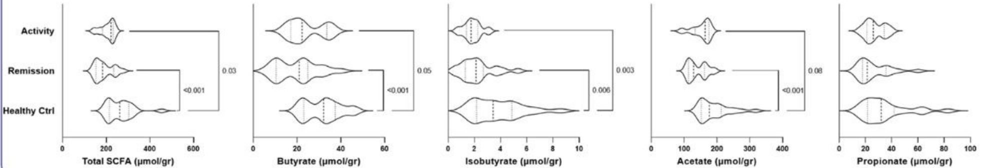


Fig. 3. INS patients show reduced levels of microbiome-derived short-chain fatty acids (SCFAs). Violin plots showing total fecal short-chain fatty acid (SCFA) levels in healthy controls and INS patients, including patients in remission and active disease. INS patients exhibited reduced total SCFA levels, mainly driven by lower butyrate abundance, supporting the presence of functional microbiome alterations in the disease.

4. INS is associated with increased circulating LPS and reduced SCFAs

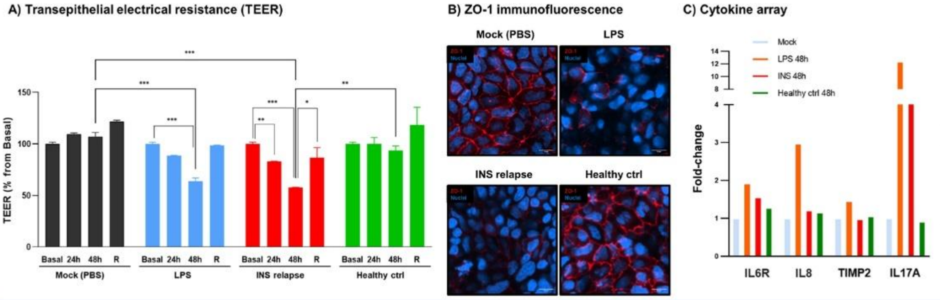


Fig. 4. Fecal supernatants from active INS patients impair epithelial barrier integrity and induce a pro-inflammatory response. A. TEER measurements in Caco-2 cells under basal conditions and after stimulation with PBS (mock), LPS, FSN from healthy controls, or FSN from active INS patients. TEER was evaluated at 24h, 48h, and after a 24h recovery period following stimulus withdrawal (R). Treatment with LPS and FSN from active INS patients induced a marked reduction in TEER, indicating impaired epithelial barrier integrity, whereas control FSN had no significant effect. TEER recovery was observed after stimulus removal. **B.** Representative immunofluorescence images showing ZO-1 expression in Caco-2 cells after stimulation. Cells treated with LPS or FSN from active INS patients displayed reduced and disrupted ZO-1 staining compared with controls. **C.** Cytokine array analysis of Caco-2 cell supernatants after stimulation revealed increased production of IL-17A in response to LPS and FSN from active INS patients, supporting the induction of a pro-inflammatory epithelial response.

CONCLUSIONS

Functional alterations of the gut microbiome contribute to epithelial barrier dysfunction and inflammation in idiopathic nephrotic syndrome (INS). Reduced levels of microbiome-derived short-chain fatty acids (SCFAs), together with increased circulating LPS and IL-17A induction, support a role for gut-derived inflammatory mediators in disease pathogenesis. These findings highlight the gut–kidney axis as a potential source of novel therapeutic targets in INS.

44. Biological Sex and Microbiota Drive DNA Methylation Patterns in the Mouse Liver

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Microbiota- and host-derived metabolites can influence epigenetic enzymes, thereby modulating DNA methylation and gene expression in host tissues, in the mammalian holobiont. Here, we used conventional and germ-free mice including both females (n=20) and males (n=20) to investigate how biological sex and microbiota status shape the host epigenetic landscape. Focusing on the liver and colon, our multi-omics results indicate that biological sex and microbiota status jointly influence DNA methylation and gene expression patterns, with an additive effect. We observed sex-specific differences in DNA methylation that were further accentuated in the presence of microbiota, particularly in males. Notably, germ-free males displayed a global DNA methylation and gene expression profiles that were more similar to those observed in conventional females. In addition, DNA methylation levels in genes involved in steroid metabolism were associated with the abundance of specific microbial taxa, including members of the Ruminococcaceae, Christensenellaceae, and Prevotellaceae families. These observations suggest that defined microbial communities may contribute to host gene regulation in a sex-dependent manner, potentially through modulation of epigenetic states.

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45. Gastric Microbiome Alterations Associated with Helicobacter Pylori Infection

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Background: Helicobacter pylori is a major risk factor for gastric cancer, although only a small proportion of infected individuals develop malignancy. Increasing evidence suggests that, beyond H. pylori, the broader gastric microbiota may contribute to disease progression. However, inconsistent findings limit understanding of microbiota dynamics in gastric carcinogenesis**.**
Methods: Seven gastric biopsy samples were analysed, including samples from ulcer, neoplastic and non-severe gastric conditions. DNA was extracted and the bacterial 16S rRNA gene was amplified and sequenced. Sequences were clustered into OTUs at 97% similarity. Alpha diversity was assessed using the Shannon index, and beta diversity using Bray–Curtis dissimilarity, together with comparative taxonomic profiling.
Results: Alpha diversity differed markedly between sample types. Ulcer samples showed extremely low diversity, consistent with near-monoculture communities, whereas non-severe samples exhibited higher diversity, indicating more complex and balanced microbiota. The neoplastic sample displayed intermediate diversity but very high richness, suggesting a disrupted yet heterogeneous ecosystem. Taxonomic analysis revealed clear shifts across conditions. Non-severe samples were dominated by diverse communities including Bacillota, Pseudomonadota and Bacteroidota. In contrast, ulcer samples were overwhelmingly dominated by Helicobacteraceae (>95%), reflecting severe dysbiosis. The neoplastic condition showed reduced dominance of H. pylori and increased presence of opportunistic taxa.
Conclusions: Gastric microbiota composition varies strongly by disease state, shifting from diverse communities in non-severe conditions to H. pylori-dominated ecosystems in ulcers, and to a more heterogeneous dysbiotic profile in gastric cancer. These findings suggest a progressive microbial restructuring during disease and support a potential role of non-H. pylori bacteria in gastric carcinogenesis.

46. Consistent Sex Differences in the Gut Microbiota of Multiple Sclerosis Patients

Irene Soler-Sáez[1], Cristina Galiana-Roselló[1], Rubén Grillo-Risco[1], Gwen Falony[2], Sara Vieira-Silva[3], Francisco García-García[1]

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Background: Multiple sclerosis (MS) is a chronic immune-mediated neurodegenerative condition of the central nervous system. The disease is influenced by host factors such as sex, which affects MS epidemiology and clinical outcomes (PMID:32719651). The gut microbiota is also necessary to develop the disease, as shown in germ-free mouse models (PMID:22031325, PMID:19841183). Multiple taxa have been associated with MS in humans, although these findings lack consistency across cohorts (PMID:36901634, PMID:39586156). While sex shapes the gut microbiota across the human lifespan (PMID:33713673), its impact on MS remains unexplored.

Methods: We investigate sex differences in the gut microbiota of MS patients through an integrative approach. A systematic data search identified six 16S rRNA gene sequencing studies, comprising a total of 337 samples. After processing each dataset, sex-associated differential abundance patterns were assessed within control and MS groups to characterise dataset-specific shifts. Individual results were then integrated using a random-effects meta-analysis. Finally, the significant findings were validated in an independent dataset.

Results: Variability across studies was substantial, with the dataset of origin as the primary source of variation. Sex-associated differences were more pronounced in MS than in controls. We identified 11 taxa showing significant differences in MS, nine enriched in females and two in males. These findings were replicated, with 82% of taxa showing concordant directionality between the meta-analysis and the validation dataset.

Conclusions: Our analysis reveals reproducible sex differences in the gut microbiota of MS patients. These findings underscore the importance of incorporating sex as a critical factor in microbiome studies, with potential implications for understanding disease heterogeneity in MS.

Back

Consistent sex differences in the gut microbiota of multiple sclerosis patients

Irene Soler-Sáez[1], Cristina Galiana-Roselló[1], Rubén Grillo-Risco[1], Gwen Falony[2], Sara Vieira-Silva[3], Francisco García-García[1]

[1]Computational Biomedicine Laboratory, Centro de Investigación Príncipe Felipe (CIPF), Valencia (Spain).
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BACKGROUND AND AIM

Multiple sclerosis (MS) is a chronic immune-mediated neurodegenerative condition of the central nervous system.

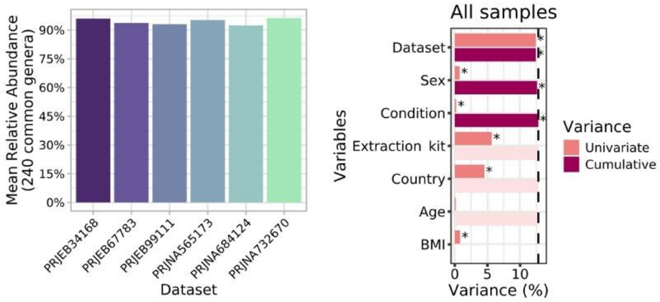
Aim. To identify consistent and reproducible sex-associated differences in the gut microbial composition of MS patients.

