

E-POSTER VIEWING - BEST POSTERS

MINING THE GUT MICROBIOTA FOR TYPE 1 DIABETES PREVENTATIVE FACTORS.

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Background and Aims: The incidence of type 1 diabetes has increased, globally. Current theory suggests an altered gut microbiome associated with modern lifestyles is altering the development of a tolerogenic immune response increasing susceptibility to autoimmune conditions. Short chain fatty acids (SCFA) are important bacterial metabolites produced by fermentation of dietary fibre. Bacterial dysbiosis prior to the onset of T1D can cause a loss of SCFA producing bacteria reducing gut barrier function and promoting a pro-inflammatory immune response. In a recent clinical trial, we administered a high amylose maize starch supplement chemically modified to deliver SCFA's acetate and butyrate in adults with T1D (HAMSAB). Here, we altered the microbiome, increased SCFA in both stool and blood and altered the immune profile to be more tolerogenic.

Methods: Here we apply a unique systems biology approach investigating stool proteomics and metabolomics to present novel insight into the response of the gut epithelium and function of the microbiota in following the HAMSAB diet. We then used a human to mouse fecal transfer model mice to test whether the HAMSAB-modified microbiota could delay diabetes progression after colonisation into germ-free NOD

Results: The stool proteome and metabolome were altered after supplementation. The post-diet microbiota from two of four trial subjects tested was able delay diabetes progression which was accompanied by similar alterations in the stool metabolome as in the trial subjects.

Conclusions: These data demonstrate that the HAMSAB associated changes in gut microbiota function were able to influence diabetes progression and support further studies into therapies targeting the gut microbiota to prevent T1D.

Disclosure: No significant relationships.

Keywords: multi-omics, Humanized-mouse-model, resistant starch, SCFA

E-POSTER VIEWING - BEST POSTERS

ASSOCIATION BETWEEN MATERNAL VITAMIN D STATUS DURING PREGNANCY AND INFANT GUT MICROBIOTA DEVELOPMENT

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Background and Aims: The first two years of life are critical for the development of the human gut microbiota, yet the influence of maternal vitamin D status on the infant microbiome development is not well understood. The aim was to investigate the effects of serum 25(OH)D at week 11-14 of pregnancy on the gut microbiota.

Methods: Fecal samples and dietary questionnaires were collected from 328 Icelandic mothers and their infants, before and after introduction of solid foods, as well as at one and two years of age. High serum 25(OH)D was defined as ≥ 75 nmol/L and low as < 50 nmol/L.

Results: Lower alpha diversity was observed in infant gut microbiota around four months of age in infants born to mothers having high 25(OH)D in the first trimester of pregnancy. Gut microbial community composition based on beta diversity around six months was higher among infants with mothers with low 25(OH)D. Beneficial microbial taxa were more abundant in infants born from mothers with high 25(OH)D, whereas low 25(OH)D during pregnancy was associated with a higher abundance of non-beneficial or potentially harmful microbial taxa in the infant gut microbiota.

Conclusions: Our results suggest an association between maternal serum 25(OH)D status during early pregnancy and the infant gut microbiota development during the first months of life. As differences were also detected in the postpartum maternal gut microbiota between high and low groups, it is still unclear if maternal vitamin D status has a direct influence on the infant gut microbiota or an indirect impact through maternal microbiota modulation and vertical transmission.

Disclosure: No significant relationships.

Keywords: microbiota, serum 25(OH)D, pregnancy, Infants

E-POSTER VIEWING - BEST POSTERS

DYNAMICS OF MICROBIOME COMPOSITION FROM PLACENTA TO FETUS AND NEONATE.

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Background and Aims: The establishment of neonatal microbiome was influenced by uterine and placental environments, ultimately serving as a critical determinant of lifelong health outcomes. This study utilized metagenomic analysis of maternal-fetal pathway to delineate alterations in the microbiome composition.

Methods: The placenta tissues were collected aseptically from distinct anatomical regions, including umbilical cord, amnion, villus tissue, decidua basalis and placental bed of maternal side. A Cord blood was analyzed both umbilical artery and vein to examine bidirectional microbiomes. The 16s rRNA sequencing via Next Generation Sequencing was conducted to determine taxonomic abundance, count ratio and compositional analyses using PCoA and heatmap analysis. The trends of microbiome composition were examined utilizing the Spearman correlation method.

Results: The results revealed specific trends depending on the type of microbiome, indicating a distinct pathway of maternal-fetal transmission via placenta. Coriobacteria and Ruminococcus were found to have higher concentrations on the maternal side and lower concentrations on fetal side with statistically significant trends ($p=0.0027$, 0.01154). In contrast, Alphaprobacteria had significantly higher concentrations on the fetal side ($p=0.00675$). These analytical results suggest that the colonization of the fetal and neonatal microbiome occurs 1) prior to birth within the fetal environment, 2) in the direction from maternal blood → placenta → umbilical cord, cord blood → fetal meconium, and 3) that there may be a specific immune barrier of placenta that regulates the transfer of microbiota.

Conclusions: The maternal-fetal microbiome pathway exhibits specific trends base on the type of microbiome, providing a foundation for research aimed at promoting fetal and neonatal health.

Disclosure: No significant relationships.

Keywords: Microbiome, placenta, cord blood, composition, dynamics

E-POSTER VIEWING - BEST POSTERS

PROOF OF CONCEPT: METATRANSCRIPTOMIC ANALYSIS OF THE EQUINE PLACENTAL MICROBIOME

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Background and Aims: The existence of a placental microbiome is a controversial topic due to the inability to distinguish placental microbes from contaminants when using DNA-based methods. Here, we characterized the microbial population in equine placenta, a species with a negligible amount of maternal contamination in its placenta, using a metatranscriptomic approach.

Methods: Total RNA was extracted from healthy prepartum placentae (n=6) and placentae (n=6, 280 days) inoculated with *Streptococcus equi* subs. *zooepidemicus* (1×10^6 CFU), followed by rRNA depletion, and deep sequencing (>60Gb RNA/sample). Taxonomy was investigated using Kraken2/Bracken.

Results: We identified 1687 species, of which 887 (53.6%) were common between the groups. Control and inoculated samples had a median (\pm SD) *Strep. equi* relative abundancy of 0.007% (min-max: 0 - 0.008%) and 0.59% (min-max: 0.01 - 5.94%) (Wilcoxon; $p < 0.01$), respectively. There was a high variation in *Strep. equi* abundancy among the inoculated samples, even though the mares were challenged with the same number of CFU. The effect of pathogen abundancy on the host transcriptome was investigated using weighted gene correlation network analysis. The abundance of *Strep. equi* was positively correlated with inflammation and immune related genes ($r=0.95$, $p < 0.01$). Additionally, there was a significant correlation ($r=0.64$, $p < 0.05$) between the abundance of *Strep. equi* and the presence of inflammatory cells in the placenta.

Conclusions: In conclusion, this data supports the use of metatranscriptomics as a tool to identify bacteria in low biomass samples that can be coupled to changes in the host transcriptome.

Disclosure: No significant relationships.

Keywords: Metatranscriptome, Equine Placenta, Placental Microbiome, Host Pathogen Interaction

E-POSTER VIEWING - BEST POSTERS

MATERNAL WESTERN STYLE DIET FEEDING PROGRAMS GUT EPITHELIAL SEROTONIN RESPONSE TO MICROBES

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Background and Aims: We have previously shown that macaque offspring exposed to maternal Western style diet (WSD) display anxiety and an altered gut microbiome, independent of post-weaning diet and persistent into juvenile life. Gut microbes interact with host gut enterochromaffin cells, which secrete serotonin and affect behavior (i.e. anxiety) via the gut-brain axis. We hypothesized that gut microbes from maternal WSD-exposed offspring aberrantly alter levels of intestinal serotonin *in vitro*.

Methods: Japanese and rhesus macaques were fed WSD or control diet during gestation/lactation. Offspring intestine was biopsied during fetal development or at 3 years old (2.5 years after weaning onto control diet). Stool was collected at 3 years old. Intestinal organoids were generated from the intestine and assayed with and without macaque stool microbes for serotonin.

Results: Without microbes, maternal WSD intestinal organoids secrete decreased serotonin compared to maternal control organoids during fetal development (59.0 pg/mL vs 2426.0 pg/mL, $p=8.0e-11$) and at 3 years old (846.9 pg/mL vs 2145.4 pg/mL, $p=0.0044$). With stool microbes, maternal WSD intestinal organoids respond with decreased serotonin secretion regardless of stool origin compared to maternal control ($p=0.07$). Maternal control organoids treated with maternal WSD stool microbes display increased serotonin compared to maternal control stool microbes ($p=0.08$).

Conclusions: Our results reveal that maternal WSD feeding programs low serotonin production in the offspring gut, beginning in fetal life and persisting to juvenile life, and alters the serotonin response to stool microbial products. Maternal WSD effects a persistent miscommunication between gut cells and resident microbes that primes the gut-brain axis in early life.

Disclosure: No significant relationships.

Keywords: maternal diet, Non-human primates, Serotonin, Intestinal Organoids

E-POSTER VIEWING - BEST POSTERS

THE DIVERSITY OF MICROBIOME PROFILES IN PERIODONTAL SUBGINGIVAL POCKETS

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Background and Aims: The oral microbiota contains over 700 microbial species, with an average of 250 species per person. The oral environment consists of micro-environments with distinct microbiome compositions. In this project, we characterized the microbiome profile in two periodontal environments, shallow and deep pockets, to see if the microbiota composition differed and if they could influence one another.

Methods: The samples were collected from 1298 participants in the population-based "Hordaland Oral Health Study (HUSK-T)" cohort from Western Norway. Subgingival plaque was collected from periodontitis participants with shallow (<5 mm) and deep (≥ 5mm) pockets, as well as participants with only shallow (<5 mm) pockets. Shotgun metagenomics was used to profile taxonomic composition and diversity of microbial communities.

Results: We found that the composition of the microbiota differed significantly between shallow and deep pockets. The genera Anaeroglobus, Fusobacterium, Porphyromonas, Tannerella, Treponema, Peptostreptococcus, Eggerthia, Dialister, Megasphaera, Shuttleworthia, and Solobacterium were significantly more abundant in the deep pocket microbiota profile. Shallow pockets, on the other hand, had a higher proportion of Corynebacterium, Rothia, Neisseria, Streptococcus, and Sanguibacter. Interestingly, the microbiota composition in shallow pockets differed significantly from that of patients with only shallow pockets and those with both shallow and deep pockets, with a higher proportion of Abiotrophia, Granulicatella, Oribacterium, and Stomatobaculum.

Conclusions: Our findings indicate that periodontal pocket depth influences microbiome composition. Furthermore, the presence of a deep pocket influences the microbiome composition in the shallow pocket, implying that there is a possibility of changing the microbiota composition in oral microbiota and/or microbiota composition in oral micro-environments.

Disclosure: No significant relationships.

Keywords: Microbiome, Shotgun metagenomics, Subgingival pockets, Microbiota profile, oral microbiota

E-POSTER VIEWING - BEST POSTERS

THE CIRCULATING MICROBIAL TRANSCRIPTOME IN MYELOID MALIGNANCY PATIENTS

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Background and Aims: In solid tumors, microbiome research has recently been directed toward the tumor site itself, revealing microbial signatures in solid tissue and blood that can accurately predict tumor type and differentiate between cancer patients and healthy individuals. Recently (Woerner et al, Nature Communications 2022), we analyzed for microbial content deep DNA sequence data from the tumor site (blood and bone marrow) of patients with myeloid malignancies. In the current study, we analyze microbial RNA content in a separate large collection of patients with myeloid malignancies.

Methods: We acquired or generated RNAseq data from the blood or bone marrow of hundreds of patients with leukemia and related conditions. Viral content was assessed using a custom enrichment kit, while bacterial reads were quantified from shotgun metagenomic sequencing using bulk and single-cell sequencing, including paired pre- and post-treatment samples. Patient clinical and genomic data included disease subtype, overall survival, response to drug treatment, and host mutational and transcriptional data.

Results: We observe associations between expression of specific genera and clinical characteristics. Single-cell sequence data demonstrates cell-specific relationships between microbial and host expression. These relationships shift from diagnosis to relapse, during which there is also a substantial increase in normalized bacterial RNA burden. Targeted sequencing of viral content shows expected exogenous viral presence in positive controls. We also identify substantial expression of known oncogenic viruses in patient samples, as well as differential expression of HERVs that have previously been implicated leukemogenesis.

Conclusions: Our study provides evidence of association between microbial content and clinical characteristics in myeloid malignancy patients.

Disclosure: No significant relationships.

Keywords: leukemia, blood microbial content, clinical impact, virus and bacteria

E-POSTER VIEWING - BEST POSTERS

DEVELOPMENT OF EARLY LIFE GUT RESISTOME AND MOBILOME ACROSS GESTATIONAL AGES AND MICROBIOTA-MODIFYING TREATMENTS

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Background and Aims: Gestational age (GA) and associated level of gastrointestinal tract maturation are main factors driving the initial gut microbiota composition in preterm infants. Besides, compared to term infants, premature infants often receive antibiotics to treat infections and probiotics to restore optimal gut microbiota. How GA, antibiotics, and probiotics modulate the microbiota's core characteristics, gut resistome and mobilome, remains nascent.

Methods: We analysed metagenomic data from longitudinal observational study in six Norwegian neonatal intensive care units to describe the bacterial microbiota of infants of varying GA and receiving different treatments. The cohort consisted of probiotic-supplemented and antibiotic-exposed extremely preterm infants ($n = 29$), antibiotic-exposed very preterm ($n = 25$), antibiotic-unexposed very preterm ($n = 8$), and antibiotic-unexposed full-term ($n = 10$) infants. The stool samples were collected on days of life 7, 28, 120, and 365, and DNA extraction was followed by shotgun metagenome sequencing and bioinformatical analysis.

Results: The top predictors of microbiota maturation were hospitalisation length and GA. Probiotic administration rendered the gut microbiota and resistome of extremely preterm infants more alike to term infants on day 7 and ameliorated GA-driven loss of microbiota interconnectivity and stability. GA, hospitalisation, and both microbiota-modifying treatments (antibiotics and probiotics) contributed to an elevated carriage of mobile genetic elements in preterm infants compared to term controls. Finally, *Escherichia coli* was associated with the highest number of antibiotic-resistance genes, followed by *Klebsiella pneumoniae* and *K. aerogenes*.

Conclusions: Prolonged hospitalisation, antibiotics, and probiotic intervention contribute to dynamic alterations in resistome and mobilome, gut microbiota characteristics relevant to infection risk.

Disclosure: No significant relationships.

Keywords: Extremely preterm infants, Mobilome, Probiotics, Gestational age, Resistome

E-POSTER VIEWING - BEST POSTERS

EXPLORATION OF 2,500 FOOD METAGENOMES REVEALS UNEXPLORED MICROBIAL DIVERSITY AND SPECIES OVERLAPS WITH THE HUMAN MICROBIOME

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Background and Aims: Food microbiomes are complex communities, with pivotal functions in certain food manufacture and a fundamental part of the food we eat. However, they remain largely unexplored, including their potential impact on the human microbiome.

Methods: Within the EU Project MASTER, we integrated 583 publicly available food metagenomes from a literature survey with 1,950 newly-sequenced food microbiomes from various food types and geographical origins. We analysed this collection and built an extensive repository of food-associated microbial data called curatedFoodMetagenomicData (cFMD).

Results: We reconstructed >10,000 metagenome-assembled genomes (MAGs) that were clustered into 1,036 species-level genome bins (SGBs), with 290 of them representing previously undescribed taxa. Sensitive taxonomic profiling of food SGBs highlighted remarkable microbial diversity within and among food categories and geographic locations. Extending the analysis to 20,000 human metagenomes from diverse countries in all continents revealed considerable species overlaps between food and human microbiomes. Further investigations highlighted systematic co-occurrence of 43 common prevalent species in both food and human, with similarities reaching the strain-level suggesting events of microbial horizontal transmission.

