THE BARCELONA DEBATES ON THE HUMAN MICROBIOME ››› 2025

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ABSTRACT BOOK

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ABSTRACT BOOK

We are pleased to present the abstract book of the 11th edition of **The Barcelona Debates on the Human Microbiome**, an international meeting focused on the latest advances in microbiome research. This year, a total of 47 abstracts were accepted, showcasing innovative work from researchers around the world.

The abstracts were evaluated through a blind, peer-reviewed process conducted by the Scientific Committee composed of 12 members. This rigorous process ensures that the selected abstracts are of the highest scientific quality, contributing to a deeper understanding of the human microbiome in health and disease.

In addition, the expert panel has selected **six outstanding abstracts that will have the opportunity to be included in the programme through oral presentations**. Among these, one will be awarded the Best Abstract Award, in recognition of exceptional scientific merit and potential impact.

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ORAL PRESENTATIONS

Clinical Associations

OP01

Microviridae Bacteriophages Influence Behavioral Hallmarks Of Food Addiction Via Tryptophan And Tyrosine Signalling Pathways

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Background: Food addiction significantly contributes to the obesity pandemic. However, treatment remains challenging due to its behavioural nature, and no targeted nutraceutical or pharmacological solutions exist. Growing evidence links the gut microbiota to obesity and food addiction, yet the specific mechanisms remain largely unknown, particularly in humans. Moreover, despite viruses being the most abundant biological entities, their role in the intestinal microbiota has been largely neglected.
Methodology: We applied a systems biology approach integrating microbiome analyses (faecal shotgun metagenomics), plasma metabolomics, brain magnetic resonance imaging (MRI), and neuropsychological assessment across four human cohorts (n=88, n=29, n=942, n=835) with pre-clinical models (mice, Drosophila melanogaster) to gain deeper insights into microbiome's role in food addiction.
Results: We identified Microviridae bacteriophages, particularly Gokushovirus WZ-2015a, as being associated with food addiction, food addiction-related phenotypic traits, and obesity across multiple cohorts. Functional analyses revealed that food addiction was closely linked to microbial pathways involved in aromatic aminoacid metabolism, with Gokushovirus WZ-2015a influencing tryptophan and tyrosine metabolism, the precursors of serotonin and dopamine. Faecal microbiota and viral transplantation from human donors with high Gokushovirus WZ-2015a load induced food addiction in recipient mice, alongside alterated tryptophan, tysorine, and dopamine metabolism in the nucleus accumbens (Nac) and dorsal striatum (DS), as well as downregulation of the dopamine receptors in the prefrontal cortex (PFC). Mechanistically, lower concentrations of anthranilic acid (AA), a tryptophan-related metabolite, were linked to higher Gokushovirus WZ-2015a levels. AA supplementation in mice reduced food addiction behaviors, altered tryptophan and tyrosine metabolism in the Nac and DS, and influenced the release cycle of several neurotransmitters in the PFC, including dopamine, serotonin, GABA, and glutamate. In Drosophila, AA regulated feeding behavior, addiction-like ethanol preference, and body weight by modulating the dopaminergic system.
Conclusions: Our findings reveal that gut bacteriophages play a crucial role in regulating food addiction by modulating tryptophan and tyrosine metabolism, ultimately influencing neurotransmitter pathways associated with addictive behaviours.

OP02

Multi-omics of the Intestine-Liver-Adipose axis in multiple Studies unveils a consistent link of the gut microbiota and the antiviral response with systemic glucose metabolism

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Background: In recent years, there has been growing recognition of the significant systemic effects of microbiota diversity and composition on the predisposition to obesity, type 2 diabetes, and metabolic syndrome. However, most studies investigating the connections between gut microbiota and insulin sensitivity have used single-omics approaches focused on specific tissues and limited to individual human studies.
Methods: We aim to elucidate the interplay between gut microbiota and insulin sensitivity across multiple tissues using an integrative multi-omics and multi-tissue approach across six independent cohorts. To achieve this, we combined euglycaemic clamp measurements (available in four of the six studies) with additional markers of glucose metabolism and insulin resistance, including glycated haemoglobin (HbA1c) and fasting glucose.
Results: Several genera and species from the Proteobacteria phylum were consistently negatively associated with insulin sensitivity in four studies (ADIPOINST, n=15; IRONMET, n=121, FLORINASH, n=67 and FLOROMIDIA, n=24). Transcriptomic analysis of the jejunum, ileum, and colon revealed T cell-related signatures positively linked to insulin sensitivity. Proteobacteria in the ileum and colon were positively associated with HbA1c but negatively with the number of T cells. Jejunal deoxycholic acid was negatively associated with insulin sensitivity. Transcriptomics of subcutaneous adipose tissue (ADIPOMIT, n=740) and visceral adipose tissue (VAT) (ADIPOINST, n=29) revealed T cell-related signatures linked to HbA1c and insulin sensitivity, respectively. VAT Proteobacteria were negatively associated with insulin sensitivity. Multiomics and multi-tissue integration in the ADIPOINST and FLORINASH studies linked faecal Proteobacteria with jejunal and liver deoxycholic acid, as well as jejunal, VAT and liver transcriptomic signatures involved in the actin cytoskeleton, insulin and T cell signalling. Fasting glucose was consistently linked to interferon-induced genes and antiviral responses in the intestine and VAT. Studies in Drosophila melanogaster validated these human insulin sensitivity-associated changes.
Conclusion: This study to offers comprehensive insights into the microbiome-gut (jejunum, ileum, and colon)-adipose-liver axis and its impact on systemic insulin action in the context of obesity and insulin resistance.

OP03

Unveiling the Porphyromonadaceae-TFF1 and Xanthomonadaceae-ITGAM interaction as Critical Factor in Post-Operative Recurrence of Crohn's Disease: A Transcriptome-Bacteriome Integrative Perspective

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Introduction: Crohn's disease (CD) is a chronic inflammatory condition of the gastrointestinal tract with high postoperative recurrence (POR) rates—up to 90% within a year—despite intestinal resection. Existing clinical tools lack predictive accuracy for recurrence, highlighting the need for molecular and microbial biomarkers. We performed multiomic integrated transcriptomic and bacteriomic analyses of paired inflamed and uninflamed ileocecal tissues from CD patients, stratified by POR status, to identify early predictive signatures and mechanisms of relapse.
Methods: Ileal tissue samples were collected during surgery from 20 CD patients and 10 CD-free controls, with a validation cohort of 54 additional patients. Samples included both inflamed and uninflamed regions. POR was assessed every six months via ileocolonoscopy using the Rutgeerts score. Tissue-based gene expression and microbiome profiles were integrated through correlation analysis and pathway enrichment to identify key host–microbe interactions and pathways involved in disease POR.
Results: We identified a novel immune interaction within the inflamed mucosa of recurrent CD patients involving Xanthomonadaceae family, specifically Stenotrophomonas maltophilia, and ITGAM, a gene involved in neutrophil activity revealed by pathway enrichment analyses. Although Xanthomonadaceae abundance was not significantly altered, its correlation with ITGAM expression was uniquely observed in inflamed tissue from recurrent patients. Furthermore, the Porphyromonadaceae family, predominantly Parabacteroides gordonii, was markedly depleted in the same patient group and mucosal pathological region. This loss was linked to a downregulation of critical barrier and repair genes such as TFF1 and LSR and corroborated in a validation cohort. Moreover, Porphyromonadaceae abundance positively correlated with short-chain fatty acid (SCFA) levels, particularly propionate. Together, these findings suggest a protective role for Porphyromonadaceae in maintaining mucosal integrity and modulating host immune response.
Conclusion: Collectively, our data reveal distinct microbial–host gene interactions associated with POR. The pathogenic immune activation signature linked to Xanthomonadaceae–ITGAM, and the protective role of Porphyromonadaceae-TFF1 and SCFA provide a foundation for microbiome-informed prognostics and targeted therapeutic strategies in CD recurrence.

OP04

Gut Microbiota Variation Modulates Polyamine Production In The Context Of Multiple Sclerosis

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Polyamines are small polycationic molecules synthesised by both the human gut microbiota and host cells. Certain polyamines, known as biogenic amines (BAs), play crucial roles in host physiology, including cell proliferation, differentiation, DNA damage repair, and immune modulation (PMID:30859104). Specifically, gut microbiota-derived BAs have been previously associated with the maintenance of the intestinal barrier (PMID:33151120), the enhancement of the immunological response against tumours (PMID:33833232) and the modulation of T-lymphocyte differentiation (PMID:32407834).
In this study, we characterize gut microbiota metabolism of polyamines, and the potential link between BAs and the autoimmune disease multiple sclerosis. We analyzed shotgun metagenomic data from fecal samples of controls (N = 138) and multiple sclerosis patients (N = 145), which were published by *Thirion et al.* (PMID:36604748). After the reads were processed, the taxonomic profile was obtained using MetaPhlAn with ChocoPhlAn database annotation. Next, community typing was performed via Dirichlet-multinomial modeling. Polyamine metabolic potential was assessed by building polyamine modules based on the abundance of functiontal KEGG orthologous. We also implemented distance-based redundancy analysis (dbRDA) to describe the microbial variance explained by polyamine modules, while significant associations were identified using non-parametric statistical approaches (i.e, Dunn test, Wilcoxon test, and Spearman correlation).
Our analysis revealed that polyamine metabolic potential explains 16.4% of microbial variation (stepwise dbRDA, eight significant modules, p.value cutoff < 0.05), including BA modules for agmatine, putrescine, and spermidine production. We also identify four enterotypes based on the microbial taxonomic distribution: Bacteriodes1 (B1), Bacteroides2 (B2), Prevotella (P) and Ruminoccocus (R), which differ in their BA potential. Taxon-specific BA associations were also observed. Finally, MS samples exhibited increased potential to agmatine production, but with significant variations based on multiple sclerosis treatment. These patterns were not identified for spermidine and putrescine modules.
Our results demonstrate that gut microbiota variation may drive differential polyamine production, with implications for host-microbe interactions in MS through different abundance of BAs.

Microbiome Ecology

OP05

Spatiotemporal Dynamics of Antimicrobial Resistance in Hospital-Associated Wastewater

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Background: Antimicrobial resistance (AMR) is a mounting global health threat, particularly in low- and middle-income countries (LMICs), where hospital effluents often serve as reservoirs for antibiotic-resistant pathogens and genes. Current methods to monitor antibiotic resistance genes (ARGs) rely heavily on resource-intensive technologies like shotgun metagenomics and quantitative PCR. To address this gap, there is a need for affordable, scalable methods to monitor ARG pollution, especially in low-resource settings.
Methodology: We conducted a multi-site and multi-season study sampling wastewater from four hospital sewage networks in an Indian metropolis across three seasons: monsoon, autumn, and spring. Samples were collected from hospital outlets, community sewers, sewage treatment plant (STP) inlets, and STP effluents. Standard biochemical parameters, including Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Organic Carbon (TOC), were measured alongside metagenomic sequencing to assess bacterial community composition and ARG profiles. Bioinformatic analysis was performed using a custom Snakemake workflow, and resistome and community diversities were evaluated through multivariate statistical analyses.
Results: Hospital sewage exhibited the highest ARG load, which did not substantially decrease across the STP treatment process. Seasonal variation influenced bacterial community structures, with monsoon samples showing distinct shifts in microbial composition and increased abundance of Pseudomonas species. The resistome composition varied significantly by season, hospital site, and sampling point. ARG profiles revealed higher abundances of aminoglycoside and beta-lactam resistance genes in hospital outlets compared to community or STP samples. Importantly, BOD and TOC levels showed a positive correlation with ARG load across all sample types and seasons. The inStrain analysis showed Klebsiella pneumoniae and Escherichia coli strains being spread from hospital to community sewage.
Conclusions: Our findings demonstrate that traditional biochemical parameters, particularly BOD and TOC, can serve as valuable proxies for ARG surveillance in hospital sewage networks. These indicators offer a pragmatic and cost-effective alternative for resource-limited settings where advanced molecular techniques are impractical. Moreover, the persistence of high ARG loads despite sewage treatment underscores the need for improved effluent management practices. Implementing biochemical-based monitoring could facilitate early identification of AMR hotspots, helping mitigate environmental and public health risks associated with wastewater discharge.

Methods

OP06

Neuroactive Potential Signatures of the Gut Microbiome Across Prokaryotic Genomes and Neuropsychiatric Conditions Using GBM2

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Gut bacterial metabolites influence neurotransmission, immune response, and behavior. Despite >15% of the population affected by mental health conditions, the gut microbiome's neuroactive potential across disorders remains poorly understood, largely due to a lack of mechanistic studies and challenges in data interpretation.
To address this, we used GBM2 (Gut-Brain Modules 2), a computational framework that systematically profiles the neuroactive metabolic potential of genomes and metagenomes. GBM2 expands on previous tools, incorporating over 200 curated metabolic pathways related to neuroactive compounds such as short-chain fatty acids (SCFAs), and metabolites from tryptophan, tyrosine, arginine, glutamate, as well as emerging compounds from vitamin, hormone, and bile acid metabolism. It leverages Enzyme Commission (EC) and UniRef annotations, overcoming limitations of proprietary systems like KEGG Orthology. GBM2's accuracy was extensively validated with experimental data from known metabolite-producing strains.
Mapping neuroactive pathways across microbial genomes, we found that capacities once considered rare, such as cortisol cleavage, are widespread. This function was present in 85% of Clostridium scindens genomes, 74.6% of other Clostridium species (91.84% within-species consistency), and 53% of Bacillota, notably 88.52% and 91.99% of Faecalibacterium and Coprococcus species, respectively. Conversely, it was absent in Bifidobacteria and Lactobacillus spp., common psychobiotic candidates. This highlights the potential of exploring broader microbiome diversity for next-generation psychobiotics.
We then meta-analyzed 53 fecal shotgun metagenomic datasets, covering over 8,300 controls and 3,700 patients with neuropsychiatric conditions such as Alzheimer's, Parkinson's, schizophrenia, major depressive disorder, autism spectrum disorder, generalized anxiety disorder, anorexia nervosa, multiple sclerosis, and myalgic encephalomyelitis/chronic fatigue syndrome. We are currently characterizing the neuroactive microbiome signatures across and within these conditions.
By addressing enzymatic redundancies, using open-source databases, and considering moonlighting activities, GBM2 offers a comprehensive assessment of the gut-brain axis. Our work aims to guide microbiome-based therapeutic strategies for neurological and mood disorders.