METHODOLOGICAL APPROACH

- Systematic review
  6 16S rRNA gene datasets
- Individual analysis
  QC → ASVs → Taxonomy → Normalization → Differential genus abundance
- Meta-analysis and computational validation
- MS clinical features associations
- Web tool

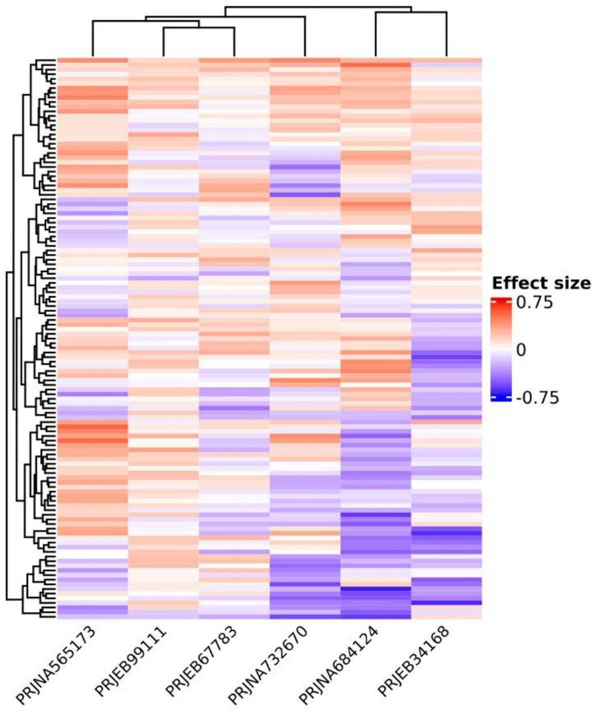
RESULTS

1. Cross-dataset heterogeneity

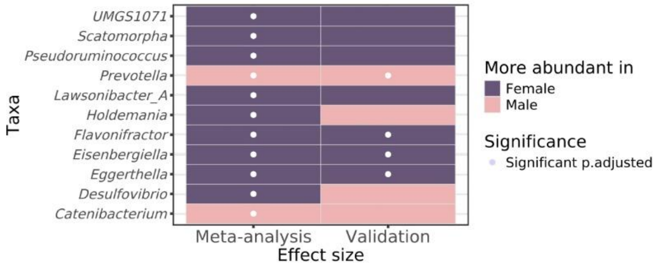


2. Individual sex-differential abundance

MS females *vs.* MS males



3. Consistent sex-differential abundance



Female-enriched ***Eggerthella*** and ***Eisenbergiella*** were associated with disease severity and MS subtypes.

Figure legends. *1. (Left) Relative abundance of taxa shared across all included datasets. (Right) Microbiome compositional variation explained by reported variables in all samples, either individually (univariate) or in a multivariate model (cumulative). BMI was excluded from the multivariate model due to missing data in two studies. 2. Columns (datasets) and rows (genera) clustered based on hierarchical clustering. The color indicates the direction of the effect size (red: higher abundance in females, blue: higher abundance in males). 3. Direction of effect (higher abundance in MS females vs. MS males) for taxa identified as significant in the meta-analysis, compared with corresponding results in the validation cohort. White dot: adjusted p < 0.05.*

CONCLUSIONS

Our analysis reveals reproducible sex differences in the gut microbiota of MS patients, with potential implications for understanding disease heterogeneity in MS.

PAPER

WEB

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Computational Biomedicine Laboratory
CIPF
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Wageningen University & Research
EMBO
The Barcelona Debates on the Human Microbiome

47. Mitigating ETEC-Induced Dysbiosis in Piglets Through Faecal Microbiota Transplantation

Carla Tort Miró[1], Marc Bravo[1], Noemí Giler[1], Clara Marín[1], Lourdes Migura[1]

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Weaning causes major social, physiological and environmental shifts in piglets, triggering microbiome disruptions that increase the risk of suffering post-weaning diarrhoea, mainly caused by enterotoxigenic Escherichia coli (ETEC). Existing approaches like antimicrobials are being reduced to limit resistance, faecal microbiota transplantation (FMT) has gained attention for its potential to reshape the gut microbial community, contributing to intestinal health. In this study, we evaluated the use of FMT both as a preventive and therapeutic intervention in post-weaning piglets experimentally challenged with ETEC.

Donor material was collected from a swine-pathogen free-farm, freeze-dried, and encapsulated. Sixty 28-day-old piglets were allocated into three groups: Control, FMT+ETEC, and ETEC. FMT was administered on arrival and days 4 and 13. ETEC was inoculated orally on days 8 and 9. Animals were monitored for diarrhoea over five weeks, and faecal samples were collected for 16S rRNA gene sequencing and, tissue-samples.

FMT+ETEC group exhibited a significant decrease in diarrhoea-score on day 22 compared to ETEC, and partial recovery of jejunal villus structures. During recovery, animals receiving FMT showed a significant increased richness and beta-diversity differences compared to ETEC. Moreover, ETEC+FMT showed a reduction in the relative abundance of Pseudomonadota phylum in day 8 and 22, suggesting reduced intestinal dysbiosis and inflammation. Additionally, an increase of beneficial taxa was observed in the ETEC+FMT group, including Clostridium species, together with an enrichment of short-chain fatty acid producers, including Phascolarctobacterium succinatutens.

Results indicate that FMT can modulate the gut microbiota enhancing the capacity of weaned piglets to be more resilient to enteric infections, supporting their value to improve intestinal health. While reinforcing the suitability of pigs as a physiological model for human research.

Back

Faecal microbiota transplantation modulates gut microbiota composition in piglets challenged with enterotoxigenic *Escherichia coli* (ETEC)

Carla Tort-Miró[1,2], Marc Bravo[1,2], Àlex Cobos[1,2,3], Noemí Giler-Baquerizo[1,2], Laura Montoro-Dasi[4], Clara Marin[4]*, Lourdes Migura-Garcia[1,2]*

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INTRODUCTION

Weaning causes major social, physiological and environmental shifts in piglets (Figure 1), triggering microbiome disruptions that increase the risk of suffering post-weaning diarrhoea (PWD), mainly caused by enterotoxigenic *Escherichia coli* (ETEC).

Existing approaches, such antimicrobials, are being reduced to limit the spread of them. Among emerging alternative options, **faecal microbiota transplantation** (FMT) has gained attention for its potential to reshape the gut microbial community contributing to intestinal health in piglets.

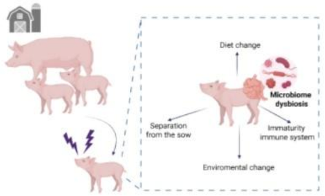

Figure 1. *Post-weaning period workflow in pig industry*

OBJECTIVE: To examine the impact of FMT as preventive and therapeutic strategy in post-weaning piglets subjected to an ETEC challenge.

STUDY DESIGN AND METHODS

A total of sixty 28-day-old piglets were transported to the experimental farm, and assigned to: Control, ETEC and FMT_ETEC.

The FMT treatment was administered on arrival at the experimental farm, and on days 4 and 13. The ETEC inoculation was delivered orally on days 8 and 9. Animals were monitored for signs of diarrhoea over a five-weeks period, and faecal samples were collected at multiple timepoints to evaluate the dynamics of the microbiota by sequencing the whole 16S rRNA gene. At the end of the study, piglets were weighed, euthanised and intestinal tissues were collected for further analysis (Figure 2).

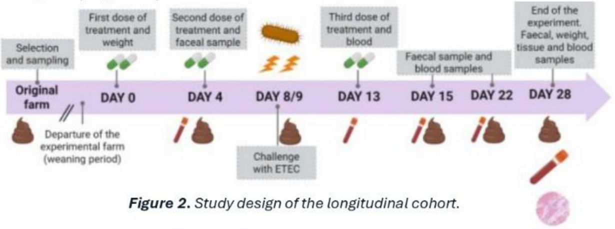

Figure 2. *Study design of the longitudinal cohort.*

RESULTS AND DISCUSSION

1. Diarrhoea score reduction in FMT treated group after ETEC challenge

Piglets from the ETEC_FMT exhibited a significant decrease in diarrhoea-scores (Figure 3A) and ETEC-F18 positive load (Figure 3B) on in the recovery period (day 22) in comparison to ETEC. Together with a partial recovery of villus structures in the jejunum for FMT+ETEC (Figure 3C).

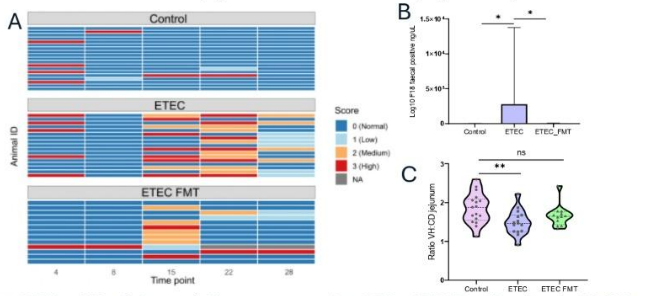

Figure 3. *(A) Effect of FMT on diarrhoea score in different treatment groups (Control, ETEC and ETEC_FMT) at different time points (4, 8, 15, 22 and 28). Fecal F18 + ETEC bacterial load in faecal samples (ng/µL) in* ***(B)*** *day 22.* ***(C)*** *Villus height:crypt (VH:CD) depth ratio observed for jejunal organ. All data represent the mean ± SEM. Asterisks denote significantly different comparison (Mann-Whitney test) for "*"$P < 0.05$, "**" for $P < 0.01$, "***" for $P < 0.001$, and "****" for $P < 0.0001$.*

2. Alpha and beta diversity responses to FMT and ETEC challenge

Alpha diversity analysis showed a significant increase in richness in ETEC_FMT compared to ETEC group during the study (Figure 4A). Also, ETEC_FMT group displayed significantly different microbiota trajectories during all the study compared to the Control and ETEC group (Figure 4B).

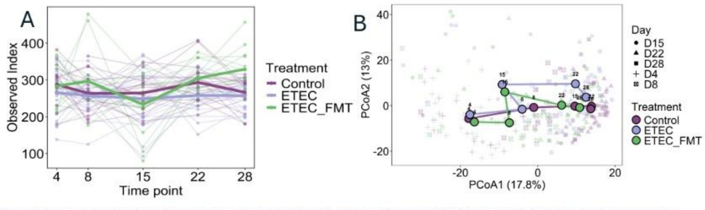

Figure 4. *Alpha and beta diversity in different groups (Control, ETEC, ETEC_FMT) and time points (4, 8, 15, 22 and 28).* ***(A)*** *Alpha diversity measured by Observed feature index (richness) studied with Lineal Mixed Model (LMM). Colour shadow indicates the proportion of each treatment.* ***(B)*** *Beta diversity estimated through Aitchison distances. Statistics was done with adonis2 test and post-hoc comparisons (not shown).*

3. Pseudomonadota reduction in FMT treated group

Significant higher abundance of Pseudomonadota phylum was observed in the ETEC group before the challenge (day 8) and during the recovery period (day 22) compared to the ETEC_FMT and Control groups, suggesting a hallmark of intestinal dysbiosis and inflammation (Figure 5A). By day 28, taxa associated with short chain fatty acids (SCFA) production were differentially abundant, distinguishing ETEC_FMT from untreated ETEC (Figure 5B)

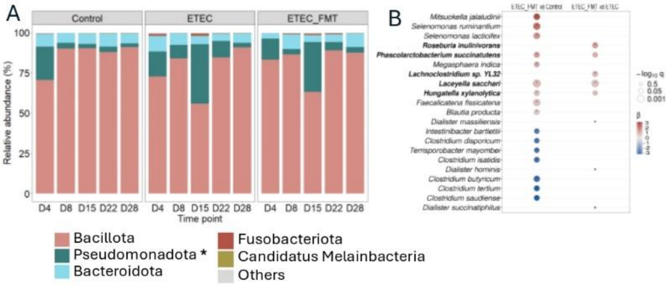

Figure 5. ***(A)*** *Changes in the relative abundance of longitudinal microbiota composition at a phylum level in different groups (Control, ETEC, ETEC_FMT) and time points (4, 8, 15, 22 and 28).* ***(B)*** *Differential abundances differing in ETEC+FMT on day 28 compared to Control and ETEC group (MaAsLin3) . Colour by β coefficient and size by $-\log_{10}(q)$. Asterisks denote significantly different comparison * $Q < 0.05$ (BH).*

CONCLUSIONS:

- FMT can modulate the gut microbiota enhancing the capacity of weaned piglets to be more resilient to enteric infections.
- FMT is a potential approach for improving intestinal health while helping to limit the use of antimicrobials in early-life management.
- Relevance of pigs as a model species, since they share important anatomical, physiological, and microbial similarities with humans, reinforcing their suitability for studying host responses to FMT.