Conclusions: cFMD is an open-access resource that will help advancement in the study of food microbiology, as well as support the use of metagenomics for food safety, certification and quality control. Our results offer valuable insights into the investigation on the formation of microbial communities inside the human body, providing the basis for thinking of food not only as prebiotics but also as probiotics.

Disclosure: This work was supported by the project MASTER-Microbiome Applications for Sustainable food systems through Technologies and Enterprise, receiving funding from the European Union's Horizon 2020 research and innovation programme (GA 818368). This manuscript

Keywords: human microbiome, food microbiome, metagenomics, strain-level analysis, metagenomic assembly

E-POSTER VIEWING - BEST POSTERS

INTRODUCTION OF PROBIOTIC-BASED SANITATION IN THE EMERGENCY WARD OF A CHILDREN'S HOSPITAL DURING THE COVID-19 PANDEMIC

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Background and Aims: The control of microbial contamination is crucial in hospitals, since hospital microbiomes can cause healthcare-associated infections (HAIs), which are particularly frequent in pediatric wards. However, the massive use of disinfectants used to prevent COVID-19 transmission has worsened the antimicrobial resistance (AMR) threat, posing further risk in the hospital environment. Instead, we previously shown that a probiotic cleaning hygiene system (PCHS) can stably decrease hospital pathogens in adult hospitals, without increasing their resistance but rather reducing AMR up to 99.9%, also halving associated HAIs and inactivating enveloped viruses, included SARS-CoV-2. Thus, here we wanted to assess the effects of PCHS in the emergency rooms (ER) of a children's hospital during the COVID-19 pandemic.

Methods: PCHS replaced chemical disinfection for 2 months in the ER ward. Surface microbial contamination was monitored before and after PCHS application by both culture-based methods (CFU count) and molecular assays, including 16S rRNA NGS and real time PCR (qPCR) microarrays for the resistome characterization. The presence of SARS-CoV-2 was also monitored by qPCR.

Results: PCHS usage was associated with a stable 80% decrease in bacterial/fungal pathogens compared to the pre-PCHS levels detected (chemical disinfection; $P < 0.01$), accompanied by an up to 99% decrease of AMR genes ($P < 0.01$). Besides, SARS-CoV-2 was stably absent in PCHS-treated environments. PCHS effect was reversed upon reintroduction of chemical disinfection, jeopardizing PCHS action.

Conclusions: Results suggest that PCHS may be successfully used to control pathogen and virus spread without simultaneous worsening the AMR concern.

Disclosure: No significant relationships.

Keywords: COVID-19, antimicrobial resistance, environmental microbiome, probiotic based-sanitation

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**UTILITY OF MULTIVARIATE DATA ANALYSIS AND PENALIZED META-REGRESSION TO EXPLORE
SOURCES OF HETEROGENEITY IN MICROBIOME META-ANALYSES**

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Background and Aims: Meta-analysis is a statistical method that quantitatively synthesizes, by calculating a combined result, the results of independent studies addressing a specific research question. The principle is simple: pooling data from several studies increases statistical power. However, a number of conditions must be assessed to ensure that the combined result is not biased and that the conclusions drawn are accurate. A key step is to explore the sources of heterogeneity and look for possible biases. Advances in bioinformatics and next-generation sequencing have led to important advances in the understanding of the role of the microbiota in health. Knowledge development is often based on the conclusions that can be drawn from all published data, i.e. meta-analyses. Yet, in microbiota studies, differences between studies (in terms of pipelines, characteristics, sequencing techniques, sample collection sites, study populations, etc.) can be very high. An exploration of the sources of heterogeneity is essential to determine whether studies, even if they address the same research question, are comparable.

Methods: Multivariate data analysis methods (such as principal components analysis for quantitative data, multiple correspondence analysis for categorical data, and factor analysis of mixed data, a mixture of the two) as well as penalized meta-regression (such as the Lasso) are applied to explore heterogeneity in microbiota meta-analyses.

Results: Data from recently published microbiome meta-analyses were re-analyzed with the developed tools.

Conclusions: In this work, we illustrate the utility of multivariate data analysis methods and penalized meta-regression in exploring sources of heterogeneity in microbiota meta-analyses. The R code is publicly available.

Disclosure: No significant relationships.

Keywords: Microbiome meta-analyses, Heterogeneity, Visual tools for data exploration, Meta-regression

012 / #265

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**LACTIC ACID BACTERIA SCREENING AND GENOMIC CHARACTERIZATION FOR POSTBIOTICS
ACTIVITY**

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Background and Aims: Recent studies suggest the viability of bacteria may not be necessary to achieve health-promoting effects. Investigations of probiotic strains have led to the characterization of specific metabolic byproducts called postbiotics. The undoubted advantage is to circumvent the problem of acquisition of antibiotic resistance genes and virulence factors, which may occur when the live strain is ingested. Observations in animal models have demonstrated the biological activity of inanimate bacteria which offer significant advantages over their live counterparts. The use of postbiotics for human health is still at a preliminary stage.

Methods: The growing volume of genomic information may facilitate systematic efforts to determine the metabolic pathways that may lead to obtain the desired postbiotic metabolites. Therefore, we aimed to screen Lactic Acid Bacteria genomes available in public repositories to identify the prevalence of genes possibly related to postbiotic activities.

Results: The results demonstrated the importance of computational biology tools for the rational discovery and identification of pathways leading to the production of bioactive molecules.

Conclusions: This work was partially funded by a grant from the Italian Ministry of Foreign Affairs and International Cooperation to the project FOODMICROHERITAGE–Quality and authenticity protection of artisanal fermented foods through the characterization and conservation of their microbial and genetic heritage and by the European Union - NextGenerationEU, NRRP - Mission 4, Component 2, Investment 1.4 - National Biodiversity Future Center - CN_00000033 (D.M. Prot. 1034 of 17/06/2022).

Disclosure: No significant relationships.

Keywords: Postbiotic, genomic characterization, health-promoting effects, Metabolites, genes

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**KLEBOTOXIN: A NOVEL TOOL FOR DETECTING KLEBSIELLA OXYTOCA SPECIES COMPLEX
MEMBERS AND THE TIL ENTEROTOXIN LOCUS**

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Background and Aims: Bacteria belonging to the *Klebsiella oxytoca* species complex (KoSC) are prevalent in the human gut microbiota early in life. These bacteria frequently carry a cluster of biosynthetic genes (*til*) for secretion of the enterotoxic peptides tilimycin and tilivalline, which can cause necrotizing enterocolitis in infants, and antibiotic-associated hemorrhagic colitis in children and adults. Specific detection of KoSC members and knowledge of their *til* biosynthetic capacity is therefore desirable, particularly for the highly vulnerable population of premature infants. To meet this need, we developed KleboToxin, a Python3-based tool for detection of KoSC members and their *til* gene status.

Methods: KleboToxin integrates multiple tools to analyze sequence data of whole-genomes (WGS) and 16S rRNA genes. For WGS, it employs a modified MetaPhlan4 and SRST2 pipeline, while for 16S data, it utilizes QIIME2 output in combination with either VSEARCH. The tool can process raw reads, employing KneadData for trimming and quality control, or pre-trimmed sequences. Furthermore, it can analyze completed reports from MetaPhlan and SRST2.

Results: To facilitate use, KleboToxin is accessible through both the command line and a user-friendly web browser interface, streamlining input and output management. This novel tool enables rapid and accurate identification of KoSC members and their *til* genes, providing essential information for assessing the potential impact of these bacteria on the health of human carriers, particularly premature infants.

Conclusions: By facilitating targeted intervention strategies, KleboToxin has the potential to improve patient outcomes and contribute to a better understanding of the complex human gut – microbiota interface.

Disclosure: No significant relationships.

Keywords: premature infants, *Klebsiella oxytoca* species complex (KoSC), Necrotizing enterocolitis, KleboToxin, Tilimycin

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**FROM LAB TO DATA: CHOOSING THE BEST METHODS FOR ACCURATE GUT MICROBIOME
ANALYSIS**

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Background and Aims: The gut microbiome plays an important role in the healthy physiological state of the human body, and imbalances in it can lead to a number of diseases, such as diabetes or IBD. Researching the gut microbiome is crucial to understand the causes of many diseases and to find a cure for these conditions. In recent years, thanks to the rapid advances in molecular biology and bioinformatics, we have been able to explore the composition and role of the microbiome in new depths. Many methods can be used to investigate the composition, functionality, transcriptome and metabolic pathways of a microbial sample. However, several methods are available to obtain these data, and different methods may give different results. It is therefore important to use the most reliable methods in these experiments.

Methods: To reach this goal, we have designed a series of experiments that aimed to compare four widely used DNA isolation methods, three sequencing platforms, several library construction techniques and bioinformatics analysis, and a bioinformatics tool developed by our group. We used the same stool sample in our experiments and a microbial control of known composition for validation.

Results: Our results include an optimization pipeline from wet-lab to the downstream data analysis and a bioinformatics tool that can be applied comprehensively to data from all library construction and sequencing methods.

Conclusions: In conclusion, we found that using the most adequate methods is essential, because the usage of suboptimal methods may lead to significant differences in the final data.

Disclosure: No significant relationships.

Keywords: Gut Microbiome, Nanopore sequencing, metagenomics, 16s rRNA sequencing, Shotgun sequencing

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**INVESTIGATING THE RELATIONSHIP BETWEEN TRAVELLER'S DIARRHOEA AND SOIL AND GUT
MICROBIOMES**

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Background and Aims: Travellers' Diarrhoea (TD) is a major issue for military personnel deployed overseas. The percentage of gastroenterology cases (medical and disease or non-battle injury admissions) that were reported to field hospitals during Operations TELIC and HERRICK were 56.9 and 43.9% respectively. The burden of such cases on military health and performance should not be underestimated, as the incapacitating nature of such admissions would put the UK at a disadvantage with regard to operational effectiveness. Having a better understanding of the gut microbiome and how it may be influenced by components within the environment may enable mechanisms to be identified that could be manipulated as prophylaxis or treatment for TD. This work investigated differences in the diversity and composition of the gut and soil microbiomes of different countries of frequent deployment.

Methods: The results suggest that there are individual factors in different countries contributing to the risk of infection.

Results: The results suggest that there are individual factors in different countries contributing to the risk of infection.

Conclusions: Additionally, they indicate that local populations are frequently exposed to pathogens commonly causing TD but symptoms do not develop, possibly due to the adaptation of the microbiome through protective mechanisms. These mechanisms are currently being investigated.

Disclosure: No significant relationships.

Keywords: gut, soil, travellers diarrhoea, computer algorithm

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**METABOLITE AND MICROBIOME STABILITY IN HUMAN FECAL SAMPLES IN DIFFERENT
STORAGE CONDITIONS**

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Background and Aims: Stool metabolome provides functional insights of the gut microbiome. There is a growing demand for an optimized sampling protocol, which could offer stable samples at ambient temperature storage and reliable results of the gut metabolome and microbiome. This study aimed to explore how different sampling conditions influence the metabolite stability and microbiome profiles.

Methods: Samples were collected from four healthy adult volunteers. For metabolomics, three different storage conditions were tested: EtOH 95%, OMNI_{met}GUT and crude feces without preservative. These sample types were compared to immediately frozen samples. Six different storage conditions were tested: immediate freeze in -80°C, 24h at +4°C, 24h at room temperature (RT), 36h RT, 48h RT and 7 days RT. Metabolome was analyzed with GC-TOF-MS and LC-MS. Analyses were focused on short chain fatty acids (SCFA), bile acids, lipids, endocannabinoids and untargeted metabolites. Next generation sequencing was performed with 16S V3V4 rRNA-sequencing with Illumina MiSeq with same samples, stored in 5 timepoints: 0, 1d, 2d and 7d (=days) RT in OMNI_{gene}GUT, EtOH 95% and those were compared to immediately frozen sample.

Results: The gut metabolites were stable with both storage solvents and immediately frozen samples. SCFA concentrations altered in the crude samples compared to other sampling conditions. Microbiome was preserved sufficiently based on relative abundances and beta-diversity with both OMNI_{gene}GUT and EtOH 95%. Alpha-diversity was slightly decreased with EtOH 95%.

Conclusions: Gut metabolites remained stable within EtOH 95% and OMNI_{met}GUT. The microbial metabolite SCFA remained unstable in the crude samples. For microbiome, OMNI_{gene}GUT preserved samples partially better than EtOH 95%.

Disclosure: No significant relationships.

Keywords: Metabolomics, Gut Microbiome, Ambient temperature, Sample collection, Storage condition

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**SQUEEGEE: A NEW CONTAMINATION DETECTION TOOL FOR LOW MICROBIAL BIOMASS
MICROBIOMES**

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Background and Aims: The exploration of host-related microbiomes using computational analysis has led to many discoveries relevant to human health and disease. However, the presence of impurities in metagenomic samples can potentially influence the interpretation of findings in microbiome studies, particularly in low-biomass setting. In order to be able to detect microbial contaminants when negative controls are unavailable, we developed “Squeegie”, a novel tool for identifying and analyzing microbes was developed to ensure consistency in contamination detection.

Methods: The Squeegie tool employs Kraken for taxonomic classification and to identify a set of possible contaminant species followed by Bowtie2 in multi-alignment mode to align reads from the input data to the representative genomes of the contaminant species. Pairwise Mash distance is also calculated for all samples. Squeegie then predicts potential contaminants via combining the prevalence score, Mash distance, and breadth/depth of genome coverage of the candidates. To evaluate Squeegie, we tested it on three datasets: a simulated dataset with known contaminants, a real dataset with negative controls, and Human Microbiome Project samples without negative controls but with associated DNA extraction kit contaminants.

Results: When comparing Squeegie to Decontam, Squeegie outperformed or matches Decontam's predictions at the species level using strict ground truth criteria, with respect to unweighted F-score, weighted F-score by the relative abundance in non-control samples, and cumulative relative abundance of correctly identified contaminants from negative control samples.

Conclusions: In summary, Squeegie has a high precision in identifying microbial contaminants, making it a useful computational method for detecting contaminants when negative controls are not available.

Disclosure: No significant relationships.

Keywords: bioinformatics, Microbiome, contaminant

018 / #216

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**A STATISTICAL PROGRAMMING FRAMEWORK FOR MICROBIOME DATA INTEGRATION AND
ANALYSIS**

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Background and Aims: Systematic approaches to microbiome data integration are needed to deal with the growing sample sizes and data modalities in contemporary microbiome science. Despite the recent progress in microbiome data science, extended support is needed for statistical integration of multi-modal and hierarchically structured data, as well as for general data provenance and performance optimization.

Methods: Dedicated data containers provide well-established solutions to meet these needs and they have become fundamental building blocks of modern statistical programming frameworks. Recent developments in this area have also opened up new opportunities for statistical data integration and analysis in microbiome science. The emerging microbiome data science ecosystem in R/Bioconductor extends the current state-of-the-art by taking advantage of recently introduced multi-assay data containers which provide thoroughly tested and optimized computational tools for integrating hierarchical, spatiotemporal, and multi-domain data in both sample and feature spaces. Moreover, the use of standardized data containers is supporting the integration of microbiome data science methods with genomics, transcriptomics, single-cell analysis, and other related frameworks, which is essential for improving the interoperability of tools, reducing overlapping development efforts, ensuring long-term sustainability and advancing collaborative development of microbiome data science.

Results: We have developed a package ecosystem for microbiome data science, supported by comprehensive online documentation, open demonstration data, and an active user community. We comply with recommended practices in research software, including continuous integration, unit tests, and a regular release cycle.

Conclusions: We demonstrate the available methods and utility of this emerging framework based on recent examples from human microbiome population studies.

Disclosure: LL is member in Bioconductor community advisory board.