POSTER EXHIBITION

Clinical Associations

PE01

Identification of a gut microbiota signature in patients with drug-resistant epilepsy upon ketogenic diet treatment

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Background: Epilepsy is a neurological disease characterized by seizures that affects 50 million people world-wide. Around 30% of the patients do not respond to the current drugs. Ketogenic diet (KD), highly enriched in fats, is an established therapy for drug-resistant epilepsy (DRE) that shows a reduction of >50% of seizures frequency in ~50% of DRE patients. Interestingly, microbiota from DRE patients responsive to KD is different from KD-nonresponder DRE patients and it is still unclear why some patients do not respond to KD.
Methods: Our research project will address microbiota differences in DRE patients, responders and nonresponders to KD, and identify strains of bacteria with potential anti-seizure effect. For this purpose, fecal samples will be collected from patients with DRE before and during treatment with KD up to 6 months together with clinical and dietary information. Stool microorganism composition will be analysed through shotgun metagenomic sequencing, stool and blood metabolites (including ketone bodies) will be measured through targeted and untargeted metabolomics, and biochemical and inflammatory parameters (e.g., C reactive protein, procalcitonin, cytokines) from serum samples will be analyzed.
Results: We expect that >30-50% of the patients under KD will have >50% reduction of seizures, ~30% will experience <50% reduction of seizure frequency and ~5-10% will not show any improvement or eventually will get worse upon KD introduction. Healthy relatives and patients with DRE without KD will be analyzed as controls. The ultimate goal of this project is to describe which microbes are associated with KD-dependent epilepsy amelioration and to test their effect in vivo in preclinical animal models of epilepsy. Identification of those microbes could be used as probiotics for a potential treatment for KD-non-responder DRE patients, as well as alternative to KD, which cannot be kept long-term.
Conclusions: We believe that these results will shed light for diet-microbiota based treatments for other neurological diseases and metabolic disorders.

PE02

Exploring the Link Between Gut Microbiome and Asthma Risk in Angolan Adults

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Background: Asthma is one of the most prevalent chronic respiratory diseases worldwide, and although its prevalence is higher in high-income countries, most asthma-related deaths are in low- and middle-income countries. The lung and intestine are connected through intricate communication pathways that influence each other's equilibrium. Metabolites generated by the gut microbiome, along with intestinal immune cells and immune factors, travel through the bloodstream to the lungs, where they contribute to immune function. The main aim of this study was to perform a case-control study to investigate the association between asthma and gut microbiome in an adult Angolan population from Luanda.
Methodology: A case-control cohort study was conducted in Luanda, the capital of Angola, from June 2022 to June 2023. The study included 157 adult asthma patients, diagnosed based on the Global Initiative for Asthma (GINA) criteria, followed at the outpatient pulmonology clinic of the Military Hospital in Luanda, along with 157 healthy volunteers. Microbial DNA was extracted using the ZymoBIOMICS™ DNA Miniprep Kit and FastPrep-24™ homogenizer, following the manufacturer's guidelines. Library preparation for sequencing was carried out according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol, targeting the V3-V4 hypervariable regions of the bacterial 16S rRNA gene. Sequencing was performed on the NextSeq550 instrument (Illumina) using 2×151 bp paired-end reads. Microbiome taxonomic profiles were generated using the EzBioCloud MTP pipeline and the EzBioCloud 16S database PKSSU4.0.
Results: Comparing microbiome composition between asthma patients and healthy controls revealed a significant decrease in Order Bifidobacteriales and Phylum Actinobacteria, alongside an increase in Order Clostridiales and Phylum Firmicutes in the asthma group. At the genus level, Bifidobacterium, Eubacterium, Collinsella, and Bulleidia were significantly lower, while Clostridium, Christensanella, Eubacterium, Coprococcus, and Agathobaculum were higher. Additionally, significant differences were observed between controlled and uncontrolled asthma patients, suggesting a potential link between the microbiome and disease severity
Conclusions: Preliminary findings from the study reinforce the link between the gut microbiome and asthma. However, further research is needed to address key questions before more effective strategies for asthma treatment and prevention can be developed.

PE03

Characterization of the Oral and Intestinal Microbiota in Individuals with Serrated Polyposis Syndrome

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Background: The pathogenesis of serrated polyposis syndrome (SPS), the most common polyposis syndrome, remains unknown, and to date, the oral and intestinal microbiota of these patients has not been characterized. The primary objective of this study was to describe and compare the composition of the oral and gut microbiota in individuals with SPS versus individuals without polyps, with sporadic serrated polyps, and with adenomas.
Methods: A prospective, observational, and bicentric study was conducted. Clinical and endoscopic data were collected. Additionally, saliva and stool samples were obtained from 126 individuals divided into four groups: SPS(n=32), serrated(n=30), adenoma(n=32), and control(n=32). The microbiota was analyzed using 16S rRNA amplicon sequencing, and qPCR was performed for the detection of Fn.
Results: The main clinical, dietary, and dental health characteristics are presented in Table 1. Regarding the oral microbiota, no differences were observed in alpha diversity among the groups. However, the composition of the oral microbiota in the SPS group showed significant differences compared to the other groups, as evidenced by a greater unweighted UniFrac distance ($p<0.001$).

In the gut microbiota, the adenoma group exhibited lower species richness than the control group, while no differences were found in the other groups. Statistically significant differences in beta diversity, measured using unweighted UniFrac distance, were observed between the SPS group and the adenoma and control groups (p<0.001).
Regarding taxonomy, Porphyromonas gingivalis, an oral bacterium associated with colorectal cancer (CRC) development and progression, showed higher oral abundance in the SPS group compared to the control and adenoma groups (p<0.05; FDR p=0.104). Treponema denticola and Prevotella intermedia, two oral bacteria related to CRC, were more abundant in the SPS group than in the control group (p<0.05; FDR p=0.09). Additionally, Peptoanaerobacter stomatis, an oral bacterium linked to CRC via the serrated pathway, was more prevalent in the SPS group compared to the serrated and adenoma groups (p<0.05; FDR p=0.26).
No differences in the abundance of Fn were found, either in saliva or stool samples.
Conclusions: The oral and gut microbiota of individuals with SPS exhibit a distinct bacterial composition compared to individuals without polyps and those with adenomas. Furthermore, certain oral bacteria associated with CRC are increased in SPS patients.

	SPS (n=32)	Serrated(n=30)	Adenoma (n=32)	Control (n=32)	Total (n=126)	p-value
Age, years	61 [57-67]	63 [56-66]	59,5 [54-67]	58,5 [53-64]	61 [55-66]	0,28
Sex, male	17 (53,1%)	13 (43,3%)	17 (53,1%)	17 (53,1%)	64 (50,8%)	0,83
Body mass index, kg/m²	27 [26-32]	27,8 [25-32]	28,3 [25-31]	27 [24-32]	27,7 [25-32]	0,99
Tobacco						<0,001
Active smoker	18 (56,3%)	12 (40%)	6 (18,8%)	3 (9,4%)	39 (31%)	
Former smoker	3 (9,4%)	7 (23,3%)	10 (31,3%)	18 (56,3%)	38 (30,2%)	
Non-smoker	11 (34,4%)	11 (36,7%)	16 (50%)	11 (34,4%)	49 (38,9%)	
Tobacco, pack-years	25 [17-45]	34 [17-40]	15 [9-40]	7,3 [4-33]	24 [11-40]	0,03
Medication use						
PPIs	5 (15,6%)	7 (23,3%)	7 (21,9%)	5 (15,6%)	24 (19%)	0,79
ASA or NSAIDs	10 (31,3%)	3 (10%)	2 (6,3%)	7 (21,9%)	22 (17,5%)	0,035
Statins	16 (50%)	6 (20%)	4 (12,5%)	10 (31,3%)	36 (28,6%)	0,006
Recommended physical exercise, yes	15 (46,9 %)	10 (33,3%)	20 (62,5%)	13 (40,6%)	58 (46%)	0,12
History of periodontitis, yes	11 (34,4%)	9 (30%)	7 (21,9%)	5 (15,6%)	32 (25,4%)	0,31
Consumption frequency, g/day						
Vegetables and greens	149 [83-205]	131 [106-186]	137 [87-203]	129 [83-189]	136 [88-195]	0,89
Fish and seafood	48 [29-64]	48 [29-64]	60 [43-89]	43 [25-63]	46 [31-68]	**0,047**
Red or processed meat	56 [36-75]	49 [33-104]	60 [43-94]	47 [21-74]	54 [33-80]	0,18
Cured meats	6,3 [3,6-22]	10,7 [7,2-25]	10,7 [7,2-25]	7,1 [2,1-13,3]	7,1 [3,5-18]	**0,002**
Fat and oils	29 [10-43]	29 [15-43]	29 [16-35]	26 [10-43]	29 [13-43]	0,94
Processed foods	4 [0,7-10]	5,3 [5,3-15]	5,3 [5,3-15]	5,3 [0-11]	5,3 [2,7-11]	**0,046**

PE04

Mapping Health Inequalities: Girostudi, a Novel Cohort Study in Sparsely Populated Regions of Girona Linking Exposome, Microbiome, and Wellbeing

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Background: L'Observatori, a unit of Dipsalut (Public Local Health Administration), aims to leverage data on social determinants of health and planetary health model to inform equitable municipal public health policies. Currently, municipal-level data in these areas are scarce, non-public, or lack interoperability. Therefore, L'Observatori launched Girostudi, a 10-year longitudinal cohort, designed to collect socioeconomic, environmental, health, and wellbeing data specifically in sparsely populated regions of Girona. It is expected that 3.650 people aged between 16 to 79 years old will be recruited, with a 10-year follow-up. A core objective is to elucidate the complex interplay between environmental exposures, the human microbiome, and health outcomes in these populations.
Methodology: Data will be collected via online questionnaires using a technological platform and through human biomonitoring (blood, stool, urine, hair, nail, and saliva samples). Sampling will employ a probabilistic, stratified, two-stage cluster design, with primary and secondary sampling units selected via simple random sampling. Microbiome variables will be analysed using massive DNA sequencing techniques. Diversity, evenness, richness, and dominance metrics will be used to determine the overall state of the microbial ecosystem. The collected data will enable the development of descriptive indicators and multilevel inferential models tailored to each study area.
Results: Girostudi, launching in 2025, will be the first to provide statistically significant and territorially representative data for the Girona province. It will generate data on social, environmental, health, and wellbeing inequities across municipalities. This will enable the integration of exposure and outcome measures at the microbiota level, providing insights into microbiome composition, diversity, and its intricate relationship with environmental exposures and health status. It is expected to identify microbial paterns associated with factors such as diet, lifestyle and pollution.
Conclusions: Girostudi will provide a holistic view of population health in sparsely populated areas, considering the interplay between the exposome, microbiome, and health. This information will enhance our understanding of health inequalities and inform local public health equity policies and programs, such as urban renaturalization, nature contact promotion, and the encouragement of organic and local food production and consumption.

PE05

Analysis of gut bacterial community changes during Giardia duodenalis infection refractory to treatment

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Nitroimidazole antimicrobials (e.g., metronidazole, tinidazole) are first-line treatment for patients with Giardia duodenalis infection. Cases of nitroimidazole-refractory giardiasis have increased in the last two decades, especially among travellers returning from South-East Asian countries [1,2]. Interaction between G. duodenalis and host gut microbiota plays an important role in the pathophysiology of giardiasis infections, but little is known about how the parasite impacts the human gut microbiome. In fact, few longitudinal studies exist, and none focuses on patients refractory to medication. Here we present clinical data and Illumina 16S rRNA gene microbial community profiling on two adults with refractory giardiasis.
A 31-year male and a 32-year female travelling to India experienced severe gastrointestinal symptoms during and immediately after the journey. Both were diagnosed with G. duodenalis (assemblage B) infection. Treatment consisting on tinidazole 50 mg/kg/day, metronidazole 25 mg/kg/day, and quinacrine 50 mg/kg/day failed over a 129-day treatment period. Eight successive stool DNA samples (3 from the male, 5 from the female) and two post-infection negative (i.e., when symptomatology was resolved) samples (one from each patient) were taken across the whole treatment period.
A significant reduction in alpha diversity during infection was evidenced, which was sustained once infection was resolved. Post-infection gut microbial communities differ from those seen during infection, with an important individual effect. Shifts in relative abundances of bacterial taxa were observed at several taxonomic ranks. During infection, both subjects revealed an increase of Prevotella, whereas Oscillibacter was the only common taxon identified at the post-infection stage in both patients. The male patient experienced a remarkable decrease of Ruminococcus throughout the disease course and an increase of Flavobacterium after it.
This is the first longitudinal gut bacterial community study in Giardia-infected adult refractory patients. We evidenced microbial shifts that may be associated with infection, treatment effect, or microbial restoration after infection, and can potentially be used to monitor disease evolution. Future studies involving larger cohorts are required to elucidate if these changes are cause or consequences of refractory giardiasis.
References: 1.Requena-Méndez et al 2014 (PMID: 23926944); 2. Nabarro et al 2015 (PMID: 25975511).