FUNDING:

This study was funded by the National Programme (PID2021-125641OB-C22 and PID2021-125641OB-C21). We would also like to thank the CERCA programme (Government of Catalonia) for its support. Carla Tort-Miró is a PhD student at the Autonomous University of Barcelona, in the Microbiology programme, with an IRTA scholarship.

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48. Gut Microbiota Dysbiosis Shapes Brain T Cell Immunity in Accelerated Aging

Laura Peschke[1]

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The bidirectional communication between the gut microbiota, immune system, and central nervous system – the gut-brain axis – plays a vital role in maintaining brain health. Its disruption can lead to neuroinflammation and cognitive decline. However, the relationship between the gut microbiota and age-related changes in brain immunity remains unclear. In this study, we examined gut microbiota composition and T cell profiles in senescence-accelerated SAMP8 mice and senescence-resistant SAMR1 controls at young adult and aged stages. Strain, age, and sex were identified as drivers of microbiota composition variation. Microbial diversity differed between strains independently of age. Several bacterial genera displayed altered relative abundances in young SAMP8 mice, with only *Intestinimonas* remaining significantly higher in aged SAMP8. T cell profiling revealed strain- and age-specific immune signatures. In the brain, SAMP8 mice exhibited a higher $CD4^+/CD8^+$ ratio, and higher levels of pro-inflammatory cytokine-producing $CD4^+$ T cells compared to SAMR1. In contrast, the spleens of SAMP8 mice had a lower $CD4^+/CD8^+$ ratio. Only the spleens of aged SAMP8 mice showed increased T cell activation (effector/naïve ratios) and higher levels of pro-inflammatory cytokine-producing $CD4^+$ and CD8+ T cells, suggesting that brain immune alterations precede systemic involvement reflected by splenic leukocytes. Nine bacterial genera correlated with immune cell frequencies and ratios. Notably, *Intestinimonas* showed a discordant association with the $CD4^+/CD8^+$ ratio in the brain (negative) and spleen (positive). Furthermore, CD8+ T cells correlated with microbiota composition variation more strongly than strain, age and sex. Together, these findings link the gut microbiota to age-dependent immune remodeling in the brain and imply that altered microbial communities could influence pro-inflammatory T cell responses, thereby contributing to accelerated brain aging.

Back

Gut microbiota dysbiosis shapes brain T cell immunity in accelerated aging

JGU UNIVERSITÄTSmedizin. MAINZ

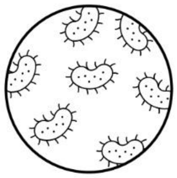
IQCB Institute for Quantitative & Computational Biosciences

Laura Peschke*, Tinh Thi Nguyen*, Jefferson Antônio Leite, Gwen Falony, Vu Thu Thuy Nguyen, Yogita Kattimani, Nishada Ramphal, Rebecca Jasser, Natalia Notarberardino Bos, Tommy Regen, Oliver Tüscher, Ari Waisman, Sara Vieira-Silva** and Kristina Endres**

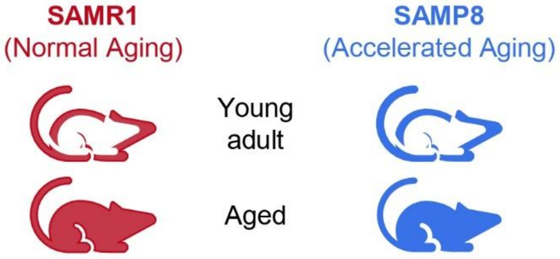
*, ** Authors contributed equally | Email: peschkel@uni-mainz.de

Accelerated aging is linked to both microbial and T cell changes

Previous studies have shown that SAMP8 mice exhibit early-onset cognitive decline and systemic inflammation.



Fecal microbiota:
16S rRNA gene sequencing



SAMR1
(Normal Aging)

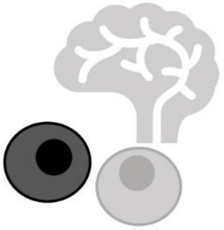
SAMP8
(Accelerated Aging)

Young adult

Aged

N = 8 per group

SAMP8: senescence-accelerated mouse prone 8
SAMR1: senescence-accelerated mouse resistant 1



T cells in brain:
Flow cytometry

Variation in gut microbiota composition is associated to mouse strain and age group.

Altered bacterial abundances in young adult mice.

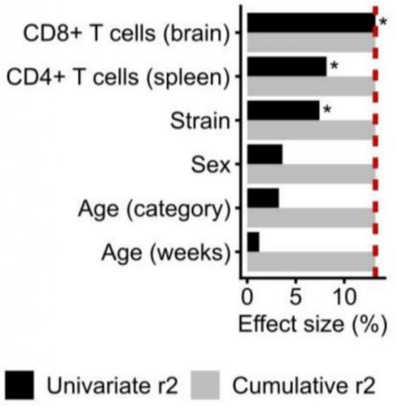
↑ *Intestinimonas* in young adult and aged SAMP8

T cell profiles are associated to mouse strain and age group.

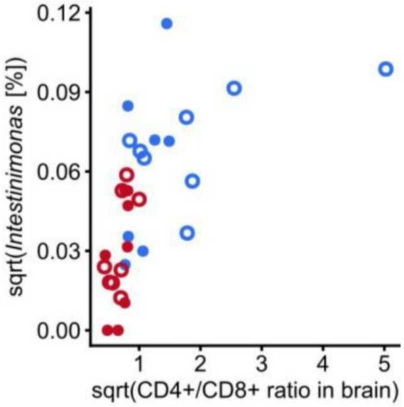
↑ $CD4^+/CD8^+$ T cell ratio
↑ pro-inflammatory $CD4^+$ T cells in young adult and aged SAMP8

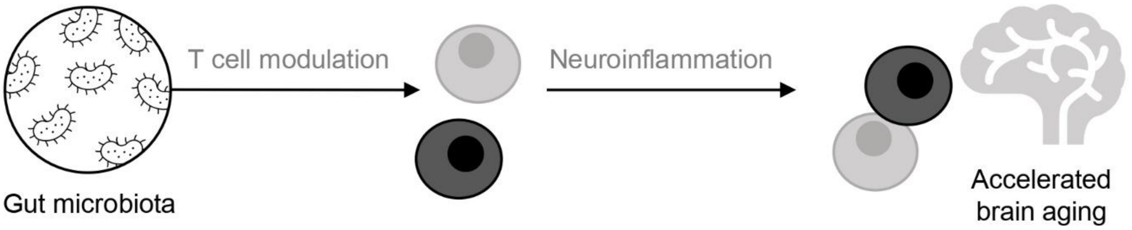
Identified changes in the gut microbiota may shape brain aging

$CD8^+$ T cell frequencies in the brain are associated with microbiota composition variation – more strongly than mouse strain or age.



Several bacteria are associated with T cell frequencies and ratios. *Intestinimonas* is strongly associated with the $CD4^+/CD8^+$ T cell ratio in the brain ($\rho = 0.77^{***}$).





Gut microbiota

T cell modulation

Neuroinflammation

Accelerated brain aging

49. Integrative Multi-Omics Insights into Gut Microbiome and Metabolome Signatures for Personalized Colorectal Cancer Risk Prediction in the Cypriot Population(The Cyprus Intestinal Health Study)

Yiorgos Apidianakis[1], Kelly Katsaounou[1]

[1] *University of Cyprus, Nicosia, Cyprus*

Background: Colorectal cancer (CRC) remains a major global health challenge, and current screening tools provide limited ability to estimate personalized, pre-morphological risk (Katsaounou et al., 2022). This study uses a multi-omics approach combining gut microbiome profiling with fecal metabolomics to identify potential microbial and biochemical biomarkers of CRC susceptibility. By examining microbial composition, predicted functional pathways, and volatile metabolite profiles, this work aims to develop non-invasive signatures that may complement colonoscopy and refine risk-stratified screening.

Method: Fecal and colonic mucosa samples were collected from participants enrolled in the Cyprus Intestinal Health Study under approved ethical protocols. Of 304 volunteers, 265 provided fecal samples ≥15 days post colonoscopy; 231 were classified as Normal Appearing (NA) and 34 as High-Risk Adenoma (HRA). Subgrouping of NA individuals identified Non-Adenoma (NAD, n = 201), Questionably Healthy (QH, n = 29), and subgrouping of HRA individuals recognized Proximal HRA (PC-HRA, n = 11), and Distal HRA (DC-HRA, n = 18). Individuals with CRC history, IBD, recent antibiotic use, or recent gastroenteritis were excluded. Microbial DNA was extracted using the PureLink Microbiome DNA Purification Kit, and the V3–V4 regions of the 16S rRNA gene were sequenced on an Illumina MiSeq platform. Taxonomic assignment used the RDP classifier, with analyses conducted at the genus level. Alpha diversity (Inverse Simpson), beta diversity (UniFrac), and differential abundance (Mann–Whitney U) were computed, and Spearman correlation with permutation-based testing assessed genus and species-level associations. Functional potential of the gut microbiome was inferred using PICRUSt2 based on 16S rRNA profiles. Predicted metabolic pathways and enzyme functions were analyzed in R using the limma voom framework, with CPM filtering and TMMwsp normalization to minimize compositional bias. The voom transformation and empirical Bayes moderation enabled robust identification of differentially enriched metabolic pathways.