Keywords: data integration, data science, Bioconductor, multi-omics

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**MULTI-OMIC CHARACTERIZATION OF A DEXTRAN SULFATE SODIUM (DSS)-RESISTANT COLITIS
PHENOTYPE AFTER DIETARY INTERVENTION WITH FRUITS AND VEGETABLES USING A
TRANSLATIONAL NUTRITIONAL PORCINE ANIMAL MODEL.**

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Background and Aims: There is a need to identify relationships that support the benefit of consuming dietary patterns rich in fruit and vegetables (FV) at recommended levels to promote health.

Methods: Thirty- six-week-old female pigs were fed an isocaloric grower diet alone (control) or supplemented with lyophilized FV equivalent to the half (Half-FV) or full (Full-FV) daily recommended amount for humans per the Dietary Guidelines for Americans (DGA). After three weeks on dietary intervention, pigs were orally treated with 4% DSS for one week and observed for recovery for five additional days.

Results: Shotgun metagenomic sequencing and metabolomic characterization from proximal colon (PC) contents, longitudinal fecal microbiota taxonomy from 16SrDNA sequencing and localized transcriptome changes in intestinal mucosa were analyzed to identify associations of dietary FV levels with responsive host clinical biomarkers. Pigs with Full-FV dietary supplementation showed resistance to DSS-induced PC mucosal damage with no alteration in crypt depth, minimal differential gene expression, better intestinal permeability relative to DSS-control group and no change in bacterial diversity when compared to non-DSS treated pigs. A FV-dose dependent increase in catechin and protocatechuic acid, two metabolites associated with fruit consumption and known for their antioxidant effects by scavenging reactive oxygen species, was observed as early as one week post intervention with FV.

Conclusions: Using the pigs as a translational nutrition animal model, we have shown that FV-supplemented diets ameliorate DSS-induced inflammatory response that leads to intestinal colitis.

Disclosure: No significant relationships.

Keywords: Multiomic, Microbiome, Metabolomics, fruits and vegetables, diet

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

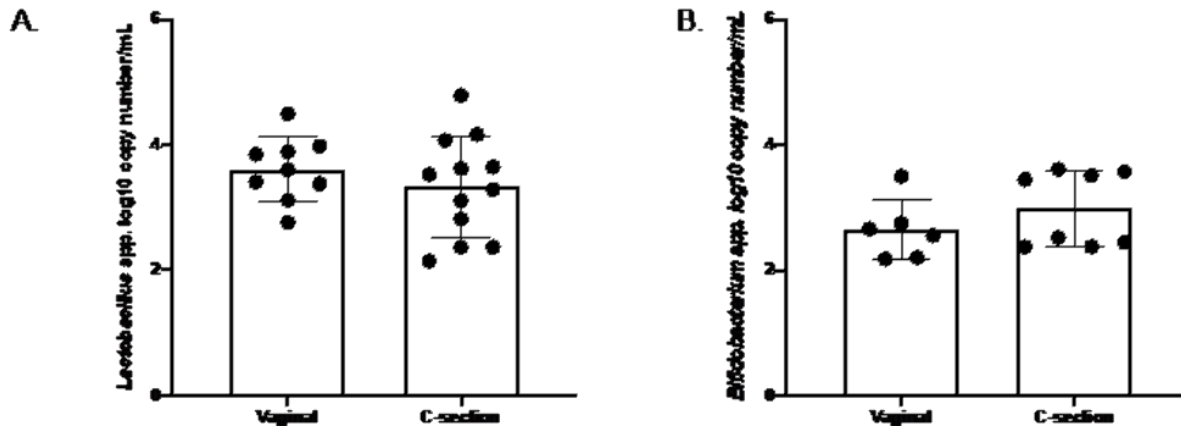
EFFECT OF ANTIBIOTIC PROPHYLAXIS AND DELIVERY MODE ON LACTOBACILLUS AND BIFIDOBACTERIUM COUNTS IN BREAST MILK

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Background and Aims: Impact of antibiotic prophylaxis and delivery mode on the breastmilk microbial composition is still poorly understood. Moreover, each country and healthcare settings may have different protocols for peripartum pre- and postpartum prophylaxis.

Methods: Herein 7 days postpartum breast milk samples were collected from 21 mothers (C-section n=12, vaginal n=9). C-section group received ampicillin-sulbactam (SAM) prophylaxis prior to skin incision. Both C-section and normal delivery groups received 7 days of cefuroxime treatment according to hospitals' regular protocol. DNA was isolated from the pellet with DNAeasy PowerSoil Pro Kit. *Lactobacillus* spp. and *Bifidobacterium* spp. were quantified using 4 µM of each genus specific PCR primers and Ampigene qPCR Green Mix. qPCR amplification was performed in a LightCycler® 480. Serial 10-fold dilutions of *L. acidophilus* ATCC 4356 and *B. lactis* BB-12 DNA were prepared for standard curve.

Results: In breast milk samples, *Lactobacillus* spp. amount was higher than *Bifidobacterium* spp. In all breast milk samples, *Lactobacillus* spp. was positive. *Bifidobacterium* spp. It was positive in 6(66.7%) of the vaginal delivery group and 8(66.7%) of the cesarean section group. *Lactobacillus* spp. in breast milk samples of cesarean section and vaginal delivery groups. amount was similar, but *Lactobacillus* spp. the amount was slightly higher (3.61 ± 0.51 vs. 3.32 ± 0.81 log₁₀ copy/mL, $p=0.45$). *Bifidobacterium* spp. The amount of breast milk samples was 2.64 ± 0.48 for the vaginal delivery group and 2.98 ± 0.60 log₁₀ copies/mL for the cesarean section ($p=0.41$).



Conclusions: Our results revealed that delivery-mode, and SAM exposure in C-section group did not significantly affect the Lactobacillus and Bifidobacterium counts. Lower Bifidobacterium levels were in accordance with the literature.

Disclosure: Our work was supported by TUBITAK with the project number 222S764 within the scope of the 1002-Quick Support-B projects and also by the Health Sciences University Scientific Research Coordinator with the project number 2022-023.

Keywords: antibiotic prophylaxis, delivery, breast milk, Lactobacillus and Bifidobacterium counts, C-section

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

AMPICILLIN-GENTAMIN TREATMENT BEFORE 1 MONTH OF LIFE IMPACTS ON MICROBIOTA AND RESISTOME DEVELOPMENT: FOLLOW-UP 2 YEARS OLD IN THE SPANISH DEMIAN COHORT

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Background and Aims: The combination of ampicillin and gentamicin has traditionally been considered a use-full first line treatment against most of all the bacterial agents causing sepsis in the neonatal period. It is known that antibiotics alter the correct gut microbiota establishment and can affect the human resistome, but it is not clear the duration of this effect. In this prospective study, we aimed at deciphering the effect of the most common antibiotic treatment at the neonatal intensive care units, ampicillin-gentamicin combination (AG), administered during the first month of life, on the gut microbiota and resistome development during the first two years of life.

Methods: Faecal samples from 20 full-term and vaginal-delivery (FTVD) neonates administered with AG were collected before to begin, at the middle of treatment and just after finishing AG treatment; also 1 month, 1 year and 2 years later on concluding treatment. A group of 20 FTVD non-antibiotic was used as control. Microbiota was analysed by 16S rRNA sequencing, metabolites by chromatography and antibiotic resistance genes (ARGs) by qPCR.

Results: Diversity and gut microbiota composition was deeply affected after treatment, specially groups such as *Bifidobacterium*. Despite, recovering over time in AG-group, differences with control group were long-lasting. Some SCFAs and amino-acids were also largely altered. ARGs were increased after AG treatment, particularly beta-lactamases.

Conclusions: Alteration on the gut microbiota establishment and resistome were observed after this common antibiotic treatment, entailing potential consequences for later health. This study lays the foundation for designing intervention strategies targeting the gut microbiota.

Disclosure: No significant relationships.

Keyword: gut microbiota, resistome, antibiotics, follow-up, early life

023 / #412

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

SIGNATURES OF THE GUT MICROBIOME ASSOCIATED WITH FEEDING HABITS IN EARLY LIFE

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Background and Aims: Along with perinatal factors including the mode of delivery and the use of antibiotics, feeding habits are one of the most important features that shape the gut microbiota in early life. As the golden standard for infant nutrition, human breast milk provides all the necessary nutrients to support the functional development and the metabolic maturation of the gut microbiota. To replicate the beneficial effects of breast milk, the inclusion of galacto-oligosaccharides and fructo-oligosaccharides (GOS-FOS) in a ratio of 9:1 in infant formulae is a common strategy.

Methods: Metagenomic shotgun sequencing allowed to investigate the impact of feeding habits on the gut microbiome of full-term infants over 6 months.

Results: The data analysis revealed the compositional signatures and metabolic capacities of exclusively breastfed and exclusively formula-fed infants.

Conclusions: The diet in early life can promote specific bacterial populations of the gut microbiota.

Disclosure: No significant relationships.

Keywords: Gut Microbiome, early life, feeding habits

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

EARLY-LIFE GUT MICROBIOME DEVELOPMENT IN HONG KONG CHINESE: A LONGITUDINAL STUDY OF THE FIRST THREE YEARS OF LIFE

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Background and Aims: The first few years of life is a critical period of gut microbiome development. While ethnicity is a major influencer on microbiome, current data on Asian, particularly Hong Kong Chinese, is scarce.

Methods: A prospective longitudinal study on 112 term Chinese infants born and grew up in Hong Kong. 16S rRNA gene sequencing was performed on 713 stool samples collected at 9 time points from birth to 3 years of age. Metadata including antenatal and postnatal events, mode of delivery, antibiotic use, illnesses, and feeding mode were collected.

Results: Alterations in the composition and alpha and beta diversity of the gut microbiota across the first three years of life were revealed. Mode of delivery, feeding mode and intrapartum antibiotics were the major determinants of gut microbiome, and their effects persisted up to 12 months. Alterations in the infant gut microbiota preceded the development of eczema. Of note, we identified that depletion of Bacteroides and enrichment of Clostridium sensu stricto 1 in the gut microbiome of infants who subsequently developed eczema around one year old. The same patterns were also observed in C-section born infants, suggesting a role of the gut microbiota in previously reported associations between C-section and increased risk of eczema.

Conclusions: Our study has facilitated understanding of the developmental dynamics and determinants of the early-life gut microbiome in Chinese population. It is worthwhile to explore whether early interventions to shape gut microbiome development could alter the risk of developing eczema in later life.

Disclosure: No significant relationships.

Keywords: Chinese, Hong Kong, Microbiome, Infant, Eczema

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

BREAST MILK MICROBIOTA IN RELATION TO MATERNAL DIET AND ALLERGY DEVELOPMENT IN CHILDREN AT 12 MONTHS OF LIFE

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Background and Aims: Breast milk (BM) is the best option for infant nutrition and development. It contains nutrients and a wide range of bioactive compounds including microbes. Although several studies have examined the relationship between different components of breastmilk and infant food allergies, limited information is available on the impact of milk microbes on infant food allergy. Our aim was to determine the human milk microbial composition in relation to maternal diet and allergy development in children (allergy-related problems, cow milk allergy) at 12 months of life.

Methods: BM samples from healthy women collected from a longitudinal cohort were included. BM microbiota of samples collected at 1 and 4 months of lactation were analysed by 16S rRNA gene sequencing. Maternal dietary information was recorded through an FFQ, and maternal-neonatal clinical characteristics were collected including the allergy-related problems at 12 months [infant atopic eczema (atopy), cow milk allergy (CMA)]

Results: We observed specific breast milk microbial clusters depending on infant health status (atopy vs CMA vs healthy). Staphylococcus and also, some strains from Firmicutes were linked to the BM groups. Higher microbial diversity was observed in CMA groups. We reported two maternal diet clusters: Cluster I (high intake of plant protein, fibre, and carbohydrates), and Cluster II (high intake of animal protein and lipids). BM microbiota was distinct according to maternal diet cluster and infant allergy health status. Differences in microbial diversity and richness were also found. Significant higher diversity was observed in CMA group compared to atopy and healthy groups, however no differences were observed for CMA cluster I vs cluster II.

Conclusions: This study provides important insights about the impact of maternal diet on milk microbes that would influence on the infant allergy development. Consumption of breastmilk with a reduced microbial richness in the first month of life is associated to maternal unbalanced diet and may play an important role in allergy development during childhood.

Disclosure: Authors acknowledge the support by the European Research Council ERC-Horizon 2020 (ERC starting grant, no. 639226). We would also like to thank the support from Mead Johnson Nutrition Award 2021

Keywords: breast milk microbiota, allergy, CMA, maternal diet

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

BIFIDOBACTERIUM ABUNDANCE EARLY IN LIFE SHAPES THE ANTIBIOTIC RESISTANCE LOAD IN THE INFANT GUT MICROBIOTA

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Background and Aims: Our aim was to study the link between Bifidobacterium abundance and antibiotic resistance load in gut microbiota of infant early in life, and to ascertain the perinatal factors that may contribute to the antibiotic resistance acquisition.

Methods: 101 infants at 1 month of age from the MAMI birth cohort were included in the study. Microbiota profiling was carried out by 16S rRNA amplicon (V3-V4 region) sequencing in Illumina Platform. Targeted antibiotic resistance genes (ARGs) including tetM, tetW, tetO, blaTEM, blaSHV and ermB were quantified by qPCR.

Results: Infant microbiota was clustered in two different groups according to the Bifidobacterium genus abundance (54% for cluster high and 9% for cluster low). Beta-Diversity showed differences between both groups ($p=0.001$). Lower abundance of Bifidobacterium was associated with higher load of antibiotic resistant genes. The most abundant ARGs were tetW and tetM, followed by beta-lactams (bla genes). Microbiota of the cluster Low was characterized by the presence of Blautia, Enterococcus, Veillonella, Phocaelcola, Streptococcus, Escherichia/Shigella, Klebsiella and Bacteroides, being these last three genera positively correlated with copies of tetO, tetM, blaTEM, blaSHV and ermB resistant genes. Random decision forest showed that antibiotic exposure during the first month of life is the factor that can explain the better this association.

Conclusions: Our results highlight the relevance of Bifidobacterium species in the early acquisition and establishment of antibiotic resistances in the gut. Further studies are needed to develop potential strategies to favour the adequate early colonization and fight against the spread of antibiotic resistances with effects on human health.

Disclosure: No significant relationships.

Keywords: infant microbiota, antibiotic resistance genes (ARGs), ANTIBIOTICS

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

IMPACT OF MATERNAL DIETARY SUPPLEMENTATION DURING LACTATION ON THE BREAST MILK MICROBIOTA AND METABOLOMIC PROFILES

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Background and Aims: For adequate infant growth and development, breastfed infants need an adequate supply of essential nutrients. Breastfeeding women have higher requirements for several nutrients, but these are not always covered by the usual diet and special dietary supplements are needed. Maternal diet influences infant development and also, modulates the breast milk nutritional and bioactive composition including lipid profile, microbiota, oligosaccharides etc.

Methods: A Randomized, double-blind clinical trial in 70 healthy lactating women in Germany was carried out during 12 weeks. Breast milk samples were collected from supplemented women (MMs) and placebo group at different time points: before intervention (V2) and 6 weeks(V3) and 12 weeks (V4) after intervention. Microbiota profile was analysed by 16S rRNA amplicon sequencing in Illumina Platform. Untargeted relative metabolomic profiles were determined by gas chromatography coupled to mass spectrometry (GC-MS) and ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF).

Results: Lactational time(V2, V3 and V4)and the intervention group(MMs vs placebo) modulated the breast milk microbial profiles. Increased relative abundances of *Rothia* genus were detected in placebo at V3 and V4 compared to MMs groups at same time. At time V4 (end of the intervention), higher microbial diversity but not in richness were observed in MMs group compared to placebo (p-value: 0.044). Metabolite profile was distinct when compared MMs and placebo at the end of the intervention, suggesting the potential effect of the intervention.

Conclusions: Conclusion: Maternal multi-micronutrient supplementation increased breast milk microbial diversity and influenced the milk metabolome profile by modulating the glycerophospholipid metabolism and phospholipid biosynthesis in healthy women.