PE06

Implications of the Human Oral Microbiome in Alzheimer's Disease Prognosis

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Background: Alzheimer's disease is the most common type of dementia. There is no cure for this disease, with only a few medicines to alleviate the symptoms, which will be more optimal if the disease is detected at an early stage. In patients with AD, chronic inflammation is the first sign of disease progression. Influencing factors in chronic inflammation are changes in the microbiome.
Previous studies have shown associations between periodontitis and Aβ-peptide plaques in the brain. Therefore, key risk factors implicated in AD include changes in the oral microbiome.
Methods: Samples were sequenced using 16S sequencing. Raw sequencing data was preprocessed by using Dada2 pipeline and taxonomy was assigned by mapping to SILVA database (v138). Afterwards, an analysis of microbiome data was carried out using the phyloseq R package. We applied different necessary filters and calculated alpha and beta diversity, applying the appropriate statistical tests in each case. After that, differential abundance analysis was carried out using different linear models in each model.
Results: To understand some alterations could precede the development of AD, we performed a microbial profiling of 594 saliva samples from patients with different degrees of AD, of which we have the evolution over four years (longitudinal study). We produced multidimensional scaling plots using Aitchison distance between the microbial profiles of samples. Significant effect was obtained depending on the diagnostic variable (PERMANOVA). Even so all samples overlap quite a lot, but the samples of the healthy group tend to cluster closer together. We next performed a differential analysis to detect taxa with differential abundance according to the diagnostic group. Some taxa were found to be differentially abundant, with a tendency to increase as the disease progresses. We used dbBact database to biologically interpret the results. Almost 70% of the species listed as differentially abundant have already been found associated with the term periodontitis in this database.
Conclusion: We compare oral microbiome samples from AD patients at different stages of disease progression. The healthy group appears to have a more differentiated sample type, it could be a breakthrough to be able to detect this disease before developing full-blown AD. However, future studies on the evaluation of the predictivity are necessary to obtain biomarkers for early diagnosis.

PE07

GCATmb: Gut Microbiome Profiles in the Catalan Population – Implications for Health, Disease, and Lifestyle Factors

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The intestinal microbiome plays a crucial role in human health, influencing both physiological and pathological processes. This study analyzes the gut microbial composition of the Catalan population within the GCAT cohort, a prospective cohort study investigating the impact of genetic and environmental factors on chronic diseases development.
We assessed microbiota composition and its associations with demographic, health, and lifestyle factors. A health score was calculated for each participant, comprising chronic disease status, risk factors (e.g., hypertension, body mass index), and lifestyle habits such as physical activity, Mediterranean diet (MD) adherence, alcohol and tobacco consumption.
Participants with lower health scores exhibited dysbiotic microbiota, characterized by reduced diversity and imbalances in microbial species. Additionally, we identified a microbial signature distinguishing individuals with optimal, intermediate, and altered microbiota profiles, with some species from the genera Bacteroides, Phocaeicola, Escherichia, and Enterocloster, which have been previously associated with disease conditions.

Among the lifestyle factors analyzed, low adherence to the MD and smoking emerged as key contributors to microbiome dysbiosis.
These findings suggest that gut microbiota profiling could serve as a biomarker for health status assessment and guide personalized medicine approaches. This study offers novel insights into the gut microbiome of the Catalan population and its potential role in preventive health strategies.

PE08

The Gut Microbiota Modulates Anastomotic Healing In Patients With Colorectal Cancer Undergoing Surgery

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Background: Anastomotic leak (AL) is a major complication in colorectal surgery and significantly increases morbidity and mortality. Our objective was to investigate the role of gut microbiota in anastomotic healing.
Methods: Preoperative fecal samples were collected from patients with colorectal cancer (CRC). Fecal microbiota transplantation (FMT) was performed in mice using samples from patients with and without AL, after which transplanted mice underwent colonic surgery. At day 6 after surgery, anastomotic healing, gut barrier integrity, and gut microbiota composition were analyzed. Bacteria of interest were isolated and assessed in vitro and in vivo.
Results: We found that, compared to mice transplanted with fecal microbiota from donors without AL, mice receiving fecal transplants from donors with AL displayed poorer anastomotic healing, increased gut permeability, and higher levels of colonic pro-inflammatory cytokines, resulting in higher AL rates. We identified a strain of Parabacteroides goldsteinii, which exerted a beneficial anti-inflammatory effect and improved anastomotic healing, and a deleterious Alistipes onderdonkii strain, which promoted inflammation and increased leakage.
Conclusions: In conclusion, gut microbiota plays an important role in surgical colonic healing in patients with CRC, paving the way toward microbiota-targeted interventions to improve anastomotic healing and prevent AL.

PE09

Airway Microbiota And Coronary Heart Disease In COPD: Hunting High And Low For Connections

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Background: Chronic obstructive pulmonary disease (COPD) and coronary heart disease (CHD) are major causes of morbidity and mortality, often co-occurring due to shared risk factors. This study aimed to investigate the association between the upper and lower airways microbiome and CHD in healthy controls and COPD patients.
Methods: 228 participants recruited from the MicroCOPD study (101 controls and 127 COPD patients, Figure 1) underwent coronary CT angiography to assess calcium score (CaSc) and significant coronary stenosis. Oral wash (OW) and bronchoalveolar lavage (BAL) samples were collected (Table 1). Microbial DNA was extracted and 16S rRNA gene sequencing was performed using the Illumina MiSeq platform. Microbiome diversity and composition were analyzed using QIIME 2. Alpha and beta diversity were assessed, and differential abundance assessed using ANCOM-BC2.
Results: Shannon diversity indicated significant differences in alpha diversity between COPD patients and controls in OW ($p<0.01$), but not BAL. Additional alpha diversity measures (Shannon diversity and Faith`s PD) showed no significant differences between CHD and non-CHD groups (both $p>0,05$). Beta diversity analysis (Bray-Curtis dissimilarity) revealed significant differences in microbial community composition between COPD patients and controls ($p<0.05$), but not between CHD and non-CHD individuals. Firmicutes were the dominant phylum across all subgroups, followed by Bacteroidetes and Actinobacteria. Several differentially abundant taxa were identified, but they represented a small fraction of the total number of taxa.
Conclusion: While COPD may alter the airway microbiome, the presence of CHD does not appear to impact microbial composition in the upper and lower airways. These findings suggest that COPD influences the airway independently of CHD. Further longitudinal studies are required to elucidate the complex interactions between respiratory and cardiovascular health and the role of the microbiome in disease progression.

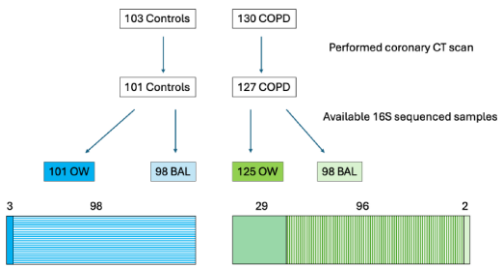

Figure 1. Flow chart over study selec5on and final sample availability

Variable	Controls (n=101, 44.3%)	COPD (n=127, 55.7%)	p*
Sex, n (%)			0.99
Women	43 (44.3)	54 (44.3)	
Men	58 (55.7)	73 (55.7)	
Age, mean (SD)	65.8 (7.9)	67.4 (7.3)	0.11
Smoking, n (%)			<0.01
Never	16 (15.8)	0	
Ex	57 (56.4)	89 (70.1)	
Daily	28 (27.7)	38 (29.9)	
Lung function			
FEV_1 in percent predicted, mean (SD)	104.2 (12.5)	56.6 (19.6)	<0.01
FVC in percent predicted, mean (SD)	112.2 (13.2)	94.4 (18.0)	<0.01
Calcium Score (CaSc)			<0.01
< 100	70 (69.3)	58 (46.6)	
≥ 100	31 (30.7)	67 (53.6)	
Significant coronary stenosis			0.18
No	79 (95.2)	89 (89.0)	
Yes	4 (4.8)	11 (11.0)	

Table 1. Baseline characteristics for the MicroCOPD study participants, for whom a coronary CT scan was taken

PE10

Colorectal Cancer-associated Microbiota Promotes Barrier Disruption, Inflammation And Dedifferentiation In Vitro

Nión Cabeza, Paula [1], Varela Eirín, Marta [1], Aja Macaya, Pablo [1], González Castellano, Inés [1], Galindo Morales, Sara [1], Bou, Germán [1], Vallejo Vidal, Juan Andrés [1], Poza Domínguez, Margarita[1,2]

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Colorectal cancer (CRC) is one of the most common cancers worldwide, and both environmental and genetic factors strongly influence its incidence. Increasing evidence suggests that microbiota plays an important role in CRC development. Gut colonization by certain microorganisms can trigger the release of toxins and promote inflammation and oxidative stress in colon cells, ultimately increasing the risk of CRC development.
We have previously demonstrated that the anaerobic pathogens Parvimonas micra, Fusobacterium animalis and Bacteroides fragilis are overrepresented in the microbiota of CRC patients. In a previous report, our group demonstrated that P. micra and F. animalis, usually located in subgingival pockets, can be translocated to the colon. This study aims to further investigate the role of these bacteria in CRC development. Infection with P. micra, F. animalis, and B. fragilis results in the upregulation of inflammatory cytokines and epithelial-to-mesenchymal transition (EMT) markers, with this effect being significantly exacerbated in the context of combined co-infections. Particularly, high levels of Cxcl10, Ccl5 and Snai2 were detected, which are associated with colorectal cancer progression and metastasis. Interestingly, the chemokine Cxcl1, whose increased expression is correlated with the invasive potential of cancer cells, was consistently overexpressed in CRC after infection. Further, Transwell in vitro assays demonstrated that the intestinal barrier integrity was compromised after co-culture of CRC and these bacteria, being this effect especially prominent in B. fragilis infection. These findings suggest that F. animalis, P. micra and B. fragilis alter the intestinal barrier, and promote EMT and a pro-inflammatory environment that may modulate the immune system and contribute to the development and/or exacerbation of CRC.

PE11

High Prevalence Of Macrolide Resistance In Streptococcus Pneumoniae Isolated From Nasopharyngeal Samples Among Naïve Six-week-old Children In Sierra Leone: A Resistome Approach

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Background: Mass administration of azithromycin has been associated with reduced childhood mortality but has also contributed to increasing antimicrobial resistance (AMR) in sub-Saharan Africa [1,2]. The resistome, representing the collection of antibiotic resistance genes within a bacterial community, can be analyzed using both phenotypic and genotypic approaches. This study aims to determine the nasopharyngeal carriage of S.pneumoniae and its level of macrolide resistance in six-week-old children in Sierra Leone. The microbiome and resistome were both analyzed, and the correlation between phenotypic and genotypic resistance to macrolides in S.pneumoniae isolates was assessed.
Methods: 936 nasopharyngeal swabs (NPS) were collected for the study. Nasopharyngeal S.pneumoniae was isolated using blood agar, identified by MALDI-TOF MS and confirmed by optochin susceptibility test and the presence of the lytA gene. The minimum inhibitory concentration (MIC) of azithromycin was measured by Etest®. From the total samples, 102 strains of S.pneumoniae were selected according to its level of resistance to azithromycin (Figure 1). These samples were analyzed through the Ion AmpliSeq™ Pan-Bacterial Research Panel from ThermoFisher to perform resistome and microbiome analysis.
Results: In the phenotypical results we found a 17.74 % of positivity for S.pneumoniae and 45.8 % these bacterial strains were considered resistant to azithromycin according to CLSI standards. In the sequenced data, we were able to differentiate significantly the two groups of positive and negative samples according to the normalized reads obtained for S.pneumoniae (p-value=2.1x10-10). Resistome data showed a correlation between the normalized reads found for the resistance genes to macrolides (according to CARD database annotation) and the groups defined by azithromycin phenotypical resistance level (Figure 2).
Furthermore, we were able to demonstrate that resistance was due to the presence of two genes: ermB and mefE, which are the ones predominantly associated with S.pneumoniae resistance to macrolides [3,4] (Figure 3).
Conclusions: This study reveals a high basal level of macrolide resistance in the described S.pneumoniae isolates, showing a significant correlation between phenotypic and genotypic macrolide resistance, driven by ermB and mefE genes.

*References: 1. https://doi.org/10.1038/s41591-019-0533-0 2. https://doi.org/10.1186/s13099-021-00478-6 3. https://doi.org/10.3389/

S. pneumoniae	Macrolide resistance level	MIC azithromycin	Sample number
Positive	High	>256	25
	Intermediate	2-32	18
	Low	0,75-1	18
	Sensitive	<0.5	17
Negative	-	-	24

Figure 1. Sample selection for genotypical analysis

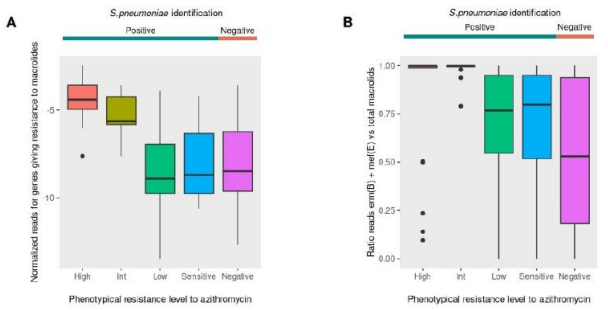

Figure 2. (A) Total macrolide resistance. Normalized read sum for all the genes that give resistance to macrolides found in the sequenced data obtained from of DNA from NPS using Ion AmpliSeq™ Pan-Bacterial Research Panel. Macrolide resistance genes have been selected according to CARD database annotation. Samples are grouped by the phenotypical resistance level to azithromycin given by the MIC values of the performed e-test analyses. (B) Specific S.pneumoniae macrolide resistance given by erm(B) and mef(E) genes. The ratio between the sum of the reads for erm(B) and mef(E) genes and the total sum of the reads that give resistance to macrolides is for the grouped samples by the phenotypical resistance level to azithromycin given by the MIC values of the performed e-test analyses. Phenotypical identification for S.pneumoniae is also included in both panels.