A subset of 25 NA and 25 HRA samples underwent fecal Volatile Organic Compound (VOC) profiling via HiSorb sorptive extraction followed by GC–MS. Normalized VOC values were analyzed using Shapiro–Wilk testing, Mann–Whitney U or t tests, and PCA, with statistical significance set at $p \leq 0.05$.

Results: Fecal 16S rRNA profiling revealed genus-level shifts consistent with early CRC associated dysbiosis. HRA individuals showed reduced abundance of beneficial butyrate-producing taxa (e.g., Faecalibacterium) and enrichment of opportunistic or pro-inflammatory genera such as Bacteroides intestinalis (Ferreira-Halder et al., 2017). Within the Normal Appearing cohort, Questionably Healthy individuals exhibited higher relative abundances of Holdemanella (Olovo et al., 2021), including H. biformis, as well as the oral-origin fermenters Streptococcus salivarius (Kaci et al., 2014) and Veillonella dispar (Zhang and Huang, 2023) compared with Non-Adenoma participants, indicating a potential mucosal homeostasis preservation in QH individuals, that may result from the production of the SCFAs butyrate and propionate from lactate metabolism (Distler and Kroncke, 1981). Colonic mucosa analysis identified region-specific alterations: Paraprevotella was increased and Odoribacter decreased in the transverse colon of HRA individuals, while Odoribacter and Desulfovibrio were elevated in NA sigmoid samples. The butyrate producer Coprococcus (Louis and Flint, 2017, Lopetuso et al., 2013) was more abundant in the sigmoid than the transverse colon of NA individuals. Stratification by adenoma location showed that those with proximal adenomas had higher abundances of Faecalibacterium, Bifidobacterium, Fusicatenibacter, Streptococcus, Anaerobranca, and Slackia compared with distal HRA cases. HiSorb GC–MS analysis identified four gut microbiota–derived volatile metabolites— acetone, methyl isovalerate, methyl valerate, and propyl propionate—elevated in NA compared with HRA fecal samples, indicating preserved microbial fermentation and an intact SCFA-producing ecosystem, whereas their reduction in HRA subjects reflects early dysbiosis and loss of metabolic diversity associated with colorectal neoplasia (Xing et al., 2022, Mochalski et al., 2014).

Conclusion: Distinct alterations in gut microbial composition, predicted metabolic capacity, and fecal volatile metabolites accompany the transition from normal mucosa to high-risk adenomatous states. The depletion of key butyrate-producing genera and enrichment of potential pro-inflammatory taxa, together with differences in regional mucosal ecology and metabolite output, underscore the intricate microbial landscape linked to CRC susceptibility. Integrating microbiome and metabolomic signatures provides a promising non-invasive framework for early CRC risk assessment and individualized screening strategies.

Back

The Cyprus Intestinal Health Study (CIHS), Decoding Microbiome: Metagenomics & Metabolomics in Colorectal Cancer Prevention

Katsaounou K[1], Nicolaou E[2], Vrachnos D[3], Vogazianos P[3], Brown C[3], Laou S[1], Tsiaoussis G[5], Agapiou A[4], Antoniades A[3], Shammas C[2], Apidianakis Y[1]

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INTRODUCTION - BACKGROUND

Colorectal cancer represents a significant global health burden, with a concerning rise in early-onset cases [1]. In Cyprus, EOCRC has risen to approximately 6% of all cases within a decade [2], highlighting an urgent need for predictive, non-invasive risk assessment tools capable of detecting sub-clinical alterations before visible morphological lesions develop. A wide range of risk factors is linked to CRC, including environmental and genetic risk factors. Gut microbiota emerges as a major risk factor for CRC development, dynamically interacting with the previous factors. The identification of early predictive multi-omics susceptibility biomarkers, can shift CRC prevention from advanced colorectal neoplasia detection toward personalized, predictive risk stratification and targeted lifestyle adjustments or precision therapeutic strategies in Cyprus and worldwide.

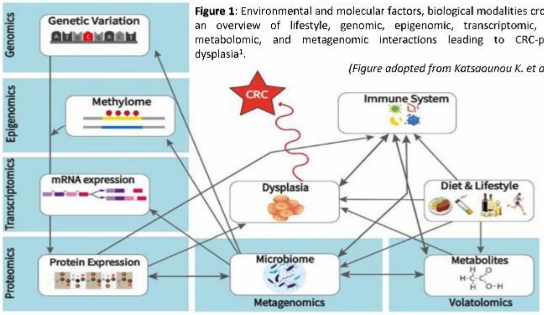


Figure 1: Environmental and molecular factors, biological modalities cross-linked in an overview of lifestyle, genomic, epigenomic, transcriptomic, proteomic, metabolomic, and metagenomic interactions leading to CRC-predisposing dysplasia[1].

(Figure adopted from Katsaounou K. et al., 2022 [3])

METHODS AND AIMS OF THE STUDY

❖ ***Cyprus Intestinal Health Study (CIHS)*** aims to deepen our understanding of the fecal and intestinal mucosal microbiota, as well as fecal VOCs, and to characterize their differences between **NLRA** and **HRA** and the sub-groups of **NA** vs **LRA** and **P-HRA** vs **D-HRA** Cypriot residents, by analyzing samples from an overall cohort of 268 NLRA and 36 HRA non-malignant tumor-bearing individuals per *Lieberman et al.,* 2012 criteria using:

- **16S rRNA gene Sequencing Analysis** of fecal and luminal bacterial DNA
- **HiSorb sorptive (GC/MS) Analysis** of fecal samples
- Gut Microbiome – VOC metabolome **Interaction Analysis**
- **Functional Potential Analysis utilizing PICRSt2 and volatolomics-derived data**

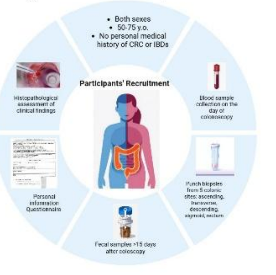
RESEARCH HYPOTHESIS

New and more reliable CRC risk biomarkers can be found by comparing fecal and colonic microbiota and linking fecal microbiota to co-existing fecal volatile organic metabolites.

CENTRAL AIM

By identifying candidate microbial and metabolic biomarkers, ***CIHS*** aims to reveal integrative, personalized omics signatures of CRC susceptibility by contributing to improved CRC risk stratification for future clinical applications by addressing the gap that conventional screening modalities (FIT, FOBT, and Cologuard) are fundamentally reactive, detecting active bleeding or cellular shedding rather than underlying predisposition.

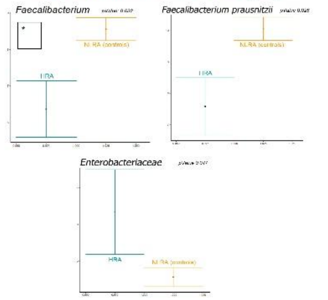
Figure 2: Schematic of Participants' Recruitment in the Cyprus Intestinal Health Study.



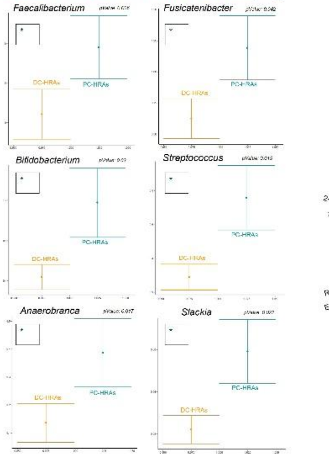
RESULTS – STATISTICAL ANALYSIS

Figure 3: Relative abundance of fecal bacteria showing significant differences between **a. HRA and NLRA** and **b. LRA and NA** groups, and luminal bacteria between **c. P-HRA and D-HRA** individuals. Significance was determined using the Mann–Whitney U test followed by a permutation test (10,000 permutations) to derive empirical p-values, adjusted for multiple testing using the Benjamini–Hochberg FDR approach. Graphs marked with (*) were statistically significant with both tests.

2a. Fecal microbiome in HRA Vs NLRA group



2c. Luminal microbiome in P-HRA Vs D-HRA group



2b. Fecal microbiome in LRA Vs NA group

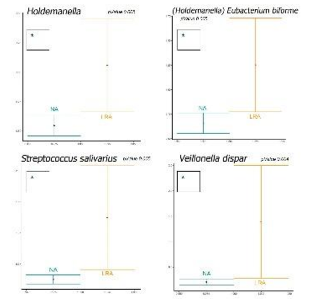
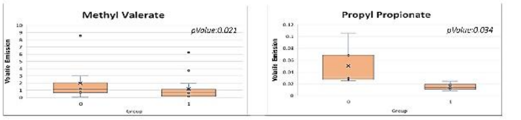


Figure 4: Relative abundance of **VOCs**, derived from Hi-Sorb analysis displaying significant differences between **25 NA and 25 HRA individuals**. The mean-value is indicated by the horizontal line dividing each box in two. The top and the bottom of the box indicate the mean-value plus/minus the standard error. Dots display data points. (Group O = NLRA, Group 1 = HRA).