Disclosure: Clinical Trial Registration: NCT04462939

Keywords: docosahexaenoic acid (DHA), maternal supplementation, breast milk, microbiota, metabolomic profile

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

GUT MICROBIOTA, SECRETORY IMMUNOGLOBULIN A AND BAYLEY-III COGNITIVE SCORES IN CHILDREN FROM THE CANADIAN CHILD STUDY COHORT.

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Background and Aims: Dysbiosis of the intestinal microbiota has been demonstrated in neurodevelopmental disorders but the underlying mechanisms are poorly understood. Gut secretory IgA (SIgA) binds pathogenic microbes, preventing mucosal penetration. It appears that gut microbes influence SIgA production and binding characteristics through short chain fatty acid metabolites, providing them with some regulatory control. Serum IgA deficiency has been noted in children with Autism Spectrum Disorders (ASD). In this study, we aimed to determine whether SIgA level in infancy is associated with gut microbiota dysbiosis and neurodevelopmental outcomes in preschool children.

Methods: For a subsample of 298 children from the Canadian CHILD study cohort, gut microbiota of fecal samples collected at 4 months was profiled using 16S rRNA sequencing, SIgA level was measured by SIgA enzyme-linked immunosorbent assay and Bayley-III cognitive scores were assessed at 1 and 2 years of age. We evaluated putative causal relationships from gut microbiota enterotypes and taxa abundances to SIgA level and Bayley-III cognitive scores in statistical mediation models.

Results: We found an indirect effect between gut microbiota at 4 months and Bayley-III cognitive scores at 2 years, mediated by SIgA at 4 months. Infants with an enriched abundance of Bacteroidetes in their gut microbiota had higher Bayley-III cognitive score at 2 years potentially mediated by a higher SIgA level (indirect path ab: 0.57 (CI95%: 0, 1.4), p=0.098).

Conclusions: Our study contributes to growing evidence that neurodevelopment is influenced by infant gut microbial composition mediated by SIgA.

Disclosure: No significant relationships.

Keywords: infant gut microbiota, secretory Immunoglobulin A, Bayley-III Scale of Infant Development

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

THE MICROBIOTA OF BULGARIAN INFANTS AS A SOURCE OF LACTOBACILLI WITH PROBIOTIC PROPERTIES

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Background and Aims: Infant fecal microbiota represents a potent source of lactobacilli well adapted to the gut environment. Therefore the aim of the present study was to isolate lactobacilli from the fecal microbiota of Bulgarian infants and to select probiotic strains for use in infant probiotic formulations.

Methods: Ninety six isolates from 12 healthy infants (1-6 month-old) were collected and identified using phenotypic and genotypic (species-specific PCR, ARDRA with enzymes HaeIII and EcoRI, 16S rRNA gene sequencing) methods. *In vitro* tests (resistance to clinically relevant antibiotics, biofilm and adhesion capacity, antagonistic and immunomodulatory activity) were applied to assess the safety and probiotic potential of 33 strains.

Results: The samples generally harbored two-three dominant *Lactobacillus* strains including species as *L. rhamnosus*, *L. paracasei*, *L. plantarum*, *L. reuteri* and *L. fermentum*. Less frequently isolated were *L. delbrueckii*, *L. helveticus* and *L. oris*. Five strains (*L. paracasei* J35, *L. paracasei* F115, *L. rhamnosus* V410, *L. reuteri* K67, *L. helveticus* I108) were selected based on their (i) antibacterial and coaggregation effects against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* and (ii) biofilm forming and/or adhesion capacity to HT-29 cell line. The strains had no potential for transfer of antibiotic resistance and were able to stimulate high IL-10 production.

Conclusions: *L. paracasei* J35, *L. paracasei* F115, *L. rhamnosus* V410, *L. reuteri* K67 and *L. helveticus* I108 strains have probiotic potential and could be included in formulations targeting infants.

Disclosure: RG, AD-C, and EK are employed by the company. The funders had no role in the design and conduct of the study, , nor the decision to present the results.

Keywords: infant microbiota, Lactobacilli, probiotic

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

FECAL MICROBIOTA TRANSPLANTATION FROM INFANTS BORN BY CESAREAN SECTION AGGRAVATES HIGH-FAT DIET INDUCED OBESITY AND METABOLIC PHENOTYPE IN MICE

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Background and Aims: Cesarean section (CS) rates are increasing worldwide and being born by CS is linked to altered early life gut microbiota composition and increased risk of several chronic inflammatory diseases later in life, including obesity. To determine the importance of CS-induced dysbiosis on obesity we transferred microbiota from CS- or vaginally-delivery (VD) infants to germ-free mice and tested their susceptibility to obesity.

Methods: Male germ-free BALB/c were colonized with human microbiota from infants either born by CS or VD and subjected to high fat diet feeding for 16 weeks. Body weight and oral glucose tolerance were measured. Samples were collected for metagenomics of the gut microbiota, serum metabolomics, RNA sequencing and qPCR for tissue gene expression and cytokine and hormone measurements on serum and adipose tissue.

Results: Mice colonized with the CS microbiome had a higher body weight and lower glucose tolerance than mice with VD microbiome. In addition, several analytes in serum and adipose tissue, including insulin, differed between the CS and VD groups as well as metabolites, adipose and liver gene expression and the microbiome. CS delivered mice supplemented with human milk oligosaccharides (HMOs) did not differ from VD mice in weight gain and cytokine expression, but still had an impaired glucose tolerance and serum insulin.

Conclusions: CS-induced gut dysbiosis affects sensitivity to high-fat diet-induced obesity in a mouse model colonized with human microbiota, but supplementing the feed with HMOs partly rescues the obese phenotype in the mouse model, leaving hope for alleviating the long-term detrimental effects of CS on the offspring.

Disclosure: No significant relationships.

Keywords: Obesity, C-section, early life microbiota, germ-free, FMT

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

INFLUENCE OF GESTATIONAL AGE ON INFANT GUT MICROBIOTA AND SHORT CHAIN FATTY ACIDS (SCFAS): THE NELA COHORT

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Background and Aims: In Spain, 1 in 13 births occurs prematurely. The births of premature infants (PTI;<37 weeks of gestation) often occur by C-section, avoiding the contact with the mother's vaginal microbiome. Moreover, in most cases the intensive use of antibiotics or sterile parental nutrition are necessary. All these factors can interfere the colonisation process and the correct development of the gut microbiota, resulting in a dysbiosis status. The state of dysbiosis has been related to respiratory, immune, and neurological problems. The aim of this work was to elucidated how gestational age can influence the infant gut microbiota (IGM) and bacterial metabolites, of 3 months old infants belonging to the NELA cohort <https://nela.imib.es/>).

Methods: IGM was analysed by q-PCR, and SCFAs were determined by gas chromatography, comparing between PTI (n=8) and full-term infants (FTI; n=188). A multifactorial analysis was performed adjusting the model with other variables such as sex, weight, mode of delivery, type of lactation, mother's BMI pre-pregnancy and mother's age.

Results: No significant differences were found in the prevalence of each of the bacteria comparing FTI vs. PTI. Regarding SCFAs, gestational age did not seem to influence in these faecal metabolites. In both multivariate analyses, no statistically significant differences were observed between groups when the profile of microbiota and SCFAs was represented.

Conclusions: Higher counts were generally observed in most of the bacteria quantified in FTI group comparing with PTI group, but only the colonisation of *Atopobium* (including *Collinsella*) (Log genomic Eq./g faeces) (median (IQR)) (3.96 (2.66-5.37) vs. 2.89 (2.65-3.14), resulted significantly higher.

Disclosure: No significant relationships.

Keywords: Gestational age, infant gut microbiota, Short chain fatty acids

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

LONGITUDINAL EVALUATION OF INFANT GUT MICROBIOME COLONIZATION IN THE FIRST 6 MONTHS: EFFECTS OF EARLY EXPOSURE TO DIETARY SUGAR AND FIBER

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Background and Aims: Background: The diversity and taxonomic composition of the gut microbiome undergoes rapid changes during the first 2-3 years of life but there are limited studies that have examined the impact of early nutrition on the developing infant's gut microbiota.

Methods: Methods: Latino mother-infant pairs from the longitudinal Mother's Milk Study (n=155) were included. The infant gut microbiota was analyzed at 6 months of age using 16S rRNA amplicon sequencing. A multivariable generalized linear regression model was performed to examine associations between dietary nutrients and microbial community abundance at 6 months of age.

Results: Results: Association analysis indicated that specific bacterial genera were correlated with total sugar and fiber intake at 6 months. Multiple bacteria at the genus level were associated with total fiber intake such Blautia (b=0.31, P_{FDR}= 0.00), Coprococcus (b= 0.20, P_{FDR}= 0.02), and Bacteroides (b=0.27, P_{FDR}= 0.04). Infants with higher consumption of total sugar exhibited a lower relative abundance of Blautia, Dorea (b=-0.20, P_{FDR}= 0.04), and f__Lachnospiraceae-Clostridium (b=-0.04, P_{FDR}=0.00).

Conclusions: Conclusion: These results indicate a significant association between dietary fiber and sugar intake and gut microbiome already evident by 6 months of age. Infants with higher fiber consumption exhibited increasing relative abundances of short-chain fatty acids-producing bacteria including Bacteroides and Lachnoclostridium which may improve metabolic outcomes. Higher sugar intake is associated with a lower abundance of bacteria with potential probiotic functions such as Blautia. Early life dietary intake may impact the developing microbiome, which may have important implications for infant growth and development.

Disclosure: No significant relationships.

Keywords: complementary diet, Latino, Infant diet, Gut Microbiome, early life nutrition

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

BIFIDOBACTERIUM BREVE-REACTIVE ANTIBODIES IN BLOOD SERUM OF CHILDREN OF MOTHERS AT RISK OF GESTATIONAL DIABETES

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Background and Aims: Gestational diabetes (GDM) is a disorder of carbohydrate metabolism that occurs during pregnancy, with dysbiosis of the gut microbiota considered a contributing factor. Specific bacterial taxa associated with GDM may be transmitted to offspring, and the accompanying dysbiosis may be related to allergy in early childhood. *Bifidobacterium breve* has been found in the gut of breastfed children and is thought to be involved in immune system activation and allergy prevention.

Methods: We aimed to determine *B. breve*-reactive IgA, IgG and IgG2 antibodies in the blood serum of children whose mothers were in the risk group for GDM during pregnancy. The study group consisted of 88 children aged 1 – 6 years. Blood samples were taken at two time-points, one year apart, during the study's paediatrician visit. *B. breve* DSM20213 was diluted to a concentration of 5×10^6 cells/ μ l and antibody reactivity was detected on an LSRFortessa flow cytometer. The obtained results were compared with children's clinical data (gender, age, breastfeeding, way of delivery and maternal GDM diagnosis). IgE sensitisation was measured with Phadiatop Infant on ImmunoCap 100 (Thermo Fisher).

Results: Reactivity to *B. breve* did not differ among children based on their mother's GDM diagnosis. We detected mainly IgA type antibodies that reacted with *B. breve*, especially in younger children who were breastfed, delivered vaginally and were IgE-negative. With increasing age, IgA type reactivity decreased and IgG type reactivity increased. We could not detect *B. breve* polysaccharide-specific IgG2 antibodies.

Conclusions: *B. breve* may have good potential to participate in allergy prevention.

Disclosure: Funded by EU HEDIMED and Estonian PRG712 project

Keywords: allergy, Infant, Bifidobacterium breve, breastfeeding, gestational diabetes mellitus

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

GUT MICROBIOME CONTRIBUTION TO PREDICTION OF POSTPRANDIAL GLYCEMIA IN PATIENTS WITH GESTATIONAL DIABETES

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Background and Aims: We aimed to evaluate the contribution of microbiome features in postprandial glycaemic responses (PPGR) in women with gestational diabetes mellitus (GDM) and healthy pregnant women.

Methods: We obtained stool samples for 96 pregnant women (65 GDM, 31 control) who consented to continuous glucose monitoring (CGM) for 7 days and provided relevant food diaries. Stool samples were collected within 1-2 weeks after recruitment (28.8±3.6 weeks). 16S rRNA gene sequence analysis was carried out after sequencing on a MiSeq platform. PPGR models (based on meal and patient characteristics) with and without microbiome composition were evaluated. The Shapley additive explanations method was implemented to evaluate feature importance.

Results: The contribution of microbiome features to the accuracy of PPGR prediction depended on the choice of prediction algorithm and method of diary filtering. Inclusion of the microbiome data slightly increased the accuracy for predicting peak glycaemic levels (mean absolute error 0.484 mmol/L vs. 0.497 mmol/L for the models with and without microbiome data) and correlation between actual and predicted values (R=0.718 vs. R=0.704) based on the full dataset (N=96), but did not change the accuracy in the more strictly filtered dataset (N=45). However, all models included bacterial traits among the top 10% of the most impactful features on the PPGR prediction. In the strictly filtered dataset the greatest contribution to PPGR was made by the abundance of bacteria of the genera Ruminococcus and Bacteroides.

Conclusions: Microbiome features are among the top 10% of the most impactful parameters on the PPGR prediction in pregnant women with GDM.

Disclosure: No significant relationships.

Keywords: Microbiome, gestational diabetes, blood glucose prediction, personalized nutrition, pregnancy

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

LONG-TERM EFFECTS OF CONTINUOUS ANTIBIOTIC PROPHYLAXIS ON THE GUT MICROBIOME PROFILE IN INFANTS WITH VESICoureTERAL REFLUX

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Background and Aims: The development of the gut microbiota (GM) during infancy can be influenced by several factors, including antibiotic treatment, which may have long-term consequences for the host health (e.g., development of allergy, asthma, and obesity). Vesicoureteral reflux (VUR) is a typical disorder of early childhood, which is treated with continuous antibiotic prophylaxis (CAP) to limit the development of urinary tract infections and subsequent damage to the renal parenchyma. However, the use of CAP is somewhat controversial. In this context, the aim of this study was to investigate the long-term effects of CAP on the GM of children with high-grade (III-V) VUR.

Methods: Specifically, the GM of 124 children with VUR treated or not with CAP was longitudinally characterized from enrolment to 72 months by 16S rRNA amplicon sequencing. In parallel, fecal levels of short-chain fatty acids were determined over time.

Results: Comparative subgroup analysis revealed changes in the dynamics of GM diversity in CAP-exposed infants compared to the non-CAP group. From the taxonomic standpoint, the GM was characterized by an initial CAP-related dysbiosis, *i.e.*, an increased relative abundance of opportunistic pathogens and decreased proportions of health-associated taxa. The dysbiosis tended to resolve over time, also from a functional point of view, but some potentially harmful signatures persisted.

Conclusions: This study demonstrates that even a short exposure to CAP in infants with VUR can lead to long-term GM alterations, with potential risks to host health, including the development of GM-related disorders.

Disclosure: No significant relationships.

Keywords: Gut microbiota, antibiotic prophylaxis, Vesicoureteral reflux, Urinary tract infection, Short chain fatty acids

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM BREAST MILK OF BULGARIAN WOMEN AND THEIR INFANT FAECES

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Background and Aims: Knowledge of the breast milk microbiome and the gut microbiome of the newborns are of high importance, as variations in them can serve to diagnose various diseases, both early and later in human life. Furthermore, a number of factors such as host, microorganisms, medical factors and environment can influence the composition of the breast milk. The aim of this study was to isolate and identified lactic acid bacteria in mature breast milk of healthy Bulgarian women and their infant faeces.

Methods: Isolated bacteria were phenotypically characterized by Gram stain and catalase production. For further analyses, only Gram positive, catalase-negative and rod-shaped strains were selected. The identification of the isolates were confirmed by specific PCR, MALDY-TOF-MS analyses and by 16S rRNA sequence analysis.