PE12

Antibiotic Use And Antibiotic Resistance Genes In Chronic Lung Disease Patients: A Bronchoscopy Study Of The Lower Airways' Microbiome

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Background: Antibiotic use may influence the presence of antibiotic resistance genes (ARGs) in a microbiome. Whole genome sequencing (WGS) makes it possible to map the cumulative presence of ARGs in a microbial community. We aimed to compare the presence of ARGs in healthy subjects with patients with chronic lung disease, adjusted for time since antibiotic use.
Methods: Bronchoalveolar lavage (BAL) samples were collected from 100 healthy controls, 93 patients with chronic obstructive pulmonary disease (COPD), 13 with asthma, 34 with sarcoidosis and from 12 with idiopathic pulmonary fibrosis (IPF). Participants had not used antibiotics 14 days prior. WGS was performed with an Illumina NovaSeq. ARGs were identified using the National Database of Antibiotic-Resistant Organisms (NDARO) as reference.
Sample reads were normalized to reads per million. Presence of any ARG was analyzed by logistic regression analyses. Due to non-normality, sample reads were log-transformed for multivariable regression analysis comparing number of sample reads.
Results: Among the healthy controls, 38% had at least one ARG present, compared with 51%, 39%, 65% and 83% of COPD asthma, sarcoidosis, and IPF patients respectively (p=0.01). ARGs against tetracycline (33%) was the most common ARG class overall, followed by beta-lactam resistance and macrolide resistance (both 26%).
In a logistic regression analysis adjusted for sex, age, body composition, smoking, and use of antibiotics, the OR (95% CI) for having ARGs in the lower airways was 1.31 (0.71-2.43) in COPD, 1.00 (0.28-3.52) in asthma, 3.54 (1.40-8.94) in sarcoidosis, and 6.46 (1.26-33.11) in IPF, compared with controls.
Overall mean (SD) ARG sample reads were 253.4 (347.4) in the 30 subjects who had used antibiotics ≤ 3 months before bronchoscopy, compared with 125.4 (258.5) in the 222 subjects who had not (p=0.04).
In a multivariable regression analysis, the geometric mean (GM) ratio with 95% CI was 2.95 (1.04-8.36) in subjects using antibiotics ≤ 3 months before bronchoscopy compared with no use. The diagnoses sarcoidosis and IPF independently predicted higher numbers of ARGs.
Conclusion: The presence of ARGs in the lower airways' microbiome was significantly higher in patients with sarcoidosis and IPF than in controls. The number of sample reads for ARGs was significantly associated with recent antibiotic use.

PE13

Taxonomic and Functional Microbiome Analysis in Pancreatic Diseases Reveals Distinct Phage-Bacterial Interactomes and Potential Oncogenic Pathways

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Background: The microbiome, including bacterial and viral communities, may influence pancreatic disease through immune modulation and carcinogenic pathways. This study aims to characterize taxonomic and functional differences in the gut and oral microbiota of PDAC, CP, and healthy individuals to uncover potential microbial contributors to pancreatic cancer.
Methodology: Shotgun metagenomic sequencing was performed on DNA from 49 stool samples (11 PDAC, 20 CP, 18 HC) and 53 saliva samples (11 PDAC, 21 CP, 21 HC) using the Illumina NovaSeq 6000 platform. Taxonomic profiling and diversity analyses were conducted in R, and phage-bacterial interactions were explored via the CoNet app in Cytoscape. Functional profiling, currently in progress, is being performed using MetaPhlAn and HUMAnN2, with viral gene content included. This will enable assessment of differentially regulated microbial gene families and functions between patient groups.

Results: Distinct gut phage-bacterial interactomes were observed in PDAC patients. Notably, CrAssphages—particularly Burzaovirus—co-occurred with Enterobacteriaceae spp. known to induce DNA damage in pancreatic cells. Conversely, Blohavirus showed mutual exclusion with Klebsiella oxytoca, a bacterium significantly enriched in PDAC stool samples but prevalent in healthy individuals. Blohavirus, the most abundant viral genus in HCs, was found to be mutually exclusive with Burzaovirus in PDAC samples. Interestingly, PDAC patients exhibited overlapping oral and gut microbiota profiles, with Fusobacterium nucleatum maintaining consistent abundance across both niches.
Conclusions: This study highlights the complex, disease-specific interplay between bacterial and viral taxa in pancreatic diseases. Our phage-bacterial interactome findings underscore potential microbial drivers of pancreatic pathogenesis and merit further investigation. Ongoing functional analyses will clarify the role of microbial gene expression in these interactions and their contribution to carcinogenesis, offering new avenues for biomarker discovery and microbiome-targeted interventions.

PE14

Altered Gut Microbiome Composition In Multiple Sclerosis Patients Characterised By Enrichment Of Pro-inflammatory Taxa And GABA Synthesis Potential

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Background: Multiple Sclerosis (MS) is an autoimmune inflammatory neurodegenerative disease of the Central Nervous System (CNS), affecting approximately 2.9 million people worldwide. Previous studies have highlighted the potential role of the gut microbiota in MS, affecting cellular infiltration to CNS and modulating neuroactive compounds availability.
Methodology: Stool samples and metadata were collected from 17 subjects diagnosed with MS and from 20 matched healthy controls (HC) at the San Raffaele Hospital, Milan (Italy). Shotgun metagenomics (median=62.5 million reads/sample) was performed on the extracted DNA and differences in microbiota composition between MS patients and HC were investigated. Reads from stool samples were pre-processed as described in https://github.com/SegataLab/preprocessing. The microbiome taxonomic and functional profiles were retrieved with MetaPhlAn 4.1.1 and HUMAnN 3.9 respectively. Alpha and beta diversity, together with differential abundance analyses were performed in R. Gut Metabolic Modules and Gut Brain Modules were also assessed, together with differences in viruses and blastocysts prevalence.
Results: MS diagnosis was associated with microbiota composition(R2adj=2%, FDR=0.03). No statistically significant differences in alpha diversity were observed, but in agreement with literature, a trend of lower alpha diversity in MS was found. At the genus level, the butyrate-producing Roseburia genus was enriched in HC, while Gordonibacter and the inflammation-associated Flavonifractor genus were more abundant in MS patients. Although none of the assessed species survived multiple testing corrections, among the most significant ones there are pro-inflammatory species(ex. Flavonifractor plautii and Rominococcus torques) enriched in MS subjects and butyrate-producing species (ex. Roseburia sp. and Agathobaculum butyriciproducens) more abundant in HC.
An enrichment in GABA synthesis genetic pathways in MS patients (MF0031, r=0.52, FDR=0.09) was also observed. Bacteriophages tended to be more prevalent in MS subjects, while blastocysts were identified only in HC.
Conclusions: MS patients were found to display altered microbiome composition, with trends in line with the literature and reflecting the inflammatory state. Future studies enrolling bigger cohorts and including longitudinal samples are needed to better assess the differences associated with disease activity states and to further elucidate the role of microbiota in MS.

PE15

Dialister-Driven Succinate Accumulation Is Associated with Disease Activity and Postoperative Recurrence in Crohn's Disease

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Succinate, a metabolite produced by both the gut microbiota and the host, has emerged as a key player in chronic inflammation. In this study, we investigated circulating succinate as a potential biomarker for disease activity in Crohn's disease (CD) and explored its associations with gut microbiota composition, immune markers, and clinical features. We analysed plasma, stool, and peripheral blood mononuclear cells from CD patients with active or inactive disease and from matched non-IBD controls (n=31). We found significant alterations in microbial diversity and composition in active CD patients compared to inactive CD patients and non-IBD subjects, alongside elevated succinate levels (Fig1). These elevated succinate levels were associated with higher Harvey-Bradshaw Index scores, increased expression of the succinate receptor SUCNR1 in immune cells, and enrichment of the succinate-producing genus Prevotella and the pro-inflammatory phylum Proteobacteria (Fig.2). Conversely, succinate levels negatively correlated with Odoribacter, a known succinate consumer (Fig.2). Interestingly, Dialister, a slow succinate consumer, was enriched in both active and inactive CD patients and was associated with impaired succinate clearance and increased disease activity as well as postoperative recurrence in a validation cohort (Fig.3 A-C). Functional microbial analyses revealed upregulation of fumarate reductase and succinate transporters, alongside reduced NADH dehydrogenase expression, indicating disrupted succinate metabolism (Fig.3D). Altogether, these findings highlight succinate as a promising biomarker of disease activity and progression in CD, and suggest that targeting succinate metabolism or modulating the abundance of key microbial taxa may offer novel therapeutic opportunities. Altogether, these findings highlight succinate as a promising biomarker of disease activity and progression in CD, and suggest that targeting succinate metabolism or modulating the abundance of key microbial taxa may offer novel therapeutic opportunities.

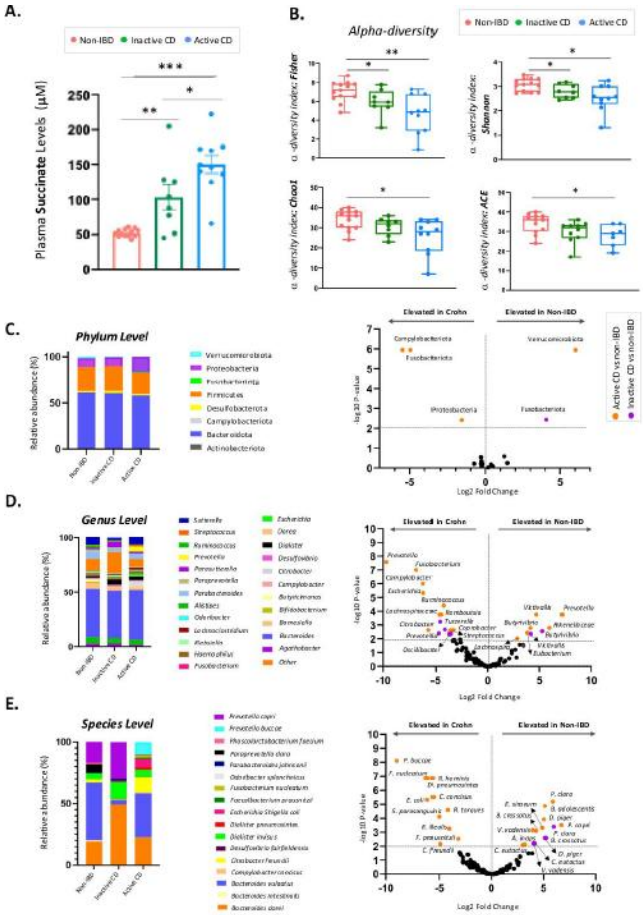

Figure 1.

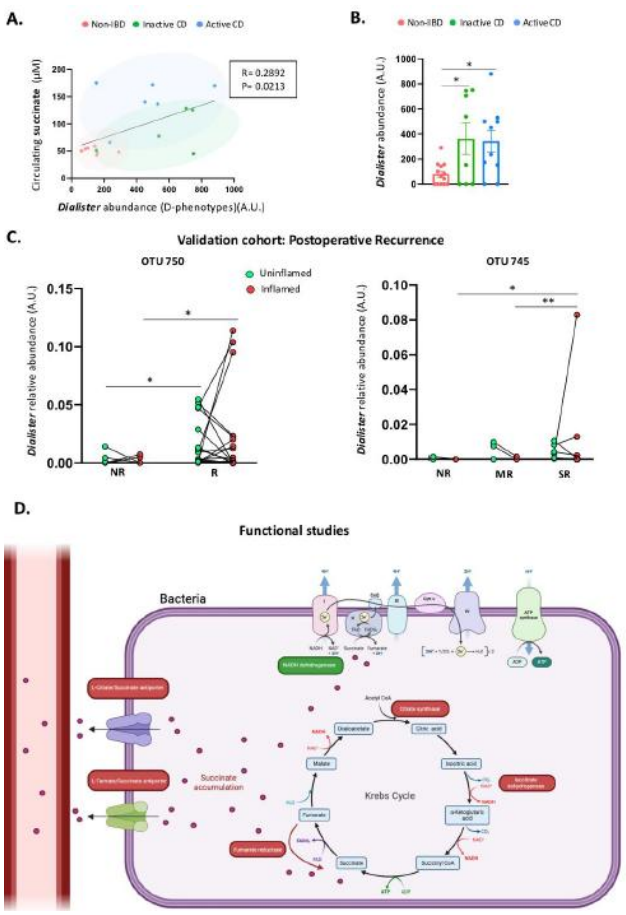

Figure 3.

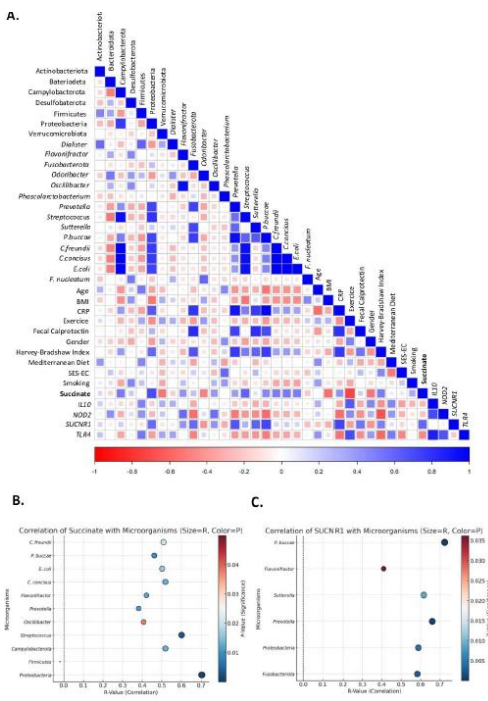

Figure 2.