DISCUSSION & PROPOSED MODEL

1. **The Early Runway: Sub-Clinical "Metabolic Priming" in LRA**: Traditional CRC studies report a depletion of *S. salivarius* and *V. dispar* in advanced carcinomas due to a shift toward protein degradation. **Pioneer Colonizers:** We capture a critical, overlooked chronological window, proving these species act as active, high-utility pioneer colonizers in early, sub-clinical adenomas (1–2 small polyps) [4]. **Trophic Chain**: *S. salivarius* ferments carbohydrates to lactate, fueling the strict lactate-utilizer *V. dispar*. This couple drives a metabolic cascade (producing acetate/propionate), engineering the exact runway required for aggressive pathobionts to engraft [5].
2. **The HRA Deterioration Cascade & Hypoxic Collapse, The Keystone Depletion:** Progression to High-Risk Adenoma (HRA) triggers a severe decline in anti-inflammatory *Faecalibacterium prausnitzii*, despite a robust predicted upregulation of its ROS-detoxifying FMN reductase. **Metabolic Bottleneck:** A predicted reduction in acetate kinase compromises butyrate biosynthesis, stalling colonocyte mitochondrial *β*-oxidation and causing luminal oxygenation. **Volatolomics Validation:** Disrupted anaerobic fermentation is physically confirmed by the significant measured depletion of methyl valerate and propyl propionate (esters of odd-chain fatty acids).
3. **Opportunistic Takeover & The Dysbiotic Loop: *Enterobacteriaceae* Proliferation**: Luminal oxygenation creates a permissive niche rapidly exploited by facultative anaerobes. **Metabolic Aggression**: *Enterobacteriaceae* outcompete commensals via predicted upregulation of carbohydrate scavenging (PTS system, +3.80 logFC) and mixed-acid fermentation. **Self-Reinforcing Loop:** Multi-omics modeling shows a complete breakdown of protective metabolic couplings, replacing a cooperative ecosystem with a pro-inflammatory, tumor-permissive pre-cancerous niche [6].

Grants and funding
EXCELLENCE/1216/0523/Research and Innovation Foundation

Figure 5: Chord diagram illustrating significant interactions ((P (interaction) < 0.05) between **bacterial genera and VOC metabolites**. Only statistically significant and biologically relevant multiple interactions are shown to improve clarity and highlight key associations between microbial genera and VOC metabolites that differentiate **HRA** from **NLRA** individuals.

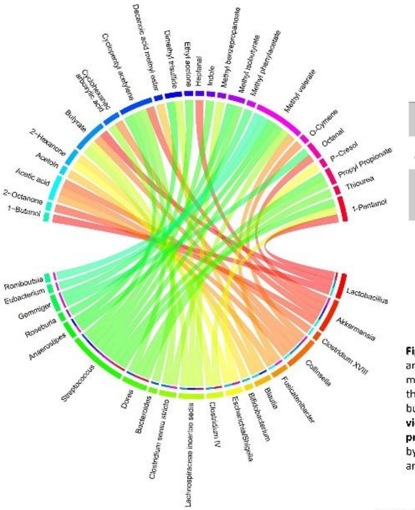
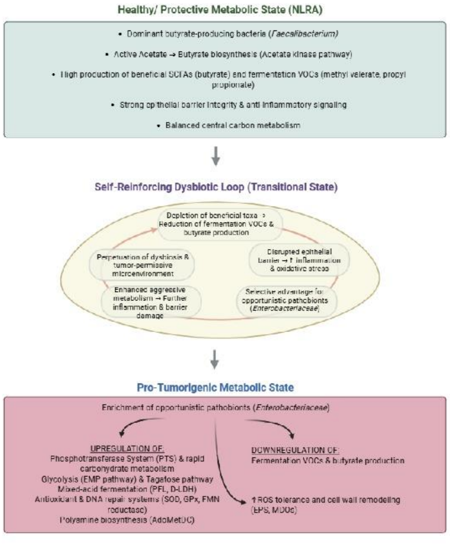


Table 1: Genus-level interactions with **propyl propionate and bacteria taxa**, showing very strong interaction effects with the gut microbiota of **HRA** individuals. Remarkably, the interaction effect between Veillonella and propyl propionate (7.89E+37) is one of the strongest positive interactions in the given dataset. Of note, **methyl valerate** showed several positive and negative interactions with both commensals and pathobionts

GENUS	VOC METABOLITE	INTERACTION ODDS RATIO (IOR) *(P VALUE)*	INTERACTION EFFECT
Dorea	Propyl propionate	8.14E-13 (0.0339)	Very strong protective
Streptococcus	Propyl propionate	2.86E+02 (0.0383)	Very strong disease risk
Veillonella	Propyl propionate	7.89E+37 (0.0101)	Extremely strong disease risk

Figure 7: Schematic representation of metabolic rewiring and the self-reinforcing dysbiotic loop in the gut microbiome of **HRA** individuals. The upper panel illustrates the **protective metabolic state** dominated by beneficial, butyrate-producing bacteria. The central loop depicts the **vicious cycle driving dysbiosis.** The lower panel shows the **pro-tumorigenic metabolic reprogramming** characterized by aggressive carbohydrate utilization, stress resistance, and gut inflammation.



Healthy/ Protective Metabolic State (NLRA)

- Dominant butyrate-producing bacteria (*Faecalibacterium*)
- Active Acetate → Butyrate biosynthesis (Acetate kinase pathway)
- High production of beneficial SCFAs (butyrate) and fermentation VOCs (methyl valerate, propyl propionate)
- Strong epithelial barrier integrity & anti inflammatory signaling
- Balanced central carbon metabolism

Self-Reinforcing Dysbiotic Loop (Transitional State)

Depletion of beneficial taxa → Reduction of fermentation VOCs & butyrate production

Disrupted epithelial barrier → ↑ inflammation & oxidative stress

Selective advantage for opportunistic pathobionts (*Enterobacteriaceae*)

Enhanced aggressive metabolism → Further inflammation & barrier damage

Perpetuation of dysbiosis & tumor-permissive microenvironment

Pro-Tumorigenic Metabolic State

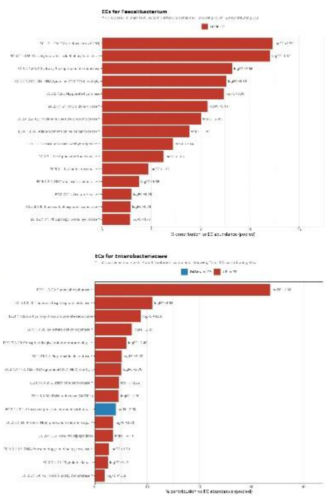
Enrichment of opportunistic pathobionts (*Enterobacteriaceae*)

UPREGULATION OF:
Phosphotransferase System (PTS) & rapid carbohydrate metabolism
Glycolysis (EMP pathway) & Tagatose pathway
Mixed-acid fermentation (PFL, D-LDH)
Antioxidant & DNA repair systems (SOD, GPx, FMN reductase)
Polyamine biosynthesis (AdoMetDC)

DOWNREGULATION OF:
Fermentation VOCs & butyrate production

↑ROS tolerance and cell wall remodeling (EPS, MDOs)

Figure 6: Top 15 functional pathways, in ***Faecalibacterium*** **(above)** and ***Enterobacteriaceae*** **(below).** logFC = $\log_2$ fold change in predicted enzyme abundance in HRA vs. NLRA groups. Positive values indicate upregulation in the HRA group. The percentage contribution of each taxa to specific enzymes reflects its predicted functional importance within the microbial community. Positive values indicate upregulation in the HRA when compared with NRLA group, while negative values indicate downregulation in the HRA group.



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Poster Session of the Barcelona Debates on the Human Microbiome, June 2026

50. Diurnal Gut Brain Communication in Alzheimer's Disease

Adriana Soria Villalba[1]

[1] *Hospital del Mar Research Institute, Cancer Research Programme, Barcelona, Spain*

Alzheimer’s disease (AD) is a devastating neurodegenerative process. Altered circadian clock-controlled behavioural processes such as a disturbed sleep–wake cycle, as well as functional deterioration in the Suprachiasmatic nucleus (SCN), home of the central clock, have been associated with AD. Decline of central clock function in the SCN of AD patients and alterations in the signalling pathways downstream of the SCN likely result in a weakening of systemic circadian clock network integrity. AD is increasingly perceived to not only affect the brain, but also to impact on peripheral tissue physiology. In turn, peripheral tissues can modulate the pathology in the brain. This is particularly true for the intestine and its role in the microbiota – gut – brain axis. AD patients develop dysbiosis at early stages of the disease and transplantation of AD-related microbiota propagates AD-like symptoms to healthy recipients, suggesting that the microbiota contributes to the development of AD pathology. Importantly, abundance, location and composition of the intestinal microbiota follows a diurnal rhythm, which is regulated by and feeds back into the host circadian clock network. Whether deteriorating circadian rhythmicity in the microbiota – gut – brain axis contributes to AD development is not known. Following a multi-omic approach, combining metagenomic, metabolomic, and transcriptomic analyses, we study how AD pathology is altering circadian rhythmicity along the microbiota-gut-brain axis in a sex-specific manner in 5xFAD mouse model. After performing faecal metagenomics around the clock, we found that the 5xFAD mice develop dysbiosis with changes in the composition of the two most common taxa, Bacteroidetes and Firmicutes; among others, compared with wild type mice. Besides, 5xFAD’s microbiota exhibits different rhythmicity in comparison with wild type mice. Finally, we also discovered sex-dependent alterations in the microbiota-regulated diurnal serum metabolism.

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Diurnal gut brain communication in Alzheimer's Disease

Soria Villalba A[1], Hermán Aroz P[1], Hrebinchuk V[1], Ramon Duaso C[2], Pérez Viltre GV[1], Alechaga Silva É[2], Pozo Mendoza O[2],
Busquets Garcia A[2], Welz PS[1]
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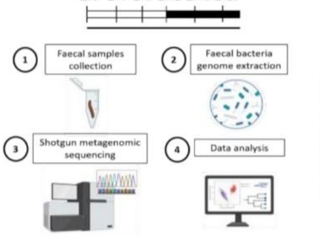
Alzheimer's disease (AD) is a devastating neurodegenerative process. AD has been associated with deterioration of the central clock, located in the Suprachiasmatic nucleus (SCN) of the brain, and this decline likely result in a weakening of the whole systemic circadian clock network integrity. AD is increasingly perceived to not only affect the brain, but also to impact on peripheral tissue physiology. In turn, peripheral tissues can modulate the pathology in the brain. This is particularly true for the intestine and its role in the microbiota – gut – brain axis. AD patients develop dysbiosis at early stages of the disease and transplantation of AD-related microbiota propagates AD-like symptoms to healthy recipients, suggesting that the microbiota contributes to the development of AD pathology. Importantly, abundance, location and composition of the intestinal microbiota follows a diurnal rhythm, which is regulated by the host circadian clock network. Whether deteriorating circadian rhythmicity in the microbiota – gut – brain axis contributes to AD development is not known. Following a multi-omic approach, combining metagenomic, metabolomic, and transcriptomic analyses, **we study how AD pathology is altering circadian rhythmicity along the microbiota-gut-brain axis in the 5xFAD mouse model.**