Results: We identified fifteen bacterial strains isolated from breast milk and infant faeces by conventional and molecular methods. A total of 54 colonies were selected based on different morphologies on MRS agar medium modified with vancomycin (10 mg/l). The results showed that most of the strains were identified as *Lacticaseibacillus rhamnosus* and *Limosilactobacillus reuteri*. and one *Lactobacillus oris*.

Conclusions: Our studies on the infant faeces microbiota suggested that *Lacticaseibacillus rhamnosus* and *Limosilactobacillus reuteri* were predominant in the studied samples. We isolated strain of *Lactobacillus oris* from breast milk. Isolated strains from mother's milk and infant's faeces may be a potential natural source for probiotic strains. Acknowledgement: This study was supported by Bulgarian National Science Fund, „Competition for financial support for basic research projects – 2022”, Grant КП-06-H6/9.

Disclosure: No significant relationships.

Keywords: breast milk, infant faeces, lactic acid bacteria, identification, MALDI-TOF

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

BACTERIAL COMPOSITION IN BREAST MILK FROM WOMEN DIAGNOSED WITH GESTATIONAL DIABETES MELLITUS IN COMPARISON TO WOMEN WITHOUT GESTATIONAL DIABETES MELLITUS: A PILOT STUDY

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Background and Aims: Background Human breast milk (HBM) is a contributing factor in modulating the infants gut microbiota. HBM contains bacteria that are directly transferred to the infant during breastfeeding. It has been shown that children of women diagnosed with gestational diabetes mellitus (GDM) have a different gut microbiota compared to children of women without GDM. Therefore, we hypothesize that mothers with GDM have a different HBM microbiota, influencing the metabolic function and capacity of the child later in life. This study aims to investigate the HBM bacterial composition in women with and without GDM.

Methods: In this case control study, a total of 47 women were included: 19 women with GDM and 28 women without GDM. A milk sample was collected from each participant 1-3 weeks postpartum and the bacterial composition was examined through 16S rRNA gene sequencing targeting the V4 region.

Results: Relatively high abundances of Streptococcus and Staphylococcus were present in the samples from both women with and without GDM. Differences in bacterial composition between women with and without GDM will be investigated through alpha diversity, beta diversity and differential abundance analysis.

Conclusions: Conclusion If a GDM-associated breast milk microbiota is present and is transferred to the child, then early modulation of the maternal or infant's microbiota profile, could be a therapeutic target to prevent later metabolic dysfunctions.

Disclosure: No significant relationships.

Keywords: GDM, breast milk microbiota, Early gut colonization

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

RECTAL AND OROPHARYNGEAL MICROBIOME DEVELOPMENT DURING THE FIRST YEAR OF LIFE. DIFFERENCES DEPENDING ON THE TYPE OF DELIVERY AND TYPE OF LACTATION.

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Background and Aims: Throughout our lives, genetic, environmental factors and lifestyle modulate our microbiome. The gestation and lactation period, as well as the first year of life, are two key moments in determining the microbiological strains of the child. The first contact of a newborn with the microbiota occurs at delivery. In a natural-birth, the baby is exposed to the maternal bacterial flora whereas babies delivered by caesarean-section have fewer beneficial bacteria in their postnatal microbiome. In the early stages of life, the bacterial environment is in continuous evolution during the first three years, to establish a more stabilized pattern throughout life. The aim of the project is to study the evolution of the oropharyngeal and rectal microbiota of the newborn in the first year of life, as well as its relationship with the type of delivery (natural or caesarean section) and type of lactation feeding.

Methods: A total of 500 DNA samples were extracted from 50 newborns. The samples were comprised of oropharyngeal and rectal smears collected on different stages of the baby's life: 7 days, 1-4-6-12 months. Delivery mode and lactation type were registered in each stage. Libraries of the regions V3, V6-7 and V9 of the bacterial 16s rRNA gene were performed and sequenced using Ion Torrent technology.

Results: The Shannon index increases in both oropharyngeal and rectal samples as the child grows. Bacterial diversity is greater in children born by natural-childbirth and formula feeding.

Conclusions: Delivery mode and lactation type affects the composition of the oropharyngeal and rectal microbiota in newborn babies.

Disclosure: No significant relationships.

Keywords: Oropharyngeal samples, Rectal samples, Delivery mode, Lactation type, Newborn

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

PROBIOTIC SUPPLEMENTATION AND RISK OF NECROTIZING ENTEROCOLITIS AND MORTALITY AMONG EXTREMELY PRETERM INFANTS - A MULTICENTER, DOUBLE-BLIND, REGISTRY-BASED RANDOMIZED CONTROLLED TRIAL: STUDY PROTOCOL

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Background and Aims: Extremely preterm (EPT) infants, defined as those born before 28 weeks' gestational age, are a vulnerable patient group at high risk of adverse outcomes. Necrotizing enterocolitis (NEC) is regarded as the most severe gastrointestinal-related morbidity in these infants with a high mortality rate. Previous randomized controlled trials (RCTs) have shown reduced incidence of NEC following probiotic supplementation, however were underpowered for EPT infants, rendering evidence for probiotic supplementation in this population insufficient to date.

Methods: The Probiotics in Extreme Prematurity in Scandinavia (PEPS) trial is a multicenter, double-blinded, registry-based RCT conducted among EPT infants (n= 1620) in Sweden and Denmark (NCT05604846). Enrolled infants will be allocated to either ProPrems® (*Bifidobacterium infantis*, *Bifidobacterium lactis*, and *Streptococcus thermophilus*) diluted in 3mL breastmilk or 3mL non-supplemented breastmilk daily until gestational week 34. The primary composite outcome is the incidence of NEC and mortality. Secondary outcomes include incidence of late-onset sepsis, hospitalization, antibiotic use, feeding tolerance, growth, and body composition.

Results: As of August 2023, 30 EPT infants have been included (86% consent rate), of which 21 have finished receiving the intervention. The average gestational age at birth is 25+4 weeks (range 22+0 to 27+6). Overall, four infants (19%) have been diagnosed with NEC, six (29%) with sepsis, and two (10%) have died.

Conclusions: Due to limited evidence, probiotic supplementation is not currently recommended in Sweden and Denmark in EPT infants. The PEPS trial will investigate the effect of supplementation to inform guidelines through sufficiently powered, high-quality evidence, with potential implications for clinical practice worldwide.

Disclosure: No significant relationships.

Keywords: Probiotics, Feeding tolerance, Necrotizing enterocolitis, Extreme prematurity, Growth failure

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

MICROBIOTA CHARACTERIZATION OF MOTHER-INFANT DYADS BELONGING TO THE NELA COHORT: SINCE DELIVERY UNTIL EARLY LIFE

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Background and Aims: Maternal microbiota forms the first microbial inoculum after birth, when the principal colonization occurs, and during lactation, when the microbial diversity increases and converges toward a stable adult-like microbiota by the end of the first 3 years of life. It has been reported that a correct infant early microbiota colonization and maturation throughout life would reduce the risk of diseases in early and later life. The aim of this study was to gaining a better understanding of the colonization process and microbial transfer from mother to child using sequencing techniques.

Methods: The rectal and breastmilk microbiota of the mother, and the meconium and faecal microbiota of the offspring belonging to the NELA cohort (<https://nela.imib.es/nela/descripcion.jsf>) has been characterized using sequencing techniques

Results: Mother's rectal microbiota was characterized by a higher proportion of Firmicutes followed by Proteobacteria. On the contrary, in breastmilk, a greater number of taxa belonging to Proteobacteria was observed. Regarding the meconium and faeces microbiota, it was observed a depletion in bacteria belonging to the Proteobacteria phylum and an increase in Firmicutes. Rectal exudates presented the greatest alpha diversity compared to the rest of the types of samples. In breastmilk the family more represented was *Oxalobacteriaceae* as well as in meconium and no significant differences were observed between the microbiota of meconium and infant faeces. With respect to beta diversity, infant faeces resulted significantly different from rectal exudates while breastmilk and meconium showed similar overall taxonomic composition.

Conclusions: The microbiota of breast milk and meconium present very similar colonization patterns.

Disclosure: No significant relationships.

Keywords: infant gut microbiota, early colonisation, Breastmilk microbiota, mother rectal microbiota, meconium

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

GESTATIONAL DIABETES MELLITUS STATUS DURING PREGNANCY AND THEIR INFLUENCE IN INFANT GUT MICROBIOTA: THE NELA COHORT

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Background and Aims: Gestational Diabetes Mellitus (GDM) is a public health problem due to its high prevalence (between 7.6% and 10.6% in Spain). Recent studies reported that GDM was associated with unique changes in the mother gut microbiota composition during pregnancy. It is known that maternal microbiota forms the first neonate's microbial inoculum, being transmitted to offspring during childbirth. Therefore, mother's microbiota is a key factor to provide a correct infant early microbiota colonization in order to reduce the risk of disease in early and later life. The aim of this study was to ascertain the influence of GDM status on the gut microbiota of infants at 3 months of age and their Short Chain Fatty Acids (SCFAs) profile,

Methods: qPCR and gas chromatography techniques were used to study gut microbiota and SCFA, respectively. A multifactorial analysis was performed adjusting the model with other variables such as sex, weight, mode of delivery and mother's age. Infants specimens belonged to NELA Cohort (<https://nela.imib.es>), and a comparison between faecal samples from children whose mothers developed GDM (n=15) and those who did not (n=180) was performed.

Results: No significant differences were observed regarding the counts or the prevalence of any of the bacteria studied between both groups. Regarding SCFAs, there was also no statistical differences. No statistically significant differences were observed between groups in both multivariate analyses adjusting the model with other confounding variables.

Conclusions: The same non-modulating effect by gestational diabetes over the gut microbiota of infants, was also clearly observed in their generated metabolites such as SCFAs.

Disclosure: No significant relationships.

Keywords: gestational diabetes mellitus, infant gut microbiota, Short chain fatty acids

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

INFLUENCE OF MODE OF DELIVERY AND INTRAPARTUM ANTIBIOTIC (IPA) ON INFANT GUT MICROBIOTA AND SHORT CHAIN FATTY ACIDS (SCFAS): THE NELA COHORT

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Background and Aims: The composition of the infant gut microbiome (IGM) has been linked to adverse long-term health outcomes. Several factors are known to impact the composition of the microbiome, including mode of delivery, gestational age, feeding method and exposure to antibiotics. The impact of intrapartum antibiotics (IPAs) on the IGM requires further research. The aim of this work was to elucidated how the mode of delivery and the use of antibiotics intrapartum can influence the IGM and bacterial metabolites of 3 months old infants belonging to the NELA cohort (<https://nela.imib.es/>).

Methods: IGM was analysed by q-PCR, and SCFAs were determined by gas chromatography, comparing between infants born by vaginal delivery not exposed to antibiotics during labour (V), infants who were (V+A), and infants born by caesarean section exposed to antibiotic during labour (C+A) and not exposed (C). A multifactorial analysis was performed adjusting the model with other variables such as infant's sex, gestational age, type of lactation, mother's BMI pre-pregnancy and mother's age.

Results: Higher counts of *Bacteroidetes* were observed in in V+A group vs. C and C+A groups. On the other hand, C+A infants presented higher counts of *Staphylococcus aureus* comparing with other groups. Regarding SCFAs, mode of delivery, taking in to account the use of IPAs did not seem to influence in these metabolites. In both multivariate analyses, no statistically significant differences were observed between groups.

Conclusions: Necessary to carry out more studies in order to know how the type of delivery or the use of antibiotics influence the pattern of colonization.

Disclosure: No significant relationships.

Keywords: infant gut microbiota, early colonisation, mode of delivery, Intra partum antibiotic, C-section

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

PREDICTING PRETERM BIRTH BASED ON VAGINAL MICROBIOTA ASSESSMENT BY STANDARDIZED REAL-TIME PCR IN THE FIRST TRIMESTER

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Background and Aims: Detecting high risk of preterm birth early makes its prevention possible. The aim of the work was to develop a mathematical predictive model for assessing the risk of preterm birth based on a quantitative analysis of the vaginal microbiota in the first trimester of pregnancy.

Methods: The study included 199 pregnant women, i.e. 41 pregnancies that ended in preterm birth, and 158 — in term birth. Vaginal microbiota was analyzed in all patients in the 1st trimester of pregnancy by quantitative real-time PCR (qPCR) using Femoflor ® 16 real-time detection kit (DNA Technology LLC, Russian Federation). Discriminant analysis was used for development of the prognostic model.

Results: Lactobacillus spp., Staphylococcus spp., Sneathia spp. / Leptotrichia spp. / Fusobacterium spp., Gardnerella vaginalis / Prevotella bivia / Porphyromonas spp., Eubacterium spp., Lachnobacterium spp. / Clostridium spp., Mobiluncus spp. / Corynebacterium spp., Ureaplasma spp. and the total bacterial load were the most significant parameters in predicting the risk of PB. The data were determined in the Lg GE/ml format. A method for predicting preterm birth was developed with the calculation of the PRIMA prognostic index (Premature Birth. Index Of Microbiological Analysis). If the PRIMA value is higher than 0 – the risk of premature birth is low, if PRIMA < 0 – the risk is high. The sensitivity and specificity of the method are respectively 70.7% and 79.75%, the effectiveness is 77.89%.

Conclusions: Evaluation of vaginal microbiota in the 1st trimester makes it possible to identify a high-risk group of PB and perform timely preventive measures.

Disclosure: No significant relationships.

Keywords: real-time PCR, Vaginal microbiota, premature birth, Femoflor-16, prediction

E-POSTER VIEWING - AS03. GUT BRAIN AXIS

EARLY CHILDHOOD GUT MICROBIOTA: ITS ASSOCIATION WITH EMOTIONAL REGULATION AND COGNITIVE ASPECTS OF EXECUTIVE FUNCTION

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Background and Aims: Human gut microbiota (GM) develops into adult-like composition around 3-5 years of age. Importantly humans show remarkable cognitive development at this time, particularly in executive function. Executive function is goal-directed cognitive process comprising emotional regulation (ER) and cognitive control (CC). Identifying factors affecting executive function development is crucial for innovating the new intervention methodology.

Methods: We examined 257 Japanese children aged 3–4 to investigate links between GM and developmental risk of executive function. We also measured their ER and CC using the mother-reported BRIEF-P questionnaire (Gioia, 2003; Ukena, 2006). Based on cutoff values, 26 children were allocated to ER-risk group, and 231 to ER-control group. Twenty were allocated to CC-risks, and 236 to CC-controls. GM was evaluated using 16S rRNA gene sequencing obtained from fecal samples. Additionally, we collected information on their dietary habits.

Results: We found that ER-risks had a higher abundance of the genera *Actinomyces* and *Sutterella* than ER-controls (q -both < 0.10). Furthermore, ER-risks had a lower green/yellow vegetables intake frequency and a higher proportion of picky eating than ER-controls (q -both < 0.10). In contrast, no such significant correlation was found in the risk of CC.

Conclusions: This study suggests the following three points. (1) Preschoolers' GM components is especially related to emotional processing, (2) ER-risk children had more inflammation-related bacteria, and (3) Dietary interventions may be especially effective for ER development support.

Disclosure: No significant relationships.

Keywords: executive function, emotion regulation, cognitive control, preschoolers, dietary habits

E-POSTER VIEWING - AS03. GUT BRAIN AXIS

P-CRESOL DERIVATIVES INTERACT WITH THE BLOOD–BRAIN BARRIER AND HIGHLIGHT THE COMPLEX NATURE OF MICROBIOTA–HOST COMMUNICATION PATHWAYS ASSOCIATED WITH THE GUT–BRAIN AXIS

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Background and Aims: A by-product of microbial fermentation of tyrosine and phenylalanine in the large intestine, p-cresol undergoes conjugation in both enterocytes and the liver, reaching systemic circulation as p-cresol sulfate (pCS) with ten-fold lower levels of p-cresol glucuronide (pCG). In metabolically healthy individuals, pCS and pCG are efficiently cleared by the kidneys, whereas in patients with renal impairment both accumulate within the blood. We sought to investigate the effects of physiologically relevant and uraemia-associated levels of these microbiota-associated metabolites on the mammalian blood–brain barrier (BBB).