PE16

Unraveling E. coli Driven Inflammatory Signatures In Crohn's Disease Via Integrative Multi-omics

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Crohn's disease (CD) is a chronic and relapsing inflammatory condition of the gastrointestinal tract characterized by exacerbated immune responses to the gut microbiota in genetically predisposed individuals. Although a causative microbe has not been identified, Escherichia coli has been proposed to contribute to CD pathogenesis. Yet, it remains unclear whether the presence of E. coli plays a role in disease heterogeneity by triggering distinct mucosal inflammatory responses. It is also unknown how the presence of specific microbiome profiles in CD associates with unique mucosal antibody repertoires.
To address these questions, we analyzed the composition of intestinal mucus-embedded microbiota through 16S rRNA sequencing in both non-IBD controls (n=31) and CD patients (n=29). Our analysis revealed a significant reduction of the alpha diversity in CD, and a marked expansion of E. coli in a subset of patients. Building on these findings, we selected a cohort (n=22) comprising non-IBD controls, and CD patients with or without mucus-embedded E. coli expansion for single-cell RNA and V(D)J sequencing to map the gut mucosal immune responses.
Our analysis confirmed that inflamed areas of CD patients display a reconfiguration of the B cell compartment and the emergence of unique inflammatory cell subsets across both immune and non-immune cellular compartments. Notably, CD patients with E. coli expansion showed distinct cellular modules driving inflammation and a unique reconfiguration of the mucosal humoral compartment characterized by a massive IgG clonal expansion.
Altogether, our preliminary findings suggest that the expansion of E. coli might be promoting a unique inflammatory signature in CD and may play a role in shaping mucosal humoral immunity.

PE17

Slow Transit Constipation Is Associated With Increased Prevalence Of Faecal Microbiota Dysbiosis

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Background: Slow Transit Constipation is a subtype of chronic constipation characterized by severely delayed colonic transit times, contributing to additional psychosocial stress. It affects predominantly women in around 4% of the global population. Despite its prevalence, its ethology and the possible involvement of the gut microbiome in associated symptoms remains underexplored.
Methodology: Faecal samples from 52 predominantly female STC patients were analysed for microbiome composition. Transit time was assessed via radiopaque marker studies and additional defecatory disorder (STC-DD) diagnosis was performed using balloon expulsion tests. Patient microbiomes were compared with a confounder-balanced subset of 600 individuals from a Belgium microbiome population survey.
Results: STC samples exhibited higher microbial loads and lower stool moisture compared to controls. Increased concentrations of fecal calprotectin and serum C-reactive protein were also detected in patients, indicating increased inflammation within the gut environment. Patient microbiomes were more diverse and comprised higher abundance of taxa previously associated with longer transit times, however abundances of key butyrate producers such as Faecalibacterium were lower. The prevalence of the dysbiotic Bact2 enterotype was elevated among patients. Transit time variation among patients was not linked to microbiome differentiation. Patients with additional defecatory disorder exhibited higher stool moisture. However, for STC-DD patients, shifts in gut microbiota composition are partly explained by differences in stool moisture.
Conclusions: While prolonged transit time has been associated with microbiome maturation, STC patients, which have prolonged transit time beyond the normal range, paradoxically exhibit higher prevalence of dysbiotic microbial communities usually associated with fast transit. Lower levels of butyrate-producing genera suggest a potential role for butyrate metabolism in STC pathophysiology.

PE18

Mapping Sex-specific Metagenomic Changes In Parkinson's Disease Through A Meta-analysis Of Multiple 16s rRNAseq Fecal And Oral Studies

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The gut microbiota plays a critical role in Parkinson's disease (PD) through the microbiota-gut-brain axis, influencing inflammation, neurotransmitter metabolism, and even protein aggregation. The "gut-first" theory has also emerged, proposing that changes in the microbiome may act as an initiating factor for PD. Moreover, sex differences have been recognized as important factors in PD research, with notable disparities between females and males reported in terms of symptoms, prevalence, pathophysiology, and even in the modulation of the microbiota–gut–brain axis. Built on the above, this work aims to identify taxa that variates during the PD progression in a sex-differential manner and explore their potential functional role through a meta-analysis of 16s rRNAseq PD datasets.
To achieve this, we performed a systematic review to identify all studies eligible for inclusion in the meta-analysis. Each study was individually analyzed following a DADA2 and phyloseq-based pipeline, which included: i) quality control, ii) ASV identification, iii) taxonomy classification, iv) exploratory analysis, v) alpha diversity comparison, and vi) differential abundance analysis with ANCOM-BC package. In the last two steps, related with comparative analysis, we conducted a PD versus healthy contrast for each sex, and also sex-specific double contrast to fully characterize the sex differential impact in the disease of the taxa analyzed. Lastly, we performed a meta-analysis based on a random effect model to extract the common signal across all the studies.
Following this methodology, we identified a total of 16 PD datasets with patient sex information, 3 from oral samples and 13 from fecal samples. After individual analysis, we performed 2 separate meta-analyses according to the sample sources. Although few statistically significant results were obtained, we observed differential sex-related trends in the abundance of taxa across most taxonomic levels. For example, in oral samples, we detected an increase of the Prevotella genus in females, whose levels are strongly and inversely correlated with PD progression, suggesting a protective role.

Considering the above, we can confirm that the study of sex differences in the field of PD metagenomics is highly relevant, although complex because of multifactorial variability of this type of data. Hence, although challenging, integrative approaches are essential to provide more robust knowledge in this field.

PE19

Gut-Vaginal Microbiome Alterations in Women with Recurrent UTI: Role of Sexual Activity

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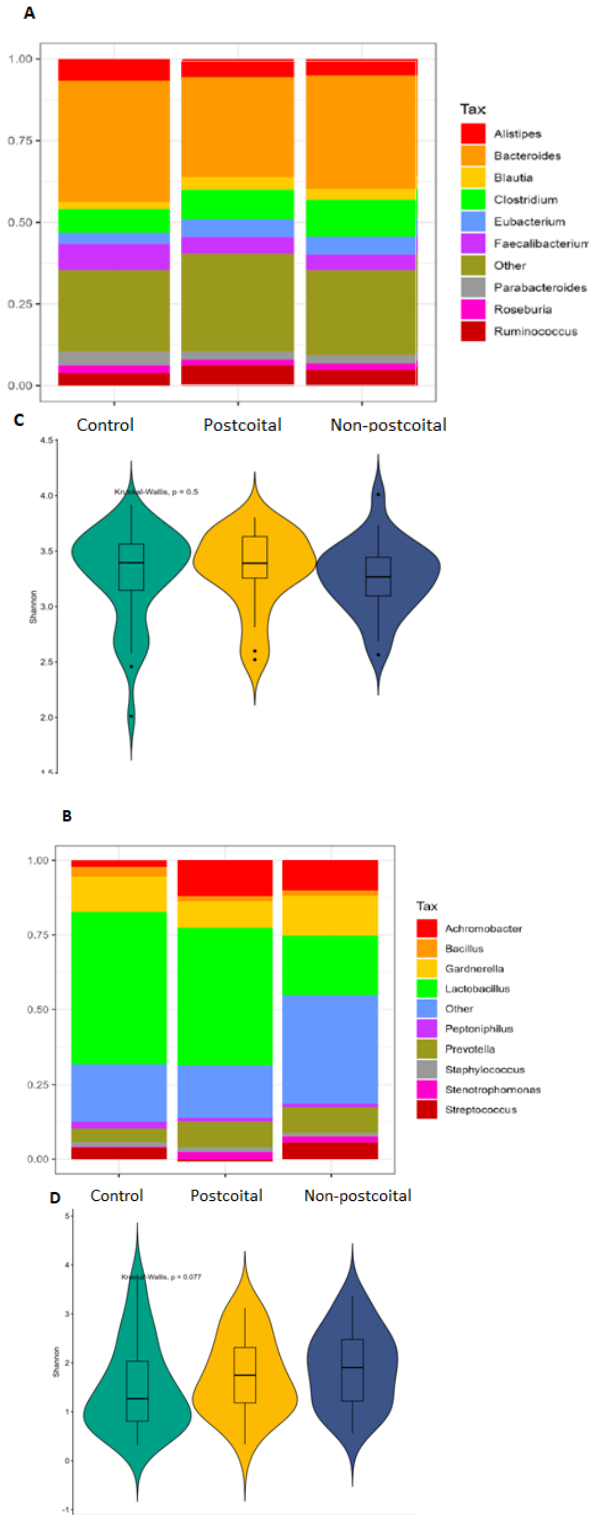
Introduction: Urinary tract infections (UTIs) represent one of the most prevalent bacterial infections. Gut-derived uropathogens can ascend via the vaginal tract and, also, colonize the bladder. Around 20% of women with an initial UTI develop recurrent UTI (rUTIs), defined as ≥3 episodes/year or 2 episodes into six months. Sexual activity is a known as risk factor, showing correlation with rUTI. This study aims to assess gut and vaginal microbiome dysbiosis in patients with rUTIs and their correlation with sexual activity.
Methodology: Shotgun metagenomic sequencing was conducted on 94 fecal and vaginal samples: 21 post-coital (episodes correlated with sexual intercourse), 29 non-postcoital (not correlated), and 44 controls. Reads were classified using GAIA. Abundance data were analyzed for diversity (Vegan), differential (ANCOM), and community state types (CSTs)(VALENCIA).
Results: Gut microbiome α-diversity was reduced in rUTI patients compared to controls, with the most pronounced decrease in the non-postcoital group. Taxonomic analysis revealed a higher abundance of taxa with proinflammatory potential, with vaginal origin, and with known uropathogens. These alterations were stronger in non-postcoital group, such as Enterobacter spp., and Clostridium spp. were notably enriched.
Vaginal microbiome α-diversity was significantly higher in rUTI patients compared to controls, especially in the non-postcoital group. A marked reduction in Lactobacillus spp. that alkalizes vaginal pH was observed in rUTI patients, whereas an increase of Gardnerella and Achromobacter correlated as dysbiosis markers. Lactobacillus crispatus dominance (CST-1), was exclusively retrieved in controls, while Bifidobacterium-dominated profiles (CST-IV) were unique to rUTI cases. Notably, Lactobacillus-type CSTs were markedly reduced in the non-post-coital group.
Conclusions: In women with rUTI, dysbiosis was characterized by taxa previously associated with gut inflammation, increased gut-vaginal microbial translocation, elevated levels of uropathogenic bacteria, and higher vaginal pH. These features suggest the presence of compromised microbial barriers, facilitating the migration of gut-associated microbes through the vaginal tract to the urinary system.
Dysbiosis was more pronounced in the No-postcoital group, suggesting that in the absence of sexual intercourse as a triggering factor, a greater degree of microbial imbalance may lower the translocation from gut for rUTI development.



A) *Compositional analysis of gut microbiomes of control (left), postcoital (center), and non-postcoital (right). The top nine genera identified are depicted and the remaining taxa detected are included in "others".* **B)** *Compositional analysis of vaginal microbiomes of control (left), postcoital (center), and non-postcoital (right). The top nine genera identified are depicted and the remaining taxa detected are included in "others".* **C)** *α-diversity analysis (Shannon index) of gut microbiomes of control (left), postcoital (center), and non-postcoital (right).* **D)** *α-diversity analysis (Shannon index) of vaginal microbiomes of control (left), postcoital (center), and non-postcoital (right).*

Diagnostics

PE20

Microbiota Transplantation as a Tool to Study New Biomarkers for Alzheimer's Disease

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Background: Alzheimer's Disease (AD) and diabetes share common pathological mechanisms, including immunological dysfunction and impaired metabolism of glucose, amyloid, and insulin. Recent evidence suggests that gut microbiota imbalances contribute to the early stages of AD. Fecal microbiota transplantation (FMT) has proven effective in animal models to understand the microbiota-disease relationship and alleviate AD pathology. However, optimal FMT protocols for AD studies remained underexplored, and the identification of reliable, non-invasive biomarkers for early AD diagnosis was urgently needed.
Methodology: The research was conducted in two phases: Optimizing FMT Protocols – Fecal microbiota samples from four groups of human donors (AD patients, diabetic patients, AD-diabetic patients, and healthy controls) were transplanted into murine recipients. Various FMT techniques were evaluated, including gut decontamination methods, administration routes, and dosing intervals. Impacts on gut microbial populations were assessed using 16S and 18S sequencing. Inflammation and anxiety-like behavior in recipient mice were also analyzed.
Microbiota and Behavioral Analysis – The optimal FMT method was applied, and behavioral patterns indicative of AD were measured. Cecum contents from transplanted mice were analyzed through 16S sequencing to identify microbial features associated with AD-related behaviors. Bioinformatics tools were used to correlate gut microbiota features with behavioral outcomes.
Results: The study successfully identified the most effective FMT protocols, which resulted in significant shifts in the gut microbiota of murine recipients. These shifts were correlated with behavioral changes indicative of AD pathology. Specific microbial features associated with the modulation of AD behaviors were identified, highlighting potential biomarkers for early AD detection.
Conclusions: This research contributed to the development of standardized FMT protocols for AD research and provided valuable insights into the role of gut microbiota in AD. The study identified microbiota features linked to AD pathology, facilitating the discovery of non-invasive, cost-effective biomarkers for early AD diagnosis and disease progression monitoring. The findings have important implications for diagnostic and therapeutic strategies in AD, offering new avenues for improving patient care.

PE21

Discovering Microbiome-derived Biomarkers For Tuberculosis Diagnosis (SULTAN Project)

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Background: Tuberculosis (TB) is a preventable and curable disease. Still, in most of the cases in endemic areas, the limitations of current TB diagnostics drive late diagnoses and excess mortality risk. We postulate and expect that individuals with TB will show unique microbial and host-derived molecular signatures in their stools. We anticipate that we can exploit the microbiota to discover new TB biomarkers.
Methods: The SULTAN project aims to discover new TB diagnostic markers by investigating the interaction between the microbiome and host in TB susceptibility. We plan to identify biomarkers derived from the microbiome and host in feces to increase diagnostic precision, validate these markers through multi-omic and aptamer-based methodologies, and explore new pathways involved in TB susceptibility.
Through a comprehensive multi-omics analysis (including metagenomics, proteomics and metabolomics) of stool samples from confirmed TB cases and suspect controls in a discovery cohort (Mozambique, n=317), together with aptamer-based enrichment strategies, we aim to identify and validate novel microbiome-biomarkers. This validation will be performed in internal (Uganda, n=100) and external (Spain, n=100) cohorts using targeted techniques (e.g., ELISA, qPCR, HPLC).
The project has secured access to the sample collection and metagenomic analyses through collaborations with the European STOOL4TB initiative (ECDTP Project), CIBER projects (IM22/INF/17 and IM23/INFEC/4) and ISCIII (PI24/00078; FOR23/00046).
Expected results: SULTAN aims to identify biomarkers for TB diagnosis and expand knowledge on the relationship between the microbiome and TB. We seek to facilitate the development of TB biomarkers and better understand the microbiome's influence on TB susceptibility.
Conclusion: There is a need for improvement in current diagnostic methods for tuberculosis, particularly affecting children and people living with HIV in high-prevalence regions. In these populations, TB is underdiagnosed, which drives an increased mortality risk. There is growing evidence on the role of the microbiota in the pathogenesis of tuberculosis, as well as the response to therapy and post-treatment outcomes. The discovery of biomarkers in the microbiota combined with omics techniques could improve diagnosis in the affected areas by this disease and prevent mortality, especially in pediatric and immunocompromised populations.