1a



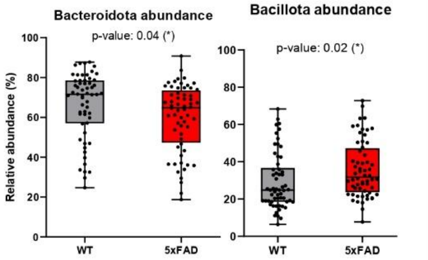
Faecal samples collection

Faecal bacteria genome extraction

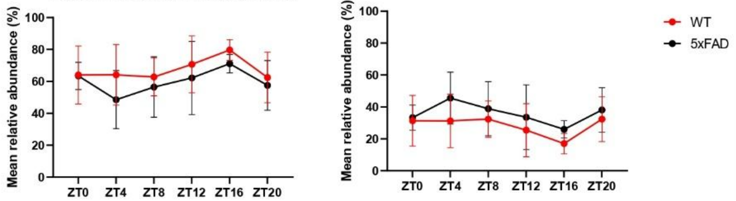
Shotgun metagenomic sequencing

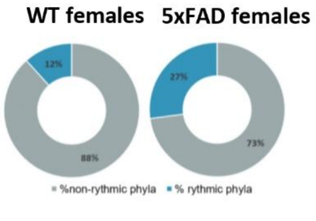
Data analysis

1b

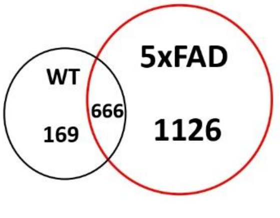


1c





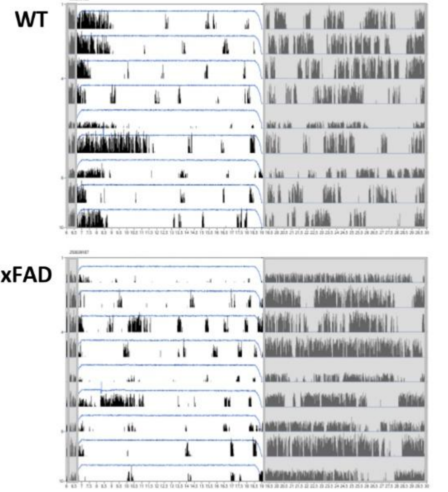
1d



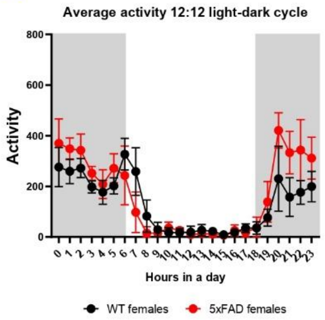
Experimental setup for establishing the diurnal microbiome by shotgun metagenomics (1a). Comparison between *wild type* and 5xFAD females of the relative abundance of the two most abundant phyla (1b). Percentage of rhythmic and non-rhythmic phyla by genotype with the time-point series of the two most abundant phyla (1c). Venn diagram of genes differentially expressed between day and night in the intestinal epithelium of both genotypes (1d).

1. 6 month-old 5xFAD female mice present increased intestinal microbiota's rhythmicity and alterations in its composition.

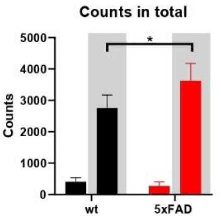
2a



2b



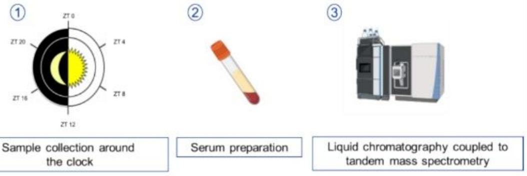
2c



Actograms of *wild type* and 5xFAD female mice under a 12:12 light-dark cycle (2a). Average activity along the day (2b). Counts in total along 8 days during the active and resting phase (2c).

2. 5xFAD female mice are hyperactive, specially during their active phase.

3a

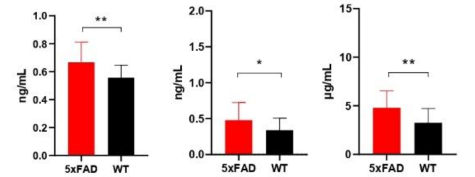


Sample collection around the clock

Serum preparation

Liquid chromatography coupled to tandem mass spectrometry

3b



Experimental setup for establishing diurnal serum metabolism (3a). Quantification of SCFAs in female mice (3b).

3. Altered daily microbiota-related serum metabolism in 5xFAD mice correlates with metagenomics.

CONCLUSIONS

- Correlating alterations in microbiome composition and microbiota-related serum metabolites in 6 month-old 5xFAD female mice.
- 6 month-old 5xFAD female mice present increased rhythmicity in the intestinal clock (IECs) and in daily microbiome rhythmicity.
- 6 month-old 5xFAD female mice are hyperactive, with increased daily behavioural rhythms.

51. Circadian Disruption in the Colorectal Cancer Tumour Environment

George Víctor Pérez Viltre[1], Pilar Aroz Hermán[1], Adriana Soria Villalba[1], Eulàlia Gómez Armengol[1], Veronika Hrebinchuk[1], Debayan Datta[1], Élida Alechaga Silva[1], Óscar Pozo Mendoza[1], Patrick Welz[2]

[1] Hospital del Mar Research Institute Barcelona (HMRIB), Barcelona, Spain; [2] Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Background: Disruption of circadian rhythmicity, as occurs during shift work, has been linked to an increased risk to develop colorectal cancer. What's more, circadian rhythmicity is disturbed in tumours and disruption of circadian clock function in colorectal cancer cells promotes tumour growth. Importantly, the host circadian clock network is a central regulator of host-microbiota crosstalk. However, it is neither known how colorectal cancer might impact on daily host-microbiota interaction, nor how disturbed diurnal communication between the host and the
microbiome might promote disease progression.
Methods: We have applied a multi-omics approach combining metagenomics, metabolomics, and transcriptomics for determining how tumours alter circadian rhythmicity in their environment in a carcinogen-induced colorectal cancer mouse model. All experiments involving animals have
been approved by the responsible ethical committee and adhere to the relevant legislation.
Results: We find that colorectal cancer alters daily host-microbiota crosstalk, which has implications on the tumour metabolome and on the transcriptome in the tumour environment. How disturbed communication between the tumour and the microbiome around the clock might impact on the
progression of the disease will be discussed.
Conclusions: Colorectal cancer alters circadian regulation of host-microbiota crosstalk with far reaching effects on the tumour environment, which likely promotes disease progression

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Circadian Disruption in the Colorectal Cancer Tumour Environment

Pérez Viltre GV. [1], Hermán Aroz P. [1], Soria Villalba A. [1], Gómez Armengol E. [1], Hrebinchuk V. [1], Datta D. [2], Alechaga Silva É. [3], Pozo Mendoza Ó. [3], and Welz PS [1]

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Disruption of circadian rhythmicity, as occurs during shift work, has been linked to an increased risk to develop colorectal cancer. What's more, circadian rhythmicity is disturbed in tumours and disruption of circadian clock function in colorectal cancer cells promotes tumour growth. Importantly, the host circadian clock network is a central regulator of host-microbiota crosstalk. However, it is neither known how colorectal cancer might impact on daily host microbiota interaction, nor how disturbed diurnal communication between the host and the microbiome might promote disease progression. We have applied a multi-omics approach combining metagenomics, metabolomics, and transcriptomics for determining how tumours alter circadian rhythmicity in their environment in a carcinogen-induced colorectal cancer mouse model. All experiments involving animals have been approved by the responsible ethical committee and adhere to the relevant legislation. We find that colorectal cancer alters daily host-microbiota crosstalk, which has implications on the tumour metabolome and on the transcriptome in the tumour environment. How disturbed communication between the tumour and the microbiome around the clock might impact on the progression of the disease will be discussed. **Colorectal cancer alters circadian regulation of host-microbiota crosstalk with far reaching effects on the tumour environment, which likely promotes disease progression.**

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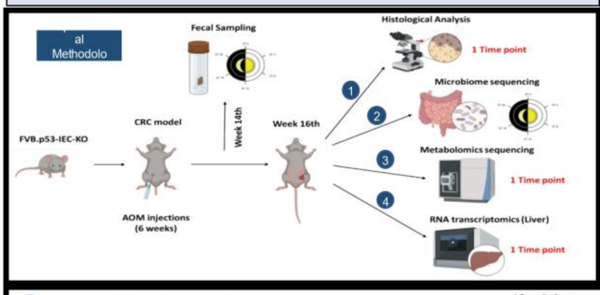
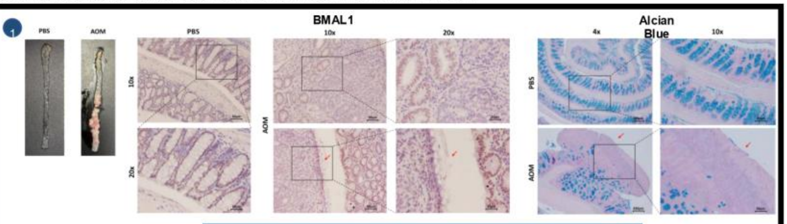
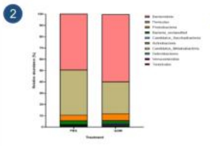
Methodolo

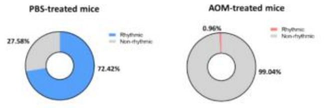




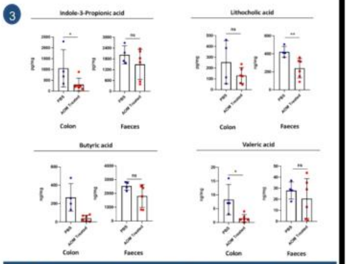
1. Tumoral regions had circadian disruption and were mostly absent for mucus