Methods: Using a combination of cell-line assays (human cerebrovascular endothelial cell line hCMEC/D3) and mouse work (e.g. PMID:35596559, 34836554), we tested the effects of pCS (0, 10, 100, 1000 µM) and pCG (0, 0.1, 1, 10, 100 µM) on the mammalian BBB and brain transcriptome.

Results: In vitro and in mice, pCG prevented the BBB-permeabilizing effects of endotoxin, acting by antagonizing the LPS receptor TLR4. In contrast, pCS increased paracellular permeability and disrupted intercellular tight junctions in a dose-dependent manner. pCS changed the whole-brain transcriptome, suppressing neuronal activity, transcription and mitochondrial respiration pathways. In vivo, the deleterious effects of pCS on the BBB were prevented by the EGFR antagonist erlotinib. Human hCMEC/D3 endothelial cells exposed to serum from haemodialysis patients, but not from healthy donors, showed an erlotinib-sensitive increase in paracellular permeability that correlated with the total serum pCS content.

Conclusions: Our data demonstrate the complexity of microbial metabolite–host communication pathways underlying the gut–brain axis, and identify means by which microbiota-associated metabolites can be targeted to improve brain function.

Disclosure: No significant relationships.

Keywords: Metabolites, transcriptome, kidney disease, neuroinflammation, mechanisms

E-POSTER VIEWING - AS03. GUT BRAIN AXIS

ARTEMISIA ASIATICA EX ON MUCINIPHILA DOMINANCE FOR MODULATION OF ALZHEIMER'S DISEASE IN MICE

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Background and Aims: Gut microbiome influence neurodevelopment and modulates behavioral and neurological disorders through bidirectional communication between the gut and the brain, the gut-brain-axis. A disruption in the gut microbiome can be the cause of Alzheimer's disease (AD) or the result of progression of AD. Artemisia asiatica ex, an extract of Artemisia asiatica Nakai (Stillen®, DA-9601), is known to treat gastric mucosal ulcers and inflammation. However, It has also been reported to improve depression by increasing brain-derived neurotrophic factor in the hippocampus. Therefore, we assumed that DA-9601 can be a potential therapeutic candidate for AD that may play role through the gut-brain axis.

Methods: Tg2576 mice were used as the animal model for AD. They were divided into four groups: wild type (WT; n=6), AD mice (Ctrl; n=6), DA-9601-administrated AD mice with 30mg/kg/day (DA_30mg; n=6) and 100mg/kg/day (DA_100mg; n=6). Changes in gut microbiome were analyzed by 16S gene sequencing. Microglial activation were evaluated by western blot analysis of Iba-1. Claudin-5, Occludin, Laminin and CD13 assay were analyzed for blood-brain barrier (BBB) integrity. Amyloid beta (A β) accumulation was analyzed by ELISA, and cognition were monitored by novel object location test.

Results: DA-9601 improved the cognitive behavior of mice (DA_30mg **p<0.01; DA_100mg **p<0.01), and decreased A β 42/40 ratio (DA_30mg ***p<0.001; DA_100mg **p<0.01). Increased Iba-1, upregulation of Claudin-5 (DA_30mg *p<0.05) and Occludin (DA_30mg **p<0.01; DA_100mg ***p<0.001) altered microglial activation and improved BBB integrity. Muciniphila species increased by DA-9601 administration (DA_30mg 47%; DA_100mg 61%)

Conclusions: DA-9601 showed improvement of AD pathology with Muciniphila dominance in the gut microbiome.

Disclosure: No significant relationships.

Keywords: Gut-brain axis, alzheimer's disease, Artemisia asiatica ex, Muciniphila

E-POSTER VIEWING - AS03. GUT BRAIN AXIS

COUPLING AMONG MICROBIOMES, AUTONOMIC NERVES, AND SELF-REGULATION IN HUMAN CHILDREN AND THEIR MOTHERS

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Background and Aims: The autonomic nervous system (ANS) modulates the gut–brain axis. The gut microbiota is associated with vagal activity, which affects self-regulation mediated by the prefrontal limbic system. The first three years after birth is a key developmental period for the gut microbiota and the ANS. Notably, during this period, children depend on their caregivers for co-regulation. Thus, we examined gut microbiome–ANS–socioemotional reactivity in mother–child pairs.

Methods: For the 78 mother-child pairs in this study, we collected fecal samples from three-year-old children and mothers, as well as three days of continuous electrocardiography from a Holter monitor. We analyzed cardiac sympathetic (CSI) and cardiac vagal (CVI) indices and measured the children's socioemotional reactivity and mothers' parenting stress using questionnaires.

Results: Children's microbiome (Shannon α) diversity was related to their CVI ($r = 0.42$, $p = 0.003$) and CSI ($r = -0.44$, $p = 0.001$), and their CVI was related to socioemotional reactivity, specifically negative emotion expression ($r = -0.31$, $p = 0.028$) and positive emotion expression during play ($r = 0.316$, $p = 0.024$). Furthermore, Shannon α was correlated between child–mother pairs ($r = 0.54$, $p < .001$). The children whose mothers showed high psychological stress showed lower CVI and significant differences in some gut microbiota (e.g., *Colidextribacter*, *Blautia*) than the control children (all $ps < 0.05$).

Conclusions: We identified functional relationships among children's microbiome–ANS–self-regulation and mothers' mental status that suggest the need for creating personalized child care supports for both mother and child.

Disclosure: No significant relationships.

Keywords: Gut Microbiome, children, autonomic nervous system, self-regulation, parenting stress

E-POSTER VIEWING - AS03. GUT BRAIN AXIS

GUT MICROBIOTA ANALYSIS OF A PATIENT WITH POST-SSRI SEXUAL DYSFUNCTION: CASE REPORT AND LITERATURE REVIEW

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Background and Aims: Selective serotonin reuptake inhibitors may exert antibacterial activity with detrimental effect on the gut microbiota. The resulting increase in gut permeability can lead to sexual dysfunction mediated through microglia activation and cytokine release, known as post-SSRI sexual dysfunction (PSSD).

Methods: Selective serotonin reuptake inhibitors may exert antibacterial activity with detrimental effect on the gut microbiota. The resulting increase in gut permeability can lead to sexual dysfunction mediated through microglia activation and cytokine release, known as post-SSRI sexual dysfunction (PSSD).

Results: Our analysis showed low richness and diversity of the gut microbiota. The taxonomic analysis revealed a dominance of the phylum *Firmicutes* (82.69%) over phylum *Bacteroidetes* (14.26%). The relative proportion of phylum *Proteobacteria* (1.7%) was low. On the genus level the taxonomic analysis revealed high relative abundances of saccharolytic genera and low relative abundances of proteolytic genera such *Bacteroides*. The proportions of anti-inflammatory species *Lactobacillus* (0.0%), *Akkermansia muciniphila* (0.0%) and *Faecalibacterium prausnitzii* (3.36%) were low.

Conclusions: The conducted analysis revealed substantial perturbances of the gut microbiota. Although it is unclear whether the fluoxetine therapy caused those, the patient's symptoms could be associated with the gut microbiota perturbances based on literature on similar conditions. Although no causative relationship between the reported gut microbiota perturbances and symptoms of PSSD can be made, further research is necessary to investigate treatment options for PSSD based on gut microbiota profiling.

Disclosure: No significant relationships.

Keywords: SSRI, gut brain axis, sexual dysfunction, gut microbiota analysis

E-POSTER VIEWING - AS03. GUT BRAIN AXIS

GUT MICROBIOTA COMPOSITION AND DIET CONTRIBUTE TO RESISTANCE TO ANTI-SEIZURE MEDICATIONS IN EPILEPTIC PEDIATRIC PATIENTS

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Background and Aims: Microbiota-gut-brain axis dysfunction is emerging as a pathogenic mechanism in epilepsy. Epilepsy patients have intestinal dysbiosis and special diets can alter the gut microbiota composition and modulate disease outcome. We investigated gut-microbiota populations in pediatric epileptic patients and the role of diet in susceptibility or resistance to anti-seizures medications.

Methods: Children with epilepsy were sub-grouped into drug-sensitive (DS) and drug-resistant (DR). A food diary was used to evaluate nutritional habits and the Rome IV questionnaire was used to record gastro-intestinal symptoms and stool consistency. Stool samples were then processed through 16S rRNA, alpha (AD) and beta-diversity (BD) were calculated, and differential abundance analysis was performed using linear models by adjusting for carbohydrate and protein consumption.

Results: 46 patients with a median age of 6.5 years (IQR:8; range: 0-17 years) and 33 aged-matched healthy (H) children were recruited. 31 (67%) patients had developmental and epileptic encephalopathy, 7 (15%) had genetic generalized epilepsy, 7 (15%) had focal epilepsy, and 1 (2%) had progressive myoclonic epilepsy. 25 (54%) patients were classified as DS and 21 (45%) as DR. Both the DR and the DS subgroups showed a significantly different BD compared to the H group. The epilepsy groups were characterized by *Eubacterium oxidoreducens*, *Hugatella*, and *Lachnospiraceaea*. The DR group was further defined by increased abundance of *Sellimonas*, *Eubacterium ventriosum*, *Coprobacter*, *Erysipelotrichaceae*, and *Lachnospiraceae*.

Conclusions: Epileptic patients displayed unique gut metagenomic signatures compared to healthy controls, with substantial gut microbiota variation between DS vs DR patients, which could contribute to the individual's response to anti-seizures medications.

Disclosure: No significant relationships.

Keywords: Epilepsy, Microbiota-gut-brain axis, Microbiome

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

ASSOCIATION OF HABITUAL FOOD CONSUMPTION WITH SALIVA MICROBIOTA AND ENZYMATIC PROFILES IN FINNISH CHILDREN

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Background and Aims: Association of habitual food intake with blood metabolites and gut microbiome have guided our understanding on diet's contribution to human metabolism and development of chronic diseases. Recently, saliva microbiome is gaining attention as a first line of defense with contributions to human health. We aimed to understand the association of habitual diet on human metabolism through studying the saliva microbiome.

Methods: Data comprised 50 children (50% male) with a mean age of 14.2 (SD 0.3), selected from the Finnish Health in Teens (Fin-HIT) cohort. Habitual food consumption was estimated with three summary indexes indicating weekly consumption frequency of sugary products (STI), fruits, berries and vegetables (plants, PCI) and milk and ice cream (dairy, DCI). Saliva microbiota was subjected to shallow metagenome sequencing. Associations between summary indexes and enzymatic profiles were investigated.

Results: DCI associated with the highest number of enzyme profiles (136), followed by STI (67) and DCI (28) (Pearson correlation $p < 0.05$). Intriguingly, STI showed a higher positive/negative correlation ratio (5.09), while DCI and PCI showed an extremely lower ratio (0.54 and 0.33, respectively). Backtracking analysis revealed that *Veillonella dispar* and *Haemophilus parainfluenzae* were the key species contributing to the enzymatic profiles associated with PCI, DCI and STI, which highlighted the production of molybdopterin molybdotransferase and nitrate reductase.

Conclusions: The main driver of the enzymatic profiles was the consumption of sugary products, followed by dairy products and plants. Our findings highlight the role of habitual diet as a modifier of bacterial enzymatic profiles in saliva.

Disclosure: No significant relationships.

Keywords: metagenomics, food consumption, diet, saliva microbiota, enzymatic profiles

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

COMPARISON OF BRONCHOALVEOLAR LAVAGE (BAL) AND LUNG TISSUE BIOPSY (LTB) PROCEDURES IN THE EVALUATION OF RESPIRATORY TRACT MICROBIOTA IN SARCOIDOSIS

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Background and Aims: Pulmonary sarcoidosis is a systemic inflammatory disease leading to decreased respiratory capacity and may end lethally if not treated. Lung tissue biopsy (LTB) is a reliable diagnostic procedure for sarcoidosis, although invasive. LTB sample is examined microscopically for granuloma formations which are a biological marker for sarcoidosis. There is a line of evidence suggesting that granuloma formation in sarcoidosis can be triggered by alterations in the composition of respiratory microbiota. Analysis of bronchoalveolar lavage (BAL) can be an alternative less invasive procedure in sarcoidosis diagnosis. BAL is analyzed for microbial content, inflammatory markers, and other indicators of respiratory health.

Methods: Our study compared the pulmonary microbiome composition in patients with sarcoidosis. We analysed by NGS BAL (n=8) and LTB (n=7) samples. Total DNA was extracted from the samples and next-generation shotgun sequencing was performed. The host-to-microbiome DNA sequence ratio measured the quality of the analysis. Bacteriome and fungiome taxonomic composition and diversity were assessed for BAL and LTB samples.

Results: On average, the yield of microbial DNA sequences was higher in BAL than in LTB samples with a factor of 32. This was due to the predominance of bacterial reads in BAL vs. LTB (52377.78±11226.1 vs. 1633.43±1719.27), whereas no significant differences were found for the fungiome.

Conclusions: Each procedure has its own advantages and disadvantages and should be used in combination to obtain the most accurate assessment of the lung microbiota in sarcoidosis patients. This research was funded by the Bulgarian National Science Fund within National Science Program VIHREN, contract number KP-06-DV/10-21.12.2019.

Disclosure: No significant relationships.

Keywords: Bronchoalveolar lavage (BAL), lung tissue biopsy (LTB), bacteriome, sarcoidosis, fungiome

052 / #218

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

INDIVIDUAL AND GEOGRAPHIC SPECIFICITIES OF THE HUMAN BLOOD MICROBIOME

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Background and Aims: The presence of blood microbiome has been reported and recognized in healthy individuals and in physiological and pathological conditions. The origins, composition and function of blood microbiota remain to be elucidated. The individual and geographic specificities of the human blood microbiome in healthy and diseased individuals are largely unknown. Our study aimed to investigate the individual and geographic specificities of the blood microbiome.

Methods: We collected and analysed blood microbiome data from 59 healthy individuals; 20 from the USA (PRJNA46335), 11 from China (PRJNA428535), and 28 from Bulgaria. DNA extraction was performed in independent laboratories applying different methods. All blood samples were analysed by targeted 16S sequencing by Illumina technology. Kraken 2 (Silva database) and Bracken pipeline generated the OTU table. Alpha and beta diversity were calculated.

Results: The results obtained reveal statistically significant specificities between identified microbial OTUs and the geographic origin of the individuals. Detailed analysis of the Bulgarian samples emerged gender and blood type specificities. Beta diversity identified a core blood microbiome.

Conclusions: DNA samples were processed with different extraction protocols and sequencing kits which is a limitation of the study. Nevertheless, the individual and geographic specificities of the blood microbiome play an essential role in both health and disease conditions. Dysbiosis of the core microbiome could be a potential biomarker of human health. This research was funded by the Bulgarian National Science Fund within National Science Program VIHREN, contract number KP-06-DV/10-21.12.2019.

Disclosure: No significant relationships.

Keyword: blood microbiome, 16S metagenomics, geographic differences, alpha diversity, beta diversity

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

ASSOCIATION BETWEEN PRO-INFLAMMATORY ORAL BACTERIA AND AIRWAY INFLAMMATION IN A POPULATION-BASED ADULT COHORT FROM A MULTICENTER STUDY

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Background and Aims: Lipid A is the primary immunostimulatory part of the lipopolysaccharide (LPS) molecule. The inflammatory response induced by LPS depends upon the structural acyl variations of the lipid A domain. Traditional LPS quantification assays cannot distinguish between different lipid A variants, and little is known about how bacteria with different inflammation-inducing potential affect fractional exhaled nitric oxide (F_{eNO}).

Methods: Shotgun metagenomic sequencing was performed on subgingival samples from 330 ECRHS3 adult participants (median age: 53 years [range: 40-65]; 50% women) in Bergen (NO), Melbourne (AU), and Tartu (EE). Annotation of oral bacteria to pro-inflammatory hexa- and less inflammatory penta-acylated LPS-producers was based on the presence of all 9, including *LpxL* and *LpxM*, and the 8 first genes in the Raetz pathway in the whole genome sequenced bacteria, respectively (based on Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO) terms). The effects of the abundance of pro-inflammatory metagenomic species (MGS) on F_{eNO} levels were estimated using a linear mixed-effects model, with smoking status and study center as random effects.