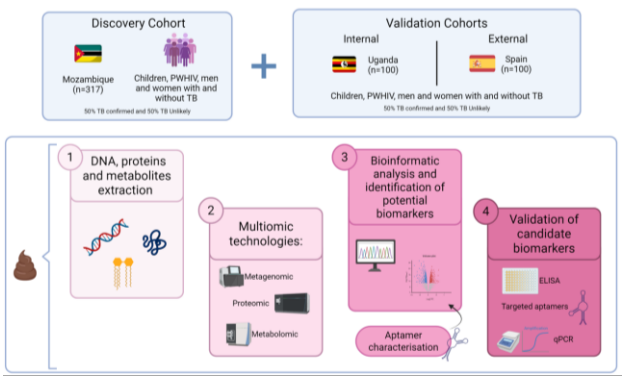

Figure 1. Discovering Microbiome-derived Biomarkers for Tuberculosis Diagnosis

PE22

Development of a GAIA 3.0 Module for Pathotyping Escherichia coli Using Shotgun Metagenomics

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Escherichia coli is a commensal bacterium located in the human gastrointestinal tract. However, certain pathogenic variants, known as E. coli pathotypes, have acquired virulence factors allowing them to cause various diseases. Among these pathotypes are EHEC, UPEC, EPEC, ETEC, EAEC, and DAEC, which are classified based on their distinct virulence mechanisms, clinical manifestations and genetic markers. Accurate identification of these pathotypes is crucial for diagnosis and public health interventions.

Despite advances in shotgun metagenomics, distinguishing E. coli pathotypes or differentiating them from closely related species like Shigella spp. is a significant challenge due to their genomic similarities. To address this, we propose a novel method developed within the GAIA 3.0 software (integrated in Sequentia Biotech multiomics environment "SequentiaHub"), which obtain not only taxonomic data from the GAIA pipeline (originally described in https://www.biorxiv.org/content/10.1101/804690v1) but also incorporates a brand-new module specifically designed to retrieve key information about these pathotypes, providing a detailed analysis of the strains.

Our method involves analyzing sequencing reads that align with a comprehensive database with over 3,500 E. coli and Shigella genomes. These reads are assembled into contigs using SPAdes, followed by a gene prediction with Prodigal and sequence alignment versus a custom virulence factor database using DIAMOND. Subsequently, the coverage of key genes associated with specific pathotypes and virulence mechanisms (e.g., stx1, stx2, eae, tir, espA, hlyA, pap, fim) is evaluated.

Finally, to test and fine-tune our method, we designed simulation tests and analyzed real metagenomic data from public databases. Moreover, statistical analyses were performed, calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The results showed a high sensitivity and specificity providing an overview of the system's performance. While the simulation was based in the core genome, in real scenarios, plasmid-borne genes, such as ehxA and toxB, would help to discriminate more accurately pathotypes improving the PPV by identifying specific virulent pathotypes.

In conclusion, this method enables efficient identification of pathogenic E. coli strains through shotgun metagenomic analysis with the potential to be applied to a wide range of pathogens in future studies.

Emerging Areas

PE23

MORELIA study: Safety, Engraftment, and Immunomodulation with FMT in Advanced Lung Cancer Treated with Immunotherapy

Laura Martín-Pedraza[1], María Eugenia Olmedo García[2], Samuel García-Huete[1], Rafael Bargiela[3], Ana Del Amo-de Palacios[1], Ana Gómez Rueda[2], Claudio Diaz[1], Rosa del Campo[4], Miguel García Pardo[2], Yolanda Lage[2], Elena Moreno[1], María Fons-Contreras[1], Manuel Ferrer[3], Pilar Garrido López[2], Sergio Serrano-Villar[1]

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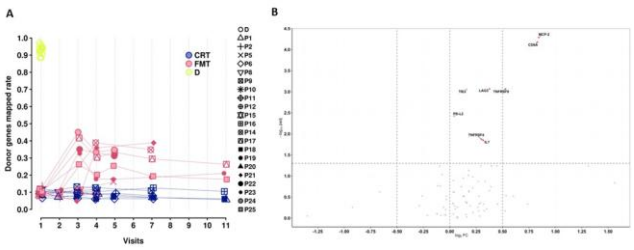
Background: Lung cancer remains one of the leading causes of mortality worldwide, and immunotherapy has revolutionized its treatment and prognosis. Preclinical models have shown that the gut microbiome modulates the efficacy of immune checkpoint inhibitors, suggesting that microbial composition may stratify patients into responders and non-responders. Fecal microbiota transplantation (FMT) has enhanced immunotherapy efficacy in animal models, but its safety and effectiveness in lung cancer remain largely unexplored.

Methodology: MORELIA is a pilot randomized, open-label clinical trial (NCT04924374) that enrolled 20 patients with stage IV non-small cell lung cancer (NSCLC) initiating anti-PD-1/PD L1 monotherapy after first-line chemotherapy. Patients were randomized to receive either oral FMT capsules (induction followed by daily maintenance through week 21) or no FMT (control). Capsules were derived from a "gold" donor with a microbiota profile previously associated with immunotherapy responsiveness. FMT recipients underwent antibiotic preconditioning. We assessed safety and tolerability of FMT, donor microbiota engraftment (shotgun metagenomic sequencing), and plasma proteomic shifts (96 immuno-oncology proteins using a proximity extension assay).

Results: FMT was well tolerated, with no severe FMT-attributable adverse events. Most FMT recipients exhibited substantial donor engraftment, acquiring approximately 40% of the donor's microbial genes, which persisted throughout the study. Proteomic analyses revealed changes in plasma proteins associated with antitumor activity, including increases in CD8A, MCP-2 (CCL8), and TNFRSF9) in the FMT group, compared to controls.

Conclusions: FMT appears safe and feasible in patients with NSCLC and shows potential immunomodulatory effects that may enhance responses to immunotherapy. These findings support further investigation in larger trials.



A. *Donor genes mapped rate of FMT patients along visits.* **B.** *Volcano Plot of immunomodulatory proteins that appear significantly increased in FMT vs CONTROL with a log2FC higher than 0.5.*

PE24

Exploring The Impact Of Microbial Metabolites On Macrophage Responses To Respiratory Bacterial Pathogens And Cigarette Smoke

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Chronic obstructive pulmonary disease (COPD) affects >250 million people worldwide causing ~3 million deaths annually. Epidemiological studies suggest that high-fibre diets reduce COPD risk; however, the underlying mechanisms of protection remain unclear. A new connection between the gut and the lungs has emerged defining the Gut-Lung axis of immune regulation that links intestinal dysbiosis to worsening of inflammatory lung conditions and respiratory infections. However, how the gut microbiome affects COPD phenotypes remains unknown. Short chain fatty acids (SCFA) derived from microbial fermentation of dietary fibre emerged as important modulators of lung function.
We show that the SCFA butyrate protects against murine pneumococcal pneumonia promoting bactericidal phenotypes in macrophages. Here we hypothesize that gut dysbiosis linked to recurrent antibiotic use in COPD patients results in loss of SCFA producing bacteria, increasing the risk for recurrent infections and exacerbations of COPD. Increased respiratory colonisation of COPD patients with bacteria including *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* has been linked to COPD exacerbations. We propose that by promoting bactericidal phenotypes in macrophages butyrate may prevent infection triggered exacerbations. Using gentamicin protection assays, we evaluate the killing capacity of human alveolar macrophages isolated from COPD patients stimulated ex vivo with butyrate and infected with pneumococci and *P. aeruginosa*. These data show that harnessing the power of the gut microbiome may aid in developing nutritional and microbiome-based therapies for improved clinical outcomes in respiratory infections and inflammatory lung disease.

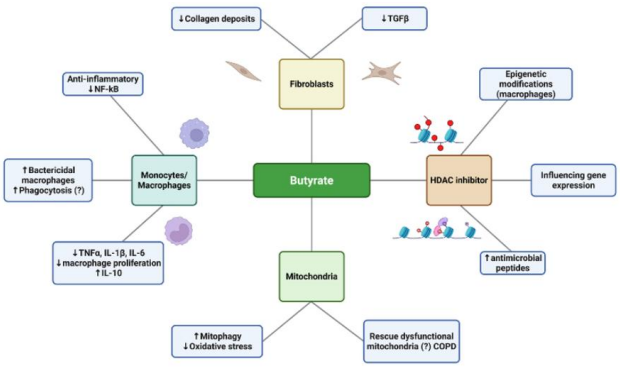

Figure 1. Pleiotropic effects of Butyrate

PE25

Microbes On The Clock: Linking Gut Dysbiosis And Immune Gene Signatures To Metabolic Disfunction In Chronodisrupted Rats

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Background: Circadian rhythms are daily biological cycles that regulate not only sleep and metabolism but also immune function and microbial composition. Disruption of these cycles contribute to detrimental metabolic outcomes. However, its impact on the gut microbiota and immune gene expression, and how these may collectively regulate systemic metabolism remains unclear.
Objective: To investigate how circadian disruption, induced by altered light-dark cycles, modifies feeding behavior and triggers downstream changes in the gut microbiota, immune gene expression, and plasma metabolic markers in chronodisrupted rats.
Methods: Fifteen Wistar rats were divided into a control cohort subjected to a standard 12-hour light/12-hour dark cycle and a chronodisrupted cohort following an 11-hour light/11-hour dark cycle over an 8-week period. Light-dark food intake pattern was monitored weekly. Plasma biochemical parameters were analyzed using commercial kits, and gene expression in PBMCs was assessed via RNA-sequencing. Elastic net regression was used to identify associations between microbial taxa, gene expression, and metabolic markers.
Results: Altering the light-dark cycle was sufficient to disrupt feeding patterns, a key regulator of circadian rhythms. This shift was accompanied by changes in specific microbial genera, including Lachnospiraceae NK4A136 group, Muribaculum, and Eubacterium siraeum group, which showed significant associations with lipid and glucose metabolism, as well as insulin levels. These findings suggest that circadian disruption influences systemic metabolism, in part through microbiota-mediated mechanisms. In parallel, transcriptomic analysis identified genes such as Tube1, Cbx2, Amhd2, and Tmpo as consistent predictors across models, highlighting their potential roles in immune-metabolic regulation under chronodisrupted conditions.
Conclusion: Our findings highlight that light cycle alteration alone can disrupt feeding rhythms and lead to systemic metabolic changes. This integrative analysis supports a microbiota-immune-metabolic axis influenced by circadian misalignment, offering insights into microbiota-host interactions in chronobiology-related metabolic disorders.

Methods

PE26

Benchmarking Alpha Diversity in Clinical Microbiome Research: What Best Reflects Dysbiosis?

Anna Sayol-Altarriba[1,2,3], Natasa Mortvanski[2,4], Andrea Aira[2,3], Elisa Rubio[3], Anna Villasante[3], Gregori Casals [5], Alex Soriano[6], José Luis Villanueva-Cañas[7], Climent Casals-Pascual[1,2,3]

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Background: Gut dysbiosis, typically described as a loss of bacterial diversity and impaired community functionality, has been associated with a range of diseases. However, it remains unclear whether dysbiosis is a biologically meaningful concept. Alpha-diversity metrics, which estimate microbial richness and evenness, are commonly used to summarize microbiome diversity, yet there is limited guidance in the literature on their optimal selection or benchmarking. This study evaluates the ability of these metrics to serve as meaningful indicators of health status, by identifying the most suitable metric(s) for assessing microbiome diversity.
Methods: We compared alpha diversity metrics (based on richness, evenness or both) across patient datasets, including the American Gut Project (AGP) and a healthy control group from the stool bank at Hospital Clínic de Barcelona (HCB). External datasets were obtained from Qiita and BioProject and processed using QIIME2. Our analysis focused on individuals with inflammatory bowel disease (IBD) and those with Clostridioides difficile infection (CDI).
Statistical analysis involved the construction of a Random Forest model to determine which metrics were most predictive of health status. A modified t-test was used to evaluate whether an individual sample's alpha diversity significantly differed from that of a control population. Spearman's correlation with Benjamini-Hochberg corrections for multiple comparisons was applied to explore associations between alpha diversity indices and short-chain fatty acids (SCFAs) within the HCB healthy cohort.
Results: Healthy gut microbiota consistently show greater richness and evenness compared to individuals with IBD or CDI. In CDI cases, richness was significantly reduced but recovered following fecal microbiota transplantation (FMT). Among diversity measures, the Gini index most effectively distinguished healthy from unhealthy samples across datasets. Combining richness and evenness metrics enhanced classification accuracy, particularly in cases of severe dysbiosis such as CDI. Additionally, SCFAs were inversely correlated with diversity measures.
Conclusion: Diversity indexes may be valuable for evaluating interventions aimed at improving gut health like FMT. The Gini index emerged as a robust evenness metric while Chao1 and Faith PD are reliable richness metrics. SCFA provide functional insights but, if considered alone, are an imperfect proxy for evaluating microbiome health?