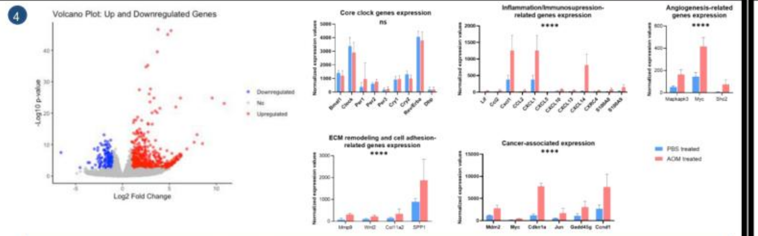
Phylum	PBS	AOM	
Bacteroidota	49.26	59.77	*
Firmicutes	40.09	28.29	*
Proteobacteria	4.94	5.99	
Bacteria_unclassified	3.19	2.38	
Candidatus_Saccharibacteria	0.69	0.79	
Actinobacteria	0.65	0.59	
Candidatus_Melainabacteria	0.21	0.84	
Deferribacteres	0.31	0.57	
Verrucomicrobia	0.00	0.78	*
Tenericutes	0.66	0.00	*



2. Microbiota of mice with CRC had alterations in abundancy, composition and rhythmicity

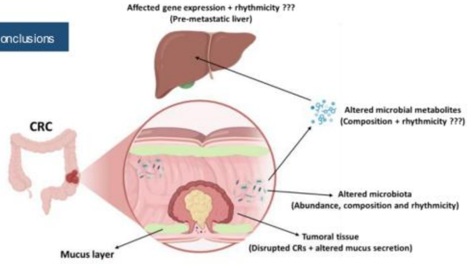


3. Microbial metabolites composition was abnormal in mice with CRC



4. The liver of AOM-treated mice showed an hepatic pre-metastatic RNA profile

Preliminary Conclusions



Universitat Pompeu Fabra Barcelona
Fundación BBVA
Generalitat de Catalunya

BEST ABSTRACT

52. Elucidating the Microbiome Metabolism of Sex Hormones and its Potential Impact on Host Health

Viviana Rossi[1], Maider Fernández-de-Mendiola[1], Albert Garcia-Valiente[1], Mireia Vallès-Colomer[1]

[1] MELIS Department, Universitat Pompeu Fabra (UPF), Barcelona, Spain

Sex hormones are bioactive molecules whose influence goes beyond reproductive health. Strains in the microbiome have been reported to be involved in the network of both endogenous and exogenous sex hormones biotransformations. These reactions have been described in a limited number of organisms, whereas a broader range is expected to hold the potential to perform sex hormone biotransformations and influence systemic sex hormone levels. Characterizing these microbial biotransformations is thus crucial for understanding the positive and negative roles of the gut and reproductive tract microbiomes in sex hormone-sensitive host health.

We developed SHM (Sex Hormone Modules), an open-source computational tool for predicting microbiome contributions to hormone regulation. SHM includes >60 modules spanning >70 biochemical reactions involved in the conjugation, deconjugation, synthesis, and degradation of endogenous and exogenous estrogens, progestogens, and androgens (including phytoestrogens and synthetic hormonal medications). In this way, it predicts whether a given strain or microbial community can biotransform each hormone, thereby increasing or decreasing systemic bioavailability and potency. We validated SHM on causal experimental evidence of microbial genes being responsible for specific enzymatic activities, demonstrating high accuracy. Next, we applied SHM to a comprehensive database of >1.6M bacterial and archaeal genomes from 179k different species, establishing the first comprehensive catalogue of microbial sex hormones metabolism. Furthermore, applying SHM to metagenomic sequencing data, we characterized differences in sex hormone biotransformation potential between males and females.

Overall, SHM is advancing our understanding of the microbiome's contribution to sex hormone metabolism, informing mechanistic research and clinical applications in physiological and pathological sex hormones-dependent conditions.

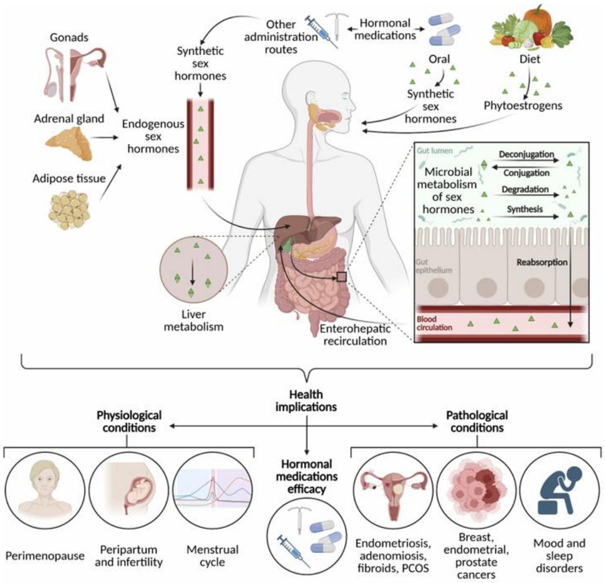


Figure 1: Graphical representation of the metabolism of sex hormones by the gut microbiome and its implications on host health. Endogenous sex hormones are produced in the gonads, adipose tissue, and adrenal glands, they travel in the blood circulation and undergo liver metabolism to be excreted with feces. In the gut lumen, they can undergo microbial biotransformation (conjugation, deconjugation, synthesis, and degradation), influencing their reabsorption via enterohepatic recirculation and systemic activity. Similarly, exogenous sex hormones (synthetic sex hormones used as medication and phytoestrogens) are introduced orally and undergo gut microbial metabolism, influencing their absorption and systemic functions. Hormonal medications that are administered via other routes leading to systemic circulation also reach the gut only after hepatic metabolism. Overall, endogenous and exogenous sex hormone metabolism can influence physiological and pathological health conditions (main examples listed in the figure) and the efficacy of hormonal medications, with major implications for healthcare. Figure created with BioRender.

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53. Host Genetics Shapes Mucin Niche Colonisation by Keystone Gut Bacteria, Influencing Metabolic Health

Felipe Morillo Sanz Dias[1]

[1] *Centre for Genomic Regulation (CRG), Barcelona, Spain*

A “polygenic-first” design for causal inference was adopted to study the caecal microbiome of HS rats, combining shallow-shotgun metagenomics with linear-mixed models to map host polygenic effects on multi-level microbiome traits. A reproducible, shallow shotgun-optimised pipeline yielded better results for detecting such effects than 16S, with heritable signals concentrating in Bacteroides and Prevotella, their shared guild, and β-diversity. Then, genome-resolved enrichments suggested potential adaptations to the crypt niche, leading to a hypothesis-driven selection of host genes. A cross-validated set of sentinel SNPs for these candidate genes explained genus-level polygenic effects and served as instruments in mediation and Mendelian randomisation analyses, contributing to a structural equation model that linked keystone bacteria, community structure, and host metabolism. The effects of these genera on fasting glucose levels had opposite signs and were balanced by indirect effects routed through community traits, suggesting that genotype-aware, niche-engineering interventions could better modulate glycaemia and other health-related traits.

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ABSTRACTS BY TOPIC

Microbiome Ecology

54. Microbes in Motion: Strain-Level Insights into the Social Transmission of the Gut Microbiome in Rats.

Kauthar Omar[1]

[1] *Centre for Genomic Regulation (CRG) / Universitat Pompeu Fabra (UPF), Barcelona, Spain*

The gut microbiome plays a crucial role in its host's health, influencing physiology, immunity, and disease susceptibility. The microbiome is seeded at birth by the mother, after which additional microbes are acquired primarily from social partners, and to a lesser extent from food and the environment. In humans and mice, it has been shown that individuals in close contact share some of their gut microbiome. Such microbial transmission can contribute to both disease transmission and the acquisition of disease resistance, highlighting the need to better understand microbial transmission dynamics.

This study aims to quantify and characterize the social transmission of commensal microbes in a laboratory rodent model. We analyzed cecal and fecal microbiomes from a large Heterogeneous Stock (HS) rat cohort (n = 2,540), alongside two negative control datasets comprising rats with no contact with the main population; BXH rats (n = 114) and rats from a high-fat diet study (n = 59). Animals were co-housed in groups of 2–3 per cage for 2–6 months. Microbiomes were profiled at species and strain levels using MetaPhlAn 4.1.1 and StrainPhlAn 4.1.1 with the ChocoPhlAn vJan25 database, and single nucleotide variants were analyzed to capture fine-scale diversity.

Incorporation of HS rat data into the ChocoPhlAn vJan25 database resulted to a mean read mapping rates of 74.28%. Transmission patterns reflected the level of contact between individual rats, with cagemates showing the highest strain sharing, followed by siblings, while rats from different populations shared ~1% of strains. Strain sharing was species-specific, with some species, for example Bifidobacterium pseudolongum showing contact level associated transmission patterns and rat-associated Lachnospiraceae species (SGB212164) showing widespread across the HS population.

Overall, these findings demonstrate that microbiome transmission is contact-dependent and species-specific, providing insight into transmission dynamics.

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55. The Early-life Exposome and Gut Microbiota Composition and Metabolic Activity from Birth to 18 Months

Pol Jimenez-Arenas[1,2,3], Alejandro J. Alcañiz[4], Oscar Pozo[5], Maria Carmen Collado[6], Ioar Rivas[1,2], Xavier Basagaña[1,2,3], Mireia Vallès-Colomer[7], Payam Dadvand[1,2,3], Jordi Sunyer[1,2,3], Mariona Bustamante[1,2,3], Mireia Gascon[1,2,3], Dolors Pelegrí-Sisó[1,2,3], Álvaro Falcon-Gracia[1,2,3], Marta Cirach[1,2,3], Adrià Cruells[8], Zoraida Garcia[1,9,6], Cecilia Persavento[1,2,3], Élida Alechaga[10], Noemí Haro[10]

[1] ISGlobal, Barcelona, Spain; [2] Universitat Pompeu Fabra (UPF), Barcelona, Spain; [3] CIBER Epidemiología y Salud Pública (CIBERESP), Spain; [4] IATA-CSIC, Valencia, Spain; [5] HMRI, Barcelona, Spain; [6] IATA-CSIC (Food Science), Spain; [7] Centre for Genomic Regulation (CRG), Barcelona, Spain; [8] MELIS-UPF, Barcelona, Spain; [9] IDAEA-CSIC, Barcelona, Spain; [10] Institutional affiliation (Alechaga & Haro)

The infant gut microbiome (GM) is a key component of the human holobiont, with important roles in host physiology and health. Although host genetics and early-life factors such as delivery mode, breastfeeding, and antibiotic exposure shape GM assembly, a substantial proportion of inter-individual variability remains unexplained. The urbanome, capturing environmental exposures linked to urban living, represents a major yet understudied determinant of early-life GM. Here, we examine how the prenatal and early postnatal urbanome influences GM development across its early-life trajectory.We analysed 631 mother-infant pairs from the Barcelona Life Study Cohort (2019-2023), integrating longitudinal exposure data (air pollution, greenness, land use, and neighbourhood socioeconomic indices) with infant GM profiling at 6 and 18 months. Gut microbiome composition and function were characterized using shotgun metagenomics, complemented by faecal short-chain fatty acid (SCFA) quantification. Analyses accounted for time-varying confounding, missing data, and differential attrition.