Results: Lipid A gene-based functional potential profiles were generated for each sample. A total of 110 MGS (42%) containing more than 4 enzymes from the Raetz pathway were found. Of these, 6 MGS were pro-inflammatory and assigned to *Aggregatibacter* (*A. sp.* and *A. aphrophilus*) and *Haemophilus* (*H. sputorum*, *H. haemolyticus*, *H. parahaemolyticus*, and *H. pittmaniae*).

Conclusions: The effect of the pro-inflammatory MGS abundances on F_{eNO} as a marker of airway inflammation was non-significant (p=0.192) in this study population.

Disclosure: No significant relationships.

Keywords: Raetz pathway, airway inflammation, Lipopolysaccharide, oral bacteria, lipid A

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

CORRELATION BETWEEN NLRP3 AND AIM2 INFLAMMASOMES AND MICROBIOTA IN ORAL PERI-IMPLANTITIS

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Background and Aims: **Background and Aims:** The activation of inflammasomes is thought to induce the inflammatory process around dental implants. No information is available in the correlation between microbiota and inflammasomes.

Methods: **Methods:** Biofilm samples were obtained from patients undergoing surgical treatment for peri-implantitis. Then, soft tissue samples from around the implant were also collected. Relative abundance of bacteria and alpha-diversity indexes were calculated after analyzing the 16S rRNA gene by next generation sequencing. The soft tissue samples were processed for evaluation of inflammasomes NLRP3 and AIM2 and their products caspase-1 and IL-1 β .

Results: **Results:** The relative abundance of specific phyla indicated that Bacteroidetes represented 44.12 (12.25) %, Firmicutes 15.31 (8.74) %, Fusobacteria 15.98 (14.55) % and Spirochaetes 10.36 (10.35) %. The expression of inflammasome NLRP3 in the lamina propria was significantly correlated with the abundance of Synergistetes and Tenericutes, while AIM2 was correlated with Spirochaetes and caspase-1 with Patescibacteria. Through a network analysis, an important cluster of variables was formed by NLRP3 and IL1 β in the lamina propria and the genera *Family_XIII_ge*, *Desulfobulbus*, *Sphaerochaeta*, *Frefibacterium*, *Odoribacter*, *Mycoplasma*, *Filifactor* and *Campylobacter*. A different cluster included the expression of caspase-1 and the genera *Parvimonas*, *Porphyromonas*, *Bacteroidia* and *Corynebacterium*. Another cluster was formed between AIM2 in the lamina propria and the genera *Flexilinea*, *Treponema_2*, *Actinobacilus*, *Pyramidobacter* and *Mogibacterium*.

Conclusions: **Conclusion:** Results from the current study indicate that inflammasomes NLRP3 and AIM2 and their downstream effectors caspase-1 and interleukin-1 β can be significantly associated with specific bacteria at the level of phyla and genera.

Disclosure: No significant relationships.

Keywords: inflammasome, AIM2, Microbiome, peri-implantitis, NLRP3

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

THE EFFECT OF INTRAPARTUM ANTIBIOTICS PROPHYLAXIS ON ORAL BACTERIOME IN NEONATES IN THE FIRST WEEK OF THEIR LIFE: A PILOT STUDY

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Background and Aims: The administration of intrapartum antibiotics prophylaxis (IAP) is commonly used for the prevention of early-onset Group-B-streptococci (GBS) infection. IAP may influence the oral microbiome in neonates. Our study aimed to compare the oral bacteriome between neonates grouped according to their exposure to antibiotics during the delivery and sampling time in the first week of their life.

Methods: In this case-control study, oral samples were collected from healthy neonates (n=66) from the CELSPAC:TNG cohort during their hospital stay. The neonates were classified into the group of those delivered by C-section or vaginally with IAP (n=33) and the control group of neonates delivered vaginally without IAP (n=33). The bacteriome profile was determined by 16S rDNA sequencing based on the V3-V4 hyper-variable region. The effect of IAP was tested separately for samples collected within 48 hours of delivery and for those collected later.

Results: Differences in alpha diversity (Shannon index, $p=0.01$) and bacterial composition ($p=0.04$, PERMANOVA) were observed in oral samples collected within 48 hours after birth, while bacteriomes in oral samples collected later were similar between IAP and control groups. The genera *Streptococcus*, *Gemella*, and *Rothia* dominated in the oral samples in all groups. Of individual genera, only the relative abundance of *Gemella* was significantly lower in samples collected within 48 hours after birth in neonates compared to the IAP group ($q=0.08$).

Conclusions: Our results illustrate the effect of IAP on oral bacteriome of neonates within the 48 hours after birth. However, this effect seems to be suppressed during the first week of their life.

Disclosure: No significant relationships.

Keywords: Oral bacteriome, Intrapartum antibiotics, neonates, 16S rDNA sequencing

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

THE ORAL RESISTOME IN A POPULATION-BASED STUDY OF ADULTS FROM BERGEN, MELBOURNE AND TARTU

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Background and Aims: The spread of antimicrobial resistance genes (ARGs) is a global public health concern. We sought to find hidden reservoirs of ARGs in subgingival samples of participants investigated as part of the European Community Respiratory Health Survey 3 (ECRHS3), an international multicenter study.

Methods: We used subgingival whole metagenome sequenced data from 330 ECRHS3 participants in Bergen (NO), Melbourne (AU) and Tartu (EE) (median age: 53 years [range: 40-65] and 50% men). The sequencing of the subgingival samples and following bioinformatic pipeline have been conducted by Clinical Microbiomics in Copenhagen (DK).

Results: Preliminary results suggest that ARGs for 18 classes of antibiotic drugs, following the CARD ontology, are detectable in the samples. Remarkably, genes encoding multidrug efflux pumps (median 0.01 relative abundance [range: 0-0.28]), tetracycline resistance (median 0.0 relative abundance [range: 0-0.17]) and β -lactam resistance (median 0.0 relative abundance [range: 0-0.16]) were the most abundant. There were no significant differences between the study centers regarding the total relative abundance of ARGs. The variation in relative abundance of ARGs was not explained by whether the participant had an antibiotic course to help breathing or for nasal/sinus problems last 12 months (24% yes, median courses: 1, range: [1-12]) or visited a hospital since last survey (approx. 10 yrs., 36% yes) or last 12 months (11% yes).

Conclusions: We hypothesize that infrequent courses of antibiotic treatment and/or hospital visits do not contribute to the emergence of antimicrobial resistance studied in gingival microbiota from the general population.

Disclosure: No significant relationships.

Keywords: Oral, Subgingival, Resistome, antimicrobial resistance

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

THE LOWER AIRWAY MICROBIOTA IN COPD AND HEALTHY CONTROLS.

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Background and Aims: Interactions between bacteria in the lower airways and COPD, smoking, and inhaled corticosteroids (ICS) has been reported. In a large cohort of non/ex-smokers or current smokers with COPD and asymptomatic controls we used bacterial 16SrRNA target gene sequencing of protected alveolar lavage (BAL) to investigate these interactions with modern techniques.

Methods: In the MicroCOPD study participant characteristics were obtained through standardised questionnaires and clinical measurements at a single centre from 2012-2015. BAL from 97 patients with COPD and 97 controls were paired-end sequenced with the Illumina MiSeq System. Data were analysed in QIIME 2 and R.

Results: Alpha-diversity was lower in COPD than controls (Pielou evenness: COPD=0.76, Control=0.80, $p=0.004$; Shannon entropy: COPD=3.98, Control=4.34, $p=0.01$). Beta-diversity differed with smoking only in the COPD cohort (Weighted UniFrac: PERMANOVA $R^2=0.04$, $p=0.03$). Nine genera were differentially abundant between COPD and controls. Genera enriched in COPD belonged to the *Firmicutes* phylum. Pack years were linked to differential abundance of taxa in controls only (ANCOM-BC log-fold difference/q-values: *Haemophilus* -0.05/0.048; *Lachnoanaerobaculum* -0.04/0.03). *Oribacterium* was absent in smoking patients with COPD compared with non-smoking patients (ANCOM-BC log-fold difference/q-values: -1.46/0.03). We found no associations between the microbiota and COPD severity or ICS.

Conclusions: The lower airway microbiota is equal in richness in COPD patients to controls, but less even. Genera from the *Firmicutes* phylum thrive particularly in COPD airways. Smoking has different effects on diversity and taxonomic abundance in COPD patients compared with controls. COPD severity and ICS use were not linked to the lower airway microbiota.

Disclosure: No significant relationships.

Keywords: Lower airways microbiome, COPD, smoking, ICS

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

HIGH-THROUGHPUT GENERATION OF PURE MICROBIAL CULTURES USING IMAGE-BASED SINGLE-CELL ISOLATION: PROOF OF CONCEPT ON VAGINAL SWABS.

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Background and Aims: Now that microbiologists (almost) sequenced the world, there is a need for mechanistic insights into host-microbiota interactions, that culture-based approaches can disentangle. This triggers the need to increase throughput in generation of microbial isolates. cellenONE is an instrument for image-based single cell isolation, initially developed for mammalian cells. It was recently augmented to allow the isolation of cells down to 0.5 µm. We aimed at testing the suitability of single bacteria isolation for high-throughput generation of culture libraries from host microbiota, with vagina as model.

Methods: Isolation conditions were first adjusted using pure bacterial cultures, for optimal isolation accuracy and cultivability. Vaginal swabs were then collected from healthy individuals, and bacterial cells were isolated from fresh or frozen samples, on solid or in liquid media, then incubated in aerobic or anaerobic conditions.

Results: We obtained very high monoclonality with both pure cultures and vaginal swab (average 91 and 85%, respectively). This could be assessed on images recorded by the instrument during isolation, guaranteeing the selection of pure isolates for downstream analysis, and avoiding the need for streaking steps. Cultivability of single vaginal swab bacteria was significantly higher from fresh sample, and in anaerobic conditions, and it was equivalent to that of classical dilution-plating. 16S rRNA gene sequencing showed diversified libraries, illustrating the method suitability for capturing microbial diversity. Finally, host and microbiota cells could be handled simultaneously in the same sample.

Conclusions: Single-cell isolation can dramatically increase the throughput of culture generation from host microbiota samples, which opens unprecedented opportunities in microbiome research.

Disclosure: Léna Carret, François Monjaret and myself are employees of Cellenion, who develops and commercialize the cellenONE instrument

Keywords: culture-based methods, culturomics, isolate repertoire, high throughput, Vaginal microbiota

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

THE ORAL MICROBIOTA OF CARIES FREE ADOLESCENTS

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Background and Aims: Adolescents are exposed to many factors that influence the oral microbiota such as puberty hormones, orthodontic treatment and novel lifestyle factors. In this study, the first samples of the oral bacterial microbiota project of the Generation R Study were analyzed. The aim was to get insight into the microbial signature of the healthy oral cavity in the adolescent population

Methods: In this cross-sectional analysis, 147 caries-free adolescents were included. DNA was isolated from dental plaque swabs and 16S rRNA sequencing was performed on the Illumina NextSeq, targeting the V3-V4 region. Sequencing data were analyzed using the DADA2 pipeline and statistical analyses were performed in R using a.o. the Phyloseq, Microbiome and ANCOM-BC packages.

Results: In all participants (mean age: 13.7 years, SD: 0.64), the most abundant genera were *Streptococcus*, *Rothia* and *Neisseria*. Diversity in alpha and beta composition differed between participants with and without orthodontic treatment (Mann-Whitney; p-value = 0.002, PERMANOVA; p-value = 0.002) and between participants with a Dutch and non-Dutch geographic ethnicity (Mann-Whitney; p-value = 0.04, PERMANOVA; p-value = 0.02). A higher abundance of *Megasphaera*, *Bifidobacterium*, *Atopobium*, *Prevotella_7* and *Lactocaseibacillus* (adj. p-value < 0.02) was found in participants with current orthodontic treatment. There were no significant differences in alpha and beta diversity or relative abundance of genera between males and females.

Conclusions: This study confirms that orthodontic treatment influences the oral microbiota of caries-free adolescents. A distinct healthy core oral microbiota was observed in this sub-population, which serves as a base to further unravel potential factors that influence the oral microbiota of adolescents.

Disclosure: This study is part of a research project supported by a TKI grant from Health-Holland (EMCLSH20015) and financial support by the Dentaid Research Center.

Keywords: oral health, adolescent, microbiota, bacteria

E-POSTER VIEWING - AS05. IT'S NOT ALL ABOUT THE GUT: VAGINAL, ORAL AND SKIN MICROBIOMES IN HEALTH AND DISEASE

THE ALTERATION IN ORAL MICROBIOTA AND SERUM LABORATORY TESTS IN PATIENTS WITH POST COVID-19 CONDITION

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Background and Aims: The number of individuals experiencing post-acute sequelae of SARS-CoV-2 infection, commonly referred to as "post COVID-19 condition," is increasing and gaining attention. However, the underlying mechanisms of its development remain unclear.

Methods: Based on a prospective follow-up study, we conducted a nested case-control study to evaluate the persistent symptoms and laboratory indicators of COVID-19 recovered patients six months after discharge from People's Hospital in Shijiazhuang, China. We also performed 16S rRNA sequencing on tongue coating samples of individuals with and without post COVID-19 condition to compare the differences in oral microbiota between the two groups, explore their correlations with laboratory indicators, and identify microbial markers.

Results: A total of 108 COVID-19 recovered patients were included, of which 54 had post COVID-19 condition. After six months of discharge, most participants showed normalized laboratory indicators. However, significant differences were observed between individuals with and without post COVID-19 condition in Hematocrit, Red blood cell count (RBC), Albumin (ALB), Serum creatinine (Crea), SARS-CoV-2 IgM antibody, SARS-CoV-2 IgG antibody, CD3 T lymphocytes, CD4 T lymphocytes, and NK cells. Compared to the asymptomatic group, the symptomatic group exhibited significant dysbiosis in the oral microbiota, including increased diversity and relative abundance of pathogenic bacteria. Furthermore, there was a significant correlation between the oral microbiota and laboratory indicators in COVID-19 recovered patients.

Conclusions: This study demonstrates that significant differences persist between individuals with and without post COVID-19 condition. Moreover, notable changes are observed in the oral microbiota of individuals with post COVID-19 condition.

Disclosure: No significant relationships.

Keywords: COVID-19, post COVID-19 condition, oral microbiota, nested case-control study, laboratory tests

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

THE EFFECTS OF VANISH SPECIES OF BACTERIA IN A MOUSE MODEL OF MULTIPLE SCLEROSIS

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Background and Aims: Lifestyle factors in industrialized nations have been associated with an increased incidence of autoimmune diseases, including multiple sclerosis (MS), and the loss of microbial gut symbionts. However, it is not known whether and how bacteria that are underrepresented in industrialized humans (VANISH species) can modulate immune-mediated pathological processes.

Methods: The objective of this work is to determine the mechanisms by which VANISH species, including *Limosilactobacillus reuteri* and *Helicobacter* species, modulate disease severity and pathology in a TCR transgenic, spontaneous, relapsing-remitting EAE (RR-EAE) mouse model of MS.

Results: Treatment of RR-EAE mice with *L. reuteri* R2lc, a hyper-inducer of the aryl hydrocarbon receptor (AhR), resulted in severe disease with high mortality (60%), while treatment with an isogenic mutant that does not produce the strain's dominant AhR agonist resulted in mild disease with low mortality (14%) (Mantel-Cox test comparing survival curves, *L. reuteri* R2lc vs the mutant: $P = 0.0684$). The *Helicobacter* species showed contrasting effects with *H. macacae* promoting a moderate disease course with low mortality (20%), while *H. pylori* promoted severe disease with high mortality (75%) (Mantel-Cox test, *H. macacae* vs *H. pylori*: $P = 0.0154$). Furthermore, *H. pylori* promoted an expansion of immune cell populations known to drive CNS inflammation, including microglia and macrophages in the brain and activated splenic T cell populations.