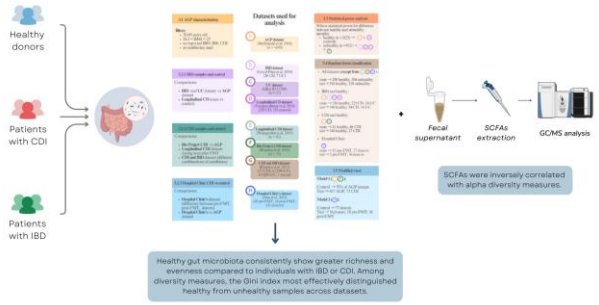

Figure 1. Benchmarking alpha diversity in clinical microbiome research: what best reflects dysbiosis?

PE27

Assessment Of Gut Microbiome Eubiosis/Dysbiosis Based On DNA Metabarcoding Data By Using Machine Learning Approaches

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The trillions of microorganisms living in and upon the human body and their theatre of activity are collectively known as the "human microbiome". This amazingly complex ecosystem evolved in synergy with the human host creating a unique biological entity defined as "holobiont". Over the past two decades, remarkable advances in metagenomics, driven by the advent of next-generation sequencing (NGS), have highlighted how maintaining a high level of gut microbial diversity (eubiosis) is essential for the well-being of the holobiont4. On the other hand, many diseases seem to be related to the disruption of this balance, leading to a condition of dysbiosis usually featured by a reduced diversity.
Given the close relationship between eubiosis/dysbiosis conditions and host health, profiling the human gut microbiota is a powerful tool for early detection and prognostic assessment of several diseases. In this scenario, unlocking the full potential of microbiota investigation by combining the strengths of metagenomics and artificial intelligence has been the target of our research activity. Specifically, we used 16S DNA metabarcoding raw data available in public repositories to carry out a robust comparative meta-analysis. More than 5,000 datasets belonging to healthy and unhealthy subjects (2,282 and 2,820 respectively) were used to train supervised learning models (i.e., Random Forest) aiming to discriminate eubiosis and dysbiosis microbial signatures.
This approach allowed us to achieve remarkable values of predictive accuracy beyond the current state of the art, even exceeding 80%. Finally, we also exploited the potential of Explainable Artificial Intelligence (XAI), and in particular the SHAP algorithm, to provide an accurate assessment of the impact of different variables on the final outcome and thus, to obtain a deeper understanding of the specific contribution of each taxon in determining health outcomes.

PE28

B-GUT Reference Genome Database Improves Biomarker Discovery And Fungal Identification In Gut Metagenomes

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Taxonomic assignment of sequencing reads is a key step in metagenomics studies, impacting all subsequent analyses. A crucial aspect determining the accuracy of this assignment is the design and quality of the underlying reference genome database. Notably, current databases generally offer a poor and non-curated representation of fungal organisms, despite their ubiquity and relevance in the human microbiome. To address this and other existing gaps we designed B-GUT, a custom database for kraken2-based classification that integrates a broad and curated set of fungal reference genomes with the telomere-to-telomere human genome reference, and previously available gut-specific, curated sets of bacterial and archaeal genomes. We identified high levels of contamination in some publicly available fungal genomes and significant cross-mapping of human reads in fungal reference genomes, highlighting the need for curation and accurate host-read filtering.
We benchmarked our genome curation method and the resulting database by using mock communities. Finally, we showcased the use of B-GUT in a colorectal cancer context. The use of B-GUT resulted in a significant improvement of the results, providing more precise taxonomic assignments and a more accurate detection of differential abundant taxa, both for bacterial and fungal organisms.

PE29

Elucidating The Transmission Of The Neuroactive Potential Of The Gut Microbiome With Microneuronet

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Medicine and Life Sciences Department Universitat Pompeu Fabra (MELIS-UPF).

Background: The intestinal microbiome plays a vital role in human health by influencing both physiological and neurological functions. A key mechanism is the microbial metabolism of neuroactive compounds, directly impacting brain activity via the gut-brain axis. Recent metagenomic and computational advances have enabled deeper insights into the role of gut bacteria producing and metabolizing neurotransmitters like serotonin and GABA. However, there is a significant gap on how these metabolic pathways are transferred between microbes and transmitted across individuals due to the absence of bioinformatic tools to systematically track them.
Objectives: The main goal is to assess the transmission of the neuroactive potential of the microbiome by developing new bioinformatics methods, with emphasis on:

1. Analysing the phylogenetic distribution of microbial genes involved in the metabolism of neuroactive compounds
2. Developing a computational method to systematically trace the transference/transmission of these genes within and between microbiomes
3. Tracing the transmission of neuroactive potential in large-scale metagenomic datasets, focusing on both within and between microbiomes

Results: The project will use GBM2 to generate an extensive catalogue of microbial genes involved in neuroactive compound metabolism, providing a reference database for understanding microbiome-brain interactions. A novel bioinformatic tool will be created to identify the transference and transmission of genes with neuroactive potential within microbiomes and between individuals; allowing a precise mapping of gene transmission in metagenomic data and tracing neuroactive gene flow between microorganisms and across hosts in large datasets. We will explore the role of social and environmental factors in the transmission of these microbial traits. Preliminar data shows the transference of genes involved in GABA synthesis among Bacteroides. In addition, single nucleotide variant rates were significantly positively associated with geographical distance among home locations, suggesting microbial GABA synthesis potential could be transmitted across closely-interacting individuals.
Conclusions: The project will reveal how neuroactive potential is transferred within and between microbiomes by developing new bioinformatics tools to trace gene exchange. It will also be the first to track neuroactive gene transmission across populations, shedding light on the gut-brain axis.

PE30

Microbiome Profiling In Colorectal Cancer Patients: A Comparison Of Nanopore And Illumina Technologies For 16S Metabarconding

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Alterations in microbiome profile have been described to contribute to a wide variety of diseases. One of them is colorectal cancer (CCR) for which a group of anaerobic bacteria has been found in tumours and faeces of early-stage patients, but not in healthy individuals. Therefore, the presence of these bacteria could be used as biomarkers for early non-invasive diagnosis.
The detection of these bacterial biomarkers typically involves analysing small regions of the 16S rRNA gene (e.g. V3V4) through short-read technologies like Illumina, obtaining genus-level results. However, recent developments in third-generation sequencing, such as Oxford Nanopore Technologies' (ONT) new R10.4.1 chemistry and improved basecalling models, are beginning to allow for a more complete and accessible species-level analysis through full-length 16S rRNA gene sequencing (spanning regions V1-V9).
This study compares both technologies on the microbiome analysis of faeces from 123 subjects. We compared Illumina-V3V4 with DADA2 and QIIME2 vs. ONT-V1V9 with Emu using multiple Dorado basecalling models and databases (SILVA vs. Emu's Default database). Results showed that different basecalling methods resulted in similar outputs but database choice with Emu had a notable effect. Emu's Default database gave a higher diversity and identified more species than using SILVA (p-value<0.05). However, it overconfidently classified at times what should be an unknown species. Bacterial abundance between of both technologies at the genus level correlated well ($R2 \geq 0.8$) but ONT-V1V9 identified more specific bacterial biomarkers for CCR. In conclusion, full 16S rRNA V1V9 sequencing through Oxford Nanopore and its new R10.4.1 chemistry achieved accurate species-level bacterial identification, facilitating the discovery of new precise disease-related biomarkers and increasing the taxonomic fidelity.

PE31

Functional Metagenomic Profiles Associated With Inflammatory And Functional Bowel Diseases.

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Background: Profiling bacterial molecular activity can provide evidence on the role of bacteria in digestive diseases, beyond the traditional taxonomic classification. Molecular functions can be described in terms of functional orthologs from the KEGG Orthologs (KOs) database. By estimating the possible metabolic roles of the microbiome, this method allows to determine which bacterial functions are more prevalent in each patient group.
Methodology: KOs were obtained from 16s rRNA gene sequencing data of stool samples using PICRUSt2. These predicted functional profiles were compared between the different clinical groups using pairwise comparations from ALDEx2, making possible to identify microbial functions that differ between groups and may be linked to inflammation and gastrointestinal symptoms.
Results: A total of 27 KOs were identified with a p-value < 0.01 in the comparison between patients with ulcerative colitis (UC) in remission and those with active UC. Comparing UC in remission and UC with irritable bowel syndrome (UC&IBS) 27 KOs were obtained with p-value < 0.001. Additionally, 40 KOs with p-value < 0.0001 were identified in comparing IBS and UC&IBS groups.
Conclusions: The identified KOs may reflect microbial pathways that can be potentially involved in inflammatory and symptom expression. The results suggest that predicted microbial functional profiles differ between both clinical groups (UC and IBS).

PE32

Study Of Adherent-invasive Escherichia Coli Host-pathogen Interactions Using Human Organoid-derived Monolayers

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Adherent-invasive Escherichia coli (AIEC) are capable to adhere and to invade intestinal epithelial cells and to survive and replicate within macrophages; however, the mechanisms associated to its pathogenicity remain not fully understood. We have infected primary differentiated organoid-derived monolayers (d-ODMs) from human intestinal epithelium to better understand AIEC pathogenicity and host response.
We established a protocol to assess the adhesion and invasion capabilities of the AIEC LF82 strain, the probiotic E. coli Nissle1917 and the commensal E. coli K12 in the d-ODMs. Bacterial genes involved in adhesion, amino acid metabolism, colanic acid biosynthesis and copper homeostasis, as well as eukaryotic genes for bacterial sensing, tight junctions, cytokines, and autophagy were quantified by qPCR at 6, 12 and 24 hours post-infection (p.i.). Additionally, at 6 hours p.i., we performed Dual-RNA sequencing of the cellular and supernatant fractions of the infected cultures.
AIEC LF82 was invasive, whereas E. coli Nissle 1917 and E. coli K12 were unable to invade intestinal epithelial cells of d-ODMs. At early infection time (6 hours p.i.), both AIEC LF82 and non-AIEC (E. coli K12) strains promoted a response in eukaryotic cells, but it was not strain-specific. Interestingly, after longer incubation (24 hours p.i.), LF82 specifically induced the expression of genes associated with barrier function, autophagy and cell receptors.
Additionally, bacterial genes related to amino acid metabolism, colanic acid biosynthesis, and fimbriae were upregulated in LF82 at 24 hours. Transcriptomic analysis revealed LF82-specific differentially expressed genes (DEGs) when compared to non-AIEC strains at 6 hours p. i.. Up to 209 genes were upregulated in LF82 in both cellular and supernatant fractions, highlighting their potential as molecular markers for AIEC detection. Furthermore, 438 DEGs were specifically in LF82 when the cellular and supernatant fractions were compared, providing insights into the pathogenic mechanisms of this AIEC strain.

Overall, d-ODM is a viable model to investigate the molecular mechanisms underlying AIEC pathogenicity and its impact on epithelial cell function.

PE33

Biome-specific Genome Catalogues Reveal Functional Potential Of Shallow Sequencing

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Background: The use of 16S rRNA metabarcoding is limited by known taxonomic biases, and especially with functional inference methods based on such data. Shallow shotgun sequencing (SSS) is a cost-effective and taxonomically high-resolution alternative to 16S-based methods, but functional inference results are limited by low sequencing depth. Currently, only a small fraction of reads in new samples from highly studied microbiomes remain taxonomically unassigned when mapping to biome-specific genome databases. Thus, we devised a functional inference method where SSS reads are mapped against such MGnify databases and extrapolated into their precalculated functional profiles.
Methods: We used three sample datasets from red junglefowl gut, mice gut, and human gut, containing matched deep shotgun metagenomic, and 16S rRNA gene amplicon sequencing data. Our Shallowmap pipeline was optimized and benchmarked both taxonomically and functionally with varying sequencing depths against full deep sequencing data (>10M reads), another SSS-based tool, and two 16S rRNA gene amplicon-based methods. An additional human gut deep shotgun sequencing data set was subsampled to 1M reads and analyzed with Shallowmap to replicate the original results.
Results: The taxonomic and functional profiles produced by Shallowmap with sequencing depth of >0.5M reads closely agreed with the results of the full deep sequencing data obtained through direct inference in all biomes. The profiles produced with amplicon sequencing were highly dissimilar to both the Shallowmap and the deep sequencing results. Furthermore, Shallowmap was able to replicate the results of a previous study on functional differences in the fecal microbiome between high and low trimethylamine N-oxide producing participants, using only <2% of the original deep sequencing data.
Conclusions: The Shallowmap tool is a powerful approach for functional prediction, which approximates the functional information of a deep-sequenced metagenome while using only a fraction of the data. SSS combined with Shallowmap could be a stand-in replacement for 16S rRNA metabarcoding with an increased taxonomic and functional resolution, and lower bias. The pipeline can be also used with e.g., samples with low microbiome read depth caused by high host DNA contamination, and further adapted to eukaryotic genomes. However, we advise caution when analysing sample types which are yet to be represented in the biome-specific genome databases.

PE34

Reproducibility Of The Food Frequency Questionnaire (FFQ) For The Study Of Intestinal Microbiota In The Spanish Population

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Understanding the role of diet in modulating the gut microbiota requires the collection of high-quality and reliable dietary data, particularly in longitudinal studies designed to track changes in microbial composition over time. In this context, accurate dietary assessment becomes essential in microbiota–cancer research. However, methodological limitations of tools such as food frequency questionnaires (FFQs) or dietary recalls present challenges regarding precision and reproducibility. It is therefore essential to translate and culturally adapt these instruments and subsequently validate them in the target populations and research settings. This study aims to assess the reproducibility of a culturally adapted FFQ designed to evaluate diet–microbiota interactions in colorectal cancer (CRC) within the Spanish population.

We conducted a single-centre observational study at Vall d'Hebron Hospital (Barcelona, Spain) to assess the reproducibility of the self-administered FFQ in patients with CRC. The questionnaire consists of 223 questions on food frequency and consumption, structured into 250 response options, and implemented on the REDCap platform using radio buttons and check boxes. Participants completed the questionnaire twice, approximately two weeks apart. Reproducibility was assessed using Spearman's rank correlation coefficient (rho), calculated with RStudio.