Although smaller in magnitude than established early-life factors, the urbanome explained a modest but statistically significant fraction of GM variation, with R^2 values ranging from 0.004 to 0.008 across exposures and time points. At 6 months, surrounding natural green space and neighbourhood income inequality were associated with taxonomic composition. At 18 months, prenatal exposure to PM2.5 and NO_2, as well as postnatal greenness and residential proximity to green spaces, explained significant variation in taxonomic composition. Across both time points, we also identified associations between urbanome exposures and specific microbial species, functional pathways, and SCFA profiles.These findings highlight modifiable urban exposures as determinants of microbiome trajectories and suggest a mechanism linking the urbanome to health, reinforcing their consideration in public health strategies.

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The urban exposome and gut microbiota in early life: a longitudinal birth cohort study

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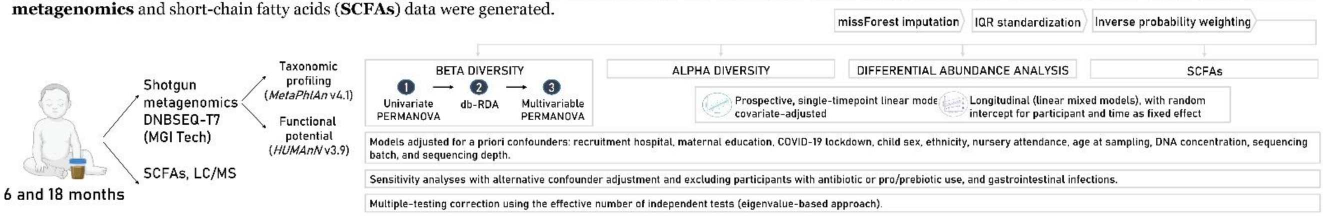
Jimenez-Arenas P *, Pelegrí-Sisó D, Falcon-Gracia Á, Cirach M, Cruells A, García-Ruiz Z, Persavento C, Alechaga É, Haro N, J. Alcañiz A, Pozo O, Collado MC, Rivas I, Basagaña X, Valles-Colomer M, Dadvand P, Sunyer J, Bustamante M, Gascon M

BACKGROUND

The gut microbiota is a key interface between environmental exposures and host physiology. Although genetics contribute to microbiome structure, environmental factors play a major role in shaping its development during early life. Established determinants such as breastfeeding, weaning, and medication use explain only a limited proportion of microbiota variability, highlighting the need to consider broader environmental influences. Urban environments, which now host most of the world's population, encompass multiple exposures —including **air pollution, green spaces, surrounding greenness, and socioeconomic status** (SES)— that may affect microbiota maturation. However, the impact of the urban exposome on the gut microbiota remains poorly understood. We investigated the **effects of the urbanome on gut microbiota** composition, functional potential, and metabolic activity.

METHODS

We conducted a prospective longitudinal analysis within the population-based **Barcelona Life Study Cohort (N = 1,080)**. Urban-related exposures, including air pollution, green spaces, surrounding greenness, and census tract socioeconomic status were assessed from conception to 18 months, in addition to 147 non-urbanome variables. **Faecal samples were collected at 6 and 18 months**, coinciding with the developmental and transitional stages of gut microbiota maturation, from which faecal **shotgun metagenomics** and short-chain fatty acids (**SCFAs**) data were generated.



missForest imputation → IQR standardization → Inverse probability weighting

6 and 18 months

Shotgun metagenomics DNBSEQ-T7 (MGI Tech)
- Taxonomic profiling (*MetaPhlAn* v4.1)
- Functional potential (*HUMAnN* v3.9)

SCFAs, LC/MS

BETA DIVERSITY: ① Univariate PERMANOVA → ② db-RDA → ③ Multivariable PERMANOVA

ALPHA DIVERSITY

DIFFERENTIAL ABUNDANCE ANALYSIS

SCFAs

Prospective, single-timepoint linear mode covariate-adjusted

Longitudinal (linear mixed models), with random intercept for participant and time as fixed effect

Models adjusted for a priori confounders: recruitment hospital, maternal education, COVID-19 lockdown, child sex, ethnicity, nursery attendance, age at sampling, DNA concentration, sequencing batch, and sequencing depth.

Sensitivity analyses with alternative confounder adjustment and excluding participants with antibiotic or pro/prebiotic use, and gastrointestinal infections.

Multiple-testing correction using the effective number of independent tests (eigenvalue-based approach).

RESULTS

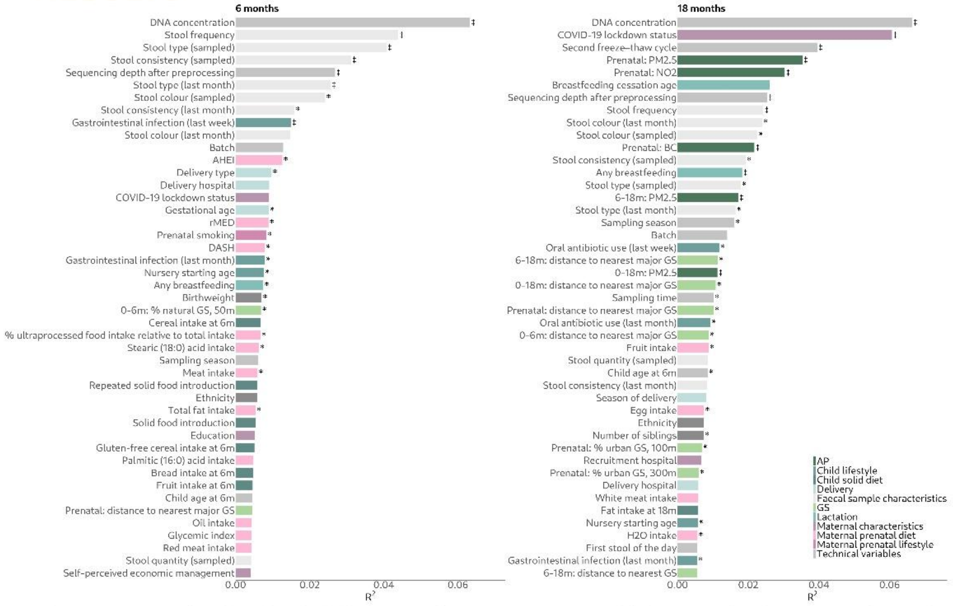
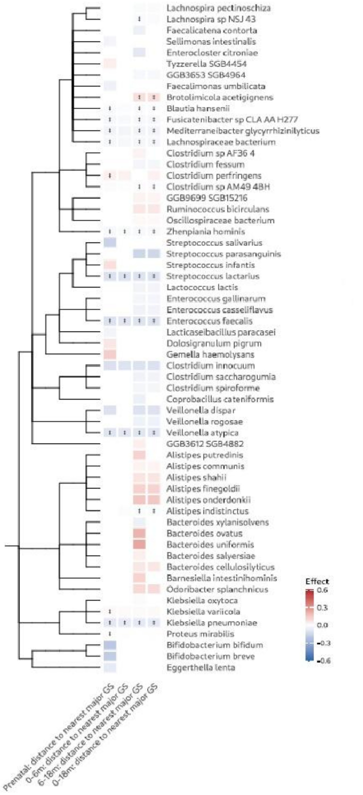


Figure 1. Variance explained (R^2) by individual exposures in bivariate PERMANOVA models of taxonomic beta diversity at 6 and 18 months.

Figure 2. Heatmap of longitudinal associations between urbanome exposures and gut microbiota taxonomic composition. Cells represent effect estimates (β).



CONCLUSIONS

1. The **urbanome explains a modest but significant proportion of gut microbiome variance**, with green spaces and air pollution being the most consistent urbanome correlates.
2. Green space exposure directly associates with taxa linked to later maturation of the gut microbiota, and inversely to early colonisers and facultative anaerobes.
3. Air pollutants, particularly PM2.5, associates with enrichment of oral-associated taxa and reduced beneficial anaerobes, such as Bifidobacterium.
4. Effects become **more evident at the transitional phase** of gut microbiota development.
5. Functional and metabolite-level associations are less consistent than taxonomic signals.

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56. Female Microbial Dynamics at Delivery and During 1 Year-postpartum: Insights from Gut, Oral and Vaginal Niches

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The microbiome is central to female's health. Physiological, immune, and metabolic shifts during key reproductive stages, including pregnancy, lactation, and menopause, can alter microbial composition and function, thereby influencing health outcomes. Despite their importance, the dynamics of the female microbiome during the postpartum period remain poorly characterized. Therefore, the aim of this study was to characterize the diversity and composition of the gut, oral, and vaginal microbiota at delivery, and to longitudinally assess gut microbiota dynamics throughout the first year postpartum.

Participants were enrolled in the MAMI cohort, and fecal samples (n= 242) were collected longitudinally at delivery, 7 days, 1 month, and 1 year postpartum, while oral (n= 98) and vaginal (n=100) samples were obtained at delivery. Microbiota profiles were determined by sequencing of the V3-V4 region of the 16S rRNA gene. Perinatal and postnatal factors were recorded as well as maternal-infant clinical metadata.

At delivery, females exhibited distinct microbial communities across the evaluated body sites. Bacteroides, Prevotella, Faecalibacterium and Subdoligranulum dominated gut microbiota, whereas oral and vaginal communities showed niche specific microbial signatures. Across the postpartum period, female gut microbiota diversity and composition showed overall stability up to 1 year after birth. The genera with the highest relative abundances included Blautia, Bifidobacterium, Subdoligranulum y Clostridia_UCG. Additionally, environmental exposures, mode of delivery, and intrapartum antibiotic administration significantly contributed to observed microbial variation.

This study provides an overview of the composition of the female microbiota at delivery and how it changes during the first year postpartum, emphasizing key drivers, and providing a starting point for future studies exploring the links between postpartum microbiota changes and maternal

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