Sixty-one patients with CRC participated, including 28 females and 33 males, with a median age of 66 years. The median interval between the two FFQ administrations was 20 days (5-50). Eleven variables were excluded due to lack of variability (standard deviation = 0), and 20 due to missing value, absence of responses at either time, or being free-text fields. Among the remaining variables, the median rho coefficient was 0.559, indicating moderate agreement. Notably, 88 items achieved a rho above 0.6, suggesting strong reproducibility across administrations. These findings indicate that the adapted FFQ consistently captures dietary patterns in this population.

Overall, the FFQ demonstrated acceptable reproducibility for assessing dietary intake in Spanish patients with CRC. The observed moderate-to-strong correlations for most items support its utility in dietary pattern analysis within microbiota and cancer research. This tool represents a valuable resource for future research on dietary exposures in Spanish populations and their relationship with microbiota composition and disease outcomes.

PE35

Zebrafish Gut Epithelium Dynamics During Inflammation

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The gut is the central scene where the enteric nervous system, the microbiome, and the local immune response interact to ensure an individual's overall health. Natural biological processes, such as inflammation, can disrupt the delicate balance of gut functions, inducing several adverse effects on the gut(1). The disturbance of intestinal homeostasis affects other organs in the body, causing, for example, neurological disorders. We aim to decipher conserved molecular mechanisms involved in intestinal inflammation and how the microbiota and its metabolites contribute. We are interested in nanoplastics as their concentration in food and water is increasing, and they are suspected of inducing intestinal inflammation(2). However, it is unknown how these particles interact with intestinal cells and how we could prevent the damage they cause.

Using zebrafish (Danio rerio) as a model organism allows us to investigate gut inflammation within a controlled and genetically tractable environment, and its impact on an entire organism. Additionally, the gut microbiota can be easily modulated in larval zebrafish, enabling the study of bacterial communities and their metabolites(1). We induce inflammation with chemicals, nanoplastics, or both and measure gut motility, permeability, and immune response during different developmental periods. By extracting RNA, we will identify potential targets and use them as markers to assess whether specific interventions or bacterial strains may help zebrafish recover from gut inflammation.

After identifying targets and some potential interventions in the zebrafish model, we will compare whether the same dynamics are conserved in the mouse model in collaboration with Björn Schröder's laboratory. The conserved mechanisms are more likely to be preserved in humans and are helpful from a therapeutic point of view.

This project, which has recently started, will enrich our knowledge of gut inflammation dynamics, the role of gut microbiota, and the impact of nanoplastics on intestinal inflammation. The results obtained from this project can have relevant future implications for developing novel intervention strategies.

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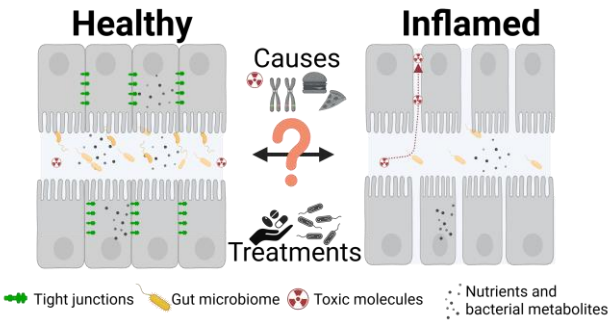

Figure 1.

Microbiome Ecology

PE36

Social Transmission Of Gut Microbiome In Heterogenous Stock Rats

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There is increasing evidence that the gut microbiome has a strong impact on the health of its host1,2. The gut microbiome is seeded at birth3,4. Over time, additional microbes are acquired from the environment and social partners. In humans and mice, it has been shown that individuals in physical contact share some of their gut microbiome5,6,7. In mice, microbial transmission has been shown to result in the transmission of disease or the acquisition of disease resistance8. These observations have generated greater interest in understanding the communities of microbiome transmitted and their corresponding transmission patterns.
In this study we aimed to quantify and characterize the social transmission of commensal microbes in a rodent model, leveraging on increased contact facilitated by natural behaviors such as allo-coprophagy and allo-grooming. To do this, we profiled the fecal and cecal microbiome of 2540 Heterogeneous Stock (HS) rats co-housed in pairs or trios for a period of 2 to 6 months. The microbial communities were profiled down to the species-level using MetaPhlAn 4.1.1 and StrainPhlAn 4.1.1 to strain-level using the latest version of the ChocoPhlan database, which includes HS rat data. This approach allowed us to characterize strain sharing events and transmission patterns in laboratory rats. This work lays the foundation for future investigations into how individual microbial traits influence transmission and the implication of microbiome transmission for the host's health and disease.
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PE37

Impact Of HPV Status And Vaccination On The Oral Microbiome Composition

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Background: Human Papillomavirus (HPV) infection is strongly associated with various cancers in the anogenital and head and neck areas. While HPV vaccination has significantly reduced the incidence of cervical cancer, its impact on the oral microbiome is less understood. This study investigates the influence of HPV presence and HPV vaccination on the oral microbiome , aiming to identify changes in micro-organisms with known roles in health and disease or potentially involved in carcinogenesis.
Methodology: From a cross-sectional study on oral HPV prevalence in Catalonia including 1610 young adults aged 18-29 years, we selected all HPV-positive samples and a random selection of HPV-negative (paired by sex and HPV vaccination status 2:1 or 3:1). We analysed 16S oral microbiome samples from 143 subjects and stratified by HPV status and HPV vaccination. Diversity analysis included alpha with raw counts and beta (PERMANOVA) with centered log-ratio transformed data. Differential abundance analysis was performed using Linear Discriminant Analysis (LinDA).
Results: No significant differences in alpha-diversity indices were detected. Beta-diversity analysis and PERMANOVA also did not identify any significant difference when stratifying the data for HPV and vaccination status. Differential abundance analysis identified microbial taxa differing significantly between the different subsets. In detail, Neisseria oralis was significantly more abundant in HPV-positive individuals, consistent with previous associations of Neisseria species with HPV-related cancers. When excluding the vaccinated subjects in the comparison among different HPV status, Neisseria perflava was identified as more abundant in the control group, in line with previous findings and suggesting an implication in health regardless of the vaccine. In the HPV-negative individuals, when comparing subjects with different vaccination status, a Streptococcus species showed increased abundance in the non-vaccinated group, species already associated with HPV+ status in other studies, suggesting a potential vaccine-related shift in oral microbiota.
Conclusions The study detects significant microbial variation correlated with HPV status and vaccination, identifying bacterial species previously associated with healthy control or with oral and cervical cancer. These findings suggest that the oral microbiome might reflect pathological changes related to HPV or protective effects conferred by vaccination. It suggests potential biomarkers for HPV-related oral health risks and vaccine efficacy. Further research incorporating clinical variables such as oral hygiene practices and comorbid conditions will enhance understanding and clinical applicability.

Therapeutics

PE38

Sex-Specific Modulation of Gut Microbiota in Wistar Rats Following Beta-Glucan Supplementation from Cava Lees

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Background: The gut microbiome plays a crucial role in host health through complex host-microbe interactions. Beta-glucans, structural polysaccharides found in yeast cell walls, have emerged as promising modulators of immune function and microbial ecology. Cava lees, a by-product of sparkling wine production composed of Saccharomyces cerevisiae cell walls, represent a rich source of beta-glucans that could be upcycled for nutritional and therapeutic applications.
Methodology: Twenty-four Wistar rats (12 males, 12 females) were randomly divided into control and treatment groups. The treatment group received daily doses of 2000 mg lees/kg body weight for 14 days. Shotgun metagenomic analysis was performed to assess microbial composition.
Results: The baseline healthy rat microbiota comprised over 9 phyla, with Bacillota (64-72%) and Bacteroidota (23-32%) being most abundant. While no significant differences were observed between male and female rats at the global community level, sex-specific variations were evident at the family level. Males exhibited higher relative abundances of Lactobacillaceae, Bacteroidaceae, Muribaculaceae, and Akkermansiaceae, while females showed higher abundances of Oscillospiraceae and Rickenellaceae. The observed increases in microbial diversity following cava lees supplementation in healthy rats suggests potential preventive benefits
Conclusions: Working with healthy individuals provides a clear understanding of the normal, baseline microbiota composition and function before any intervention. This baseline characterization is essential for accurately interpreting the effects of dietary interventions like beta-glucans from cava lees. This study demonstrates the remarkable plasticity of the gut microbiome and its responsiveness to dietary modifications. Beta-glucans from cava lees appear to create a favorable environment for beneficial bacteria, with sex-specific modulation of certain bacterial families. These findings provide a solid foundation for future translational research in humans. As a next step, carefully designed human clinical trials should investigate whether similar microbiota modulation occurs in response to cava lees supplementation across diverse human populations. Such trials should consider sex-specific responses. Furthermore, the upcycling of cava lees as a source of bioactive compounds represents an environmentally sustainable approach to developing novel prebiotic interventions.

PE39

Protein Hydrolysates as Modulators of Gut Microbiota and Blood Pressure Following Long Term Consumption in SHR

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Background: Hypertension is one of the leading causes of cardiovascular disease and premature death worldwide. The gut microbiota (GM) has emerged as a new target for its prevention due to its role in blood pressure (BP) modulation. In a previous study, we demonstrated that acute administration of two protein hydrolysates (PHs) modulated the fecal microbiota of spontaneously hypertensive rats (SHR) and exerted antihypertensive effects that were lost when treated with antibiotics, indicating a possible role of the microbiota.
Methods: Twenty-four 11-week-old male SHR rats were divided into three groups according to the treatment received orally on a daily basis for 4 weeks (n=8/group): 1) control (water), 2) animal-derived HP (H1), and 3) plant-derived HP (H2). The HPs were orally daily administered at a dose of 55 mg/kg of body weight. BP was measured with an HD-S10 sensor previously inserted in the aorta.
Results: The results showed that especially H1 but also H2, exhibited antihypertensive effects during the first two weeks of treatment. However, these effects were lost at the end of the experiment.
In terms of GM, a significant increase in the α and β diversity of microbial species was observed in H1 treated rats. Additionally, rats administrated with both PHs showed differences in GM composition compared to the control rats, increasing the Firmicutes/Bacteroidetes ratio.
Conclusions: In conclusion, HPs, especially H1, have prebiotic potential, but their antihypertensive capacity is limited. Further studies are needed to understand the underlying mechanisms and to evaluate their effects under other conditions of the metabolic syndrome.
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PE40

First Stool Bank In Catalonia, Spain: Establishment And Initial Impact

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Background: Faecal microbiota transplantation (FMT) has been introduced as an effective treatment for recurrent Clostridioides difficile infection (rCDI) in the last decade, generating a need for the establishment of stool banks. Based on this demand, in 2023 emerged the first stool bank of Catalonia, Spain, comprising two centers: Hospital Clínic de Barcelona (HC) and Hospital Universitario de Bellvitge (HUB).
Methodology: The establishment of the stool bank involved the implementation of rigorous protocols for donor recruitment, screening, and sample processing. Healthy donors underwent a comprehensive questionnaire and laboratory tests to exclude infectious diseases and other contraindications. Donors who are deemed eligible begin a two-month donation period. After this period, screening is repeated to confirm that all donations from this period are safe for processing. The stool donations are processed and two types of products are generated: faecal microbiota solution to be administered by nasogastric tube or colonoscopy, and lyophilised oral capsules (Figure1). Logistical workflows were also developed for storing, tracking, and distributing treatments to other hospitals in Catalonia, guaranteeing equity across the territory.
Results: The results from both sites of the Catalonia Stool Bank are shown in Table 1. Between the two centers, 63 FMT's were performed, 43 by oral capsules and 20 by colonoscopy. Regarding donors, 42 were active during this period, but only 29 completed the two-month donation period with a negative screening test at the beginning and at the end. The main causes of positive screening tests were enteropathogenic E. coli, and Dientamoeba fragilis. Fifteen donors left the programme before completing the period. A total of 426 stool donations were received and 321 were processed, obtaining 346 products: 68 oral capsules and 278 solutions.
Conclusions: The establishment of the first stool Bank in Catalonia in 2023, including these two centres, demonstrates a successful initial effort to address the growing need for FMT treatments in the region. The implementation of rigorous donor selection and sample processing protocols has enabled the safe production of both oral capsules and faecal microbiota solutions. The stool bank is able to produce and stock treatments for FMT for colonoscopy or oral administration and ensure its availability to all rCDI patients in Catalonia under rigorous quality procedures.

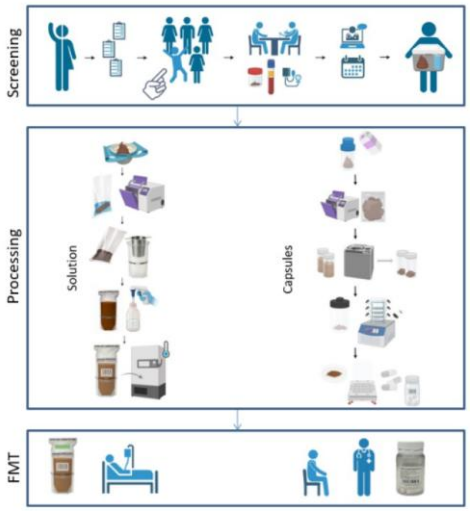

Figure 1. Stool bank workflow: donor screening, solution and capsule processing and product delivery.

Centre	HUB		HC	
FMT's performed (No.)	Capsules: 41	Colonoscopy: 3	Capsules: 2	Colonoscopy: 17
Active donors (No.)	15 (2 donors withdrew)		27 (13 donors withdrew)	
Complete donation period (No.)	11		18	
Donations received (No.)	96		330	
Donations processed (No.)	29		292	
FMT treatments obtained (No.)	Capsules: 41	Solution: 7	Capsules: 27	Solution: 271

Table 1: FMT, donor, and donation processing data from November 2023 to March 2025
[1]HUB: Hospital Universitario de Bellvitge. [2]HC: Hospital Clinic de Barcelona. [3]FMT: Faecal Microbiota Transplantation

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