Conclusions: This work shows that VANISH bacteria have strong but variable effects in a mouse model of MS that range from protective to detrimental. These findings provide critical information to inform strategies to develop microbial-based treatments for MS patients.

Disclosure: J.W. acknowledges funding for this work from the W. Garfield Weston Foundation and the support of CAIP and SFI. J.W. is an owner of a patent on *Limosilactobacillus reuteri* PB-W1 and a co-owner of Synbiotic Health, a developer of synbiotic products.

Keywords: Gut Microbiome, Immunology, *Helicobacter*, Multiple Sclerosis, *Limosilactobacillus reuteri*

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

GUT MICROBIOTA MEDIATE INFLAMMATORY AND TUMORIGENIC RESPONSE TO UNFERMENTED B-FRUCTAN FIBRES IN SELECT IBD PATIENTS

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Background and Aims: Dietary fibres are not digested in the bowel; they are fermented by microbes, typically promoting gut health. However, IBD patients have altered gut microbiota composition and we previously showed if dietary β -fructans are not fermented by gut microbes in IBD patients, these fibres can induce inflammation. This diet-induced inflammation could have serious repercussions as chronic inflammation creates a mutagenic environment that promotes progression to colorectal cancer (CRC).

Methods: Pathways associated with response to β -fructan were examined in a RCT of β -fructan supplementation (15g/day) in remission UC patients (RNA sequencing). Pathways (proteomics/ELISA) and structural changes (microscopy) were validated in IBD patient biopsies cultured *ex vivo* with β -fructans. Cell invasion, migration, and proliferation were examined using scratch wound assays and chick chorioallantoic membrane assays (CAM; n=10/treatment).

Results: CRC-related pathways (SLIT2/MAPK and SOS1) were induced and anti-tumour gene RPS27A was reduced only in biopsies from remission UC patients who entered relapse following consumption of β -fructans (endpoint versus baseline), not those in the placebo arm who relapsed. Pathways were validated in IBD patient colonic biopsies cultured *ex vivo* demonstrating oligofructose produced more significant effects than inulin; response correlated with disease severity. Inulin and oligofructose further influenced migration and invasion reflective of tumorigenic processes and risk of CRC.

Conclusions: Our findings suggest that intolerance of specific fibres in select IBD patients occurs when whose gut microbiota do not support fermentation of dietary fibres resulting in increased presence of unfermented dietary fibres in the gut which can elicit gut inflammation and tumorigenesis.

Disclosure: No significant relationships.

Keywords: Microbiome, IBD, dietary fibre, fermentation, tumorigenesis

063 / #374

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

THE MICROBIOME AFFECTS OBESITY-RELATED METABOLITES IN THE PROCESS OF AGING

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Background and Aims: The human microbiota of aged people differs from that of younger adults, displaying dysbiosis patterns associated with disease onset and progression. One of the ways that the microbiome can affect the host is via metabolites. In this study we examined the contribution of the microbiome to the metabolic profile in aged mice.

Methods: Fecal samples were collected from 8 weeks-old (young) and 18 months-old (aged) Swiss-Webster mice raised conventionally (ConV) or germ free (GF). We used liquid chromatography mass spectrometry for untargeted metabolomics to study the metabolomes of these mice, focusing on metabolites that were significantly different between young and aged mice detected only in ConV but not in GF mice, since we were interested in the influence of the microbiome on fecal metabolites in the aging process.

Results: ConV aged and young mice had more metabolite differences than the GF groups. Analyses of the differentially abundant metabolites only in ConV mice demonstrated that the microbiome mainly affects fatty acid metabolites with higher levels in aged mice. Some of the metabolites were related to different pathways such as linoleic acid metabolism and alanine, aspartate and glutamate metabolism.

Conclusions: The microbiome affects metabolites in the feces of aged mice. In a previous study, we found that the aging mouse microbiome has obesogenic characteristics. Indeed, it has been found that there is an increase in various fatty acids in the feces of aged mice, as well as an effect on the pathways associated with various diseases, inflammation and obesity.

Disclosure: No significant relationships.

Keywords: Microbiome, Aging, Metabolomics

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

GASTRIC MICROBIOTA IN GASTRIC PRECANCEROUS CONDITIONS: A PROSPECTIVE GERMAN-ITALIAN STUDY PROJECT

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Background and Aims: The human gastrointestinal tract is the residence of approximately 10^{13-14} microorganisms collectively called *microbiota*. Mounting evidence supports the microbiota's responsibility in (re)modulating the host's gene expression, including those involved in oncogenesis. Gastritis (non-atrophic/atrophic) affects the intra-gastric microenvironment, resulting in microbiota changes potentially involved in gastric cancer promotion. This study project aims to profile gastric microbiota in inflammatory/precancerous gastric conditions, distinguishing non-atrophic from atrophic gastritis.

Methods: The study population includes 61 subjects (31 Germans and 30 Italians) prospectively recruited in two tertiary University Hospitals (Munich University-Germany; Padova University-Italy). Inclusion criteria were: (i) Auto-immune atrophic-gastritis (19 subjects); (ii) non-atrophic Hp+ve gastritis (14); (iii) Atrophic-metaplastic Hp+ve gastritis (10). Eighteen subjects with no laboratory/histology abnormalities were included as controls.

Exclusion criteria included gastrointestinal surgery, oncological diseases, and immunomodulating/immunosuppressive therapies. All involved subject gave their written informed consensus.

All subjects underwent clinical examination, laboratory testing, and EGDS with biopsy samplings. Laboratory testing included: hematological profiling, Hp-status assessment, and functional serology (pepsinogen I-II, gastrin 17). Biopsy sampling strictly required obtaining samples from antral, oxyntic, and incisura-angularis mucosa; additional biopsies were obtained for microbiota molecular profiling qualitatively and quantitatively analyzed through 16S RNA gene profiling.

Results: Microbiome diversity is described in terms of alpha and beta diversities. This ongoing study highlights alpha and beta differences in gastric precancerous conditions among subjects recruited in different geographical areas.

Conclusions: We reasonably expect that an altered microbiota population in precancerous gastric conditions plays a subsidiary role in maintaining gastric mucosa inflammation entangled in cancer promotion.

Disclosure: No significant relationships.

Keywords: gastric, microbiota, precancerous, 16S RNA, gene profiling

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

LONG-TERM EFFECT OF DAAS ON GUT MICROBIOTA DYSBIOSIS AND MICROBIAL TRANSLOCATION IN HCV-INFECTED PATIENTS WITH AND WITHOUT HIV COINFECTION: A PROSPECTIVE OBSERVATIONAL STUDY.

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Background and Aims: New evidence suggests that direct-acting antivirals (DAAs) may have the potential to alleviate gut dysbiosis in hepatitis C virus (HCV) infected patients who achieve sustained virological response (SVR). However, there is limited data on the long-term effects of DAAs on gut microbial composition, short-chain fatty acids (SCFAs), and microbial translocation in patients with chronic HCV infection.

Methods: To address this, a prospective longitudinal study was conducted on 50 patients with HCV mono-infection and 19 patients with HCV/HIV coinfection who received DAAs. Fecal specimens were collected before treatment and at week 72 after treatment completion and analyzed for gut microbiota using 16S rRNA sequencing and real-time PCR for butyryl-CoA:acetateCoA transferase (BCoAT) expression. Plasma lipopolysaccharide binding protein (LBP) and intestinal fatty acid binding protein (I-FABP) were quantified using ELISA assays.

Results: The result, SVR rates were similar in mono- and coinfecting patients (94% vs. 100%). Improvement of gut dysbiosis and microbial translocation was observed in responders but not in non-responders. Responders showed significant restoration of alpha-diversity, BCoAT, and LBP in HCV-monoinfected patients with low-grade fibrosis (F0-F1), while HCV/HIV-coinfecting patients exhibited partial improvement at week 72. Plasma I-FABP did not significantly decrease in responders. The changes in microbiota induced by treatment were associated with an increase in SCFAs-producing bacteria, such as *Blautia*, *Fusicatenibacter*, *Subdoligranulum*, and *Bifidobacterium*.

Conclusions: These results provide insight into the long-term effects of successful DAAs on restoring gut dysbiosis and microbial translocation. However, early initiation of DAAs may require alteration of gut microbiota, especially enhanced SCFAs-producing bacteria, to reduce HCV-related complications.

Disclosure: No significant relationships.

Keywords: Sustained virologic response (SVR), Gut Microbiome, HCV/HIV-coinfecting, hepatitis C virus (HCV), direct-acting antivirals (DAAs)

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

LONGITUDINAL ANALYSIS OF THE SALIVARY MICROBIOME IN THE RHINESSA GENERAL STUDY POPULATION

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Background and Aims: Few studies have investigated temporal changes of the salivary microbiome in a general population setting, and its impact on respiratory health.

Methods: Saliva samples were collected at baseline (2014-15) and at follow-up (2020-21) from 75 adults as part of the RHINESSA study (www.rhinessa.net) in Bergen, Norway. Bacterial community profiling was performed by targeted amplicon sequencing of the bacterial 16S rRNA gene regions V3-V4 using an Illumina MiSeq. Bioinformatics and statistical analyses were performed using QIIME2 and R.

Results: Analyses of taxonomy, alpha- and beta-diversity showed a clear distinction between the salivary microbiota at baseline and follow-up. 19 bacterial genera were differential abundant between the two-time points; in particular, *Alloprevotella*, *Megasphaera*, and *Prevotella* were found to be more abundant at follow-up compared to baseline (log fold change = 3.01, 1.39, and 1.03 respectively). The alpha diversity indexes (Shannon index and number of ASVs) were significantly higher at follow-up compared to at baseline ($P < 0.001$). Beta diversity as assessed by Bray Curtis was also significantly different between baseline and follow-up ($p=0.001$, permonava).

Conclusions: The salivary microbiome varies from baseline to follow-up in our study population. This warrants further investigation into the influence of the salivary microbiota on respiratory health.

Disclosure: No significant relationships.

Keywords: salivary microbiome, longitudinal, general population

067 / #200

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

ABSOLUTE QUANTIFICATION OF SHORT-CHAIN FATTY ACIDS, ORGANIC ACIDS AND AMINO ACIDS IN FECES USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Background and Aims: The gut microbiome contributes to human physiology by producing beneficial metabolites. Among well-described microbial metabolites are short-chain fatty acids (SCFA) that have key roles in colonization resistance against pathogens, epithelial cell homeostasis, and immune system development. Low levels of SCFA are implicated in gut microbial dysbiosis linked to a risk of chronic infections and allergic diseases. SCFA thus provide insights into the health status of the host. However, limited knowledge of SCFA physiological concentrations prevents their utilization as diagnostic biomarkers.

Methods: We have adapted a targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for absolute quantification of 13 SCFA, together with associated Krebs cycle intermediates and 14 amino acids, using 3-nitrophenylhydrazine as a derivatization reagent. Quantifying absolute levels of metabolites in feces is challenging due to the complexity of the fecal matrix and inter-individual variation. To overcome these challenges, we have evaluated the matrix effect using germ-free animals, carefully monitored recovery and reproducibility for every step of the sample processing workflow, and normalized for variations in water content by dry weight measurement.

Results: Finally, we used the method on meconium samples from 40 healthy vaginally born infants. We detected SCFA in the nanomolar range for the low abundant SCFA (including valerate and hexanoate) and concentrations between 0.7-41.6 $\mu\text{mol/g}$ dry weight for acetate, the most abundant SCFA.

Conclusions: This highly sensitive targeted approach will allow the description of SCFA and associated metabolites at absolute levels and their concentration range in various clinical settings.

Disclosure: No significant relationships.

Keywords: Infants, Feces, Validation, SCFA, Metabolomics

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

EMPHYSEMA AND THE MICROBIOME IN COPD

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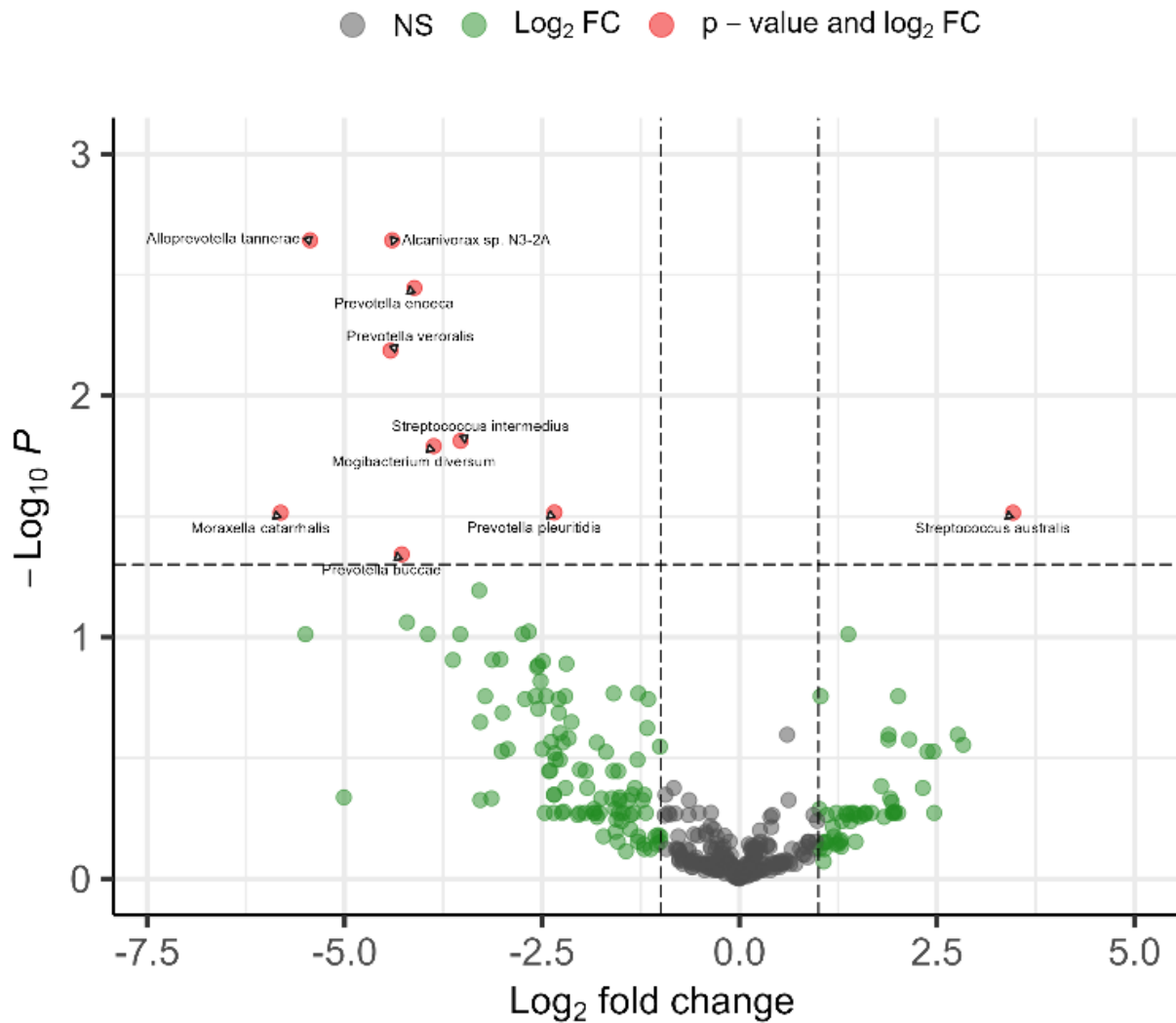
Background and Aims: COPD patients with emphysema have a worse prognosis and a higher symptom burden than COPD patients without emphysema. We aimed to evaluate the lung bacterial microbiome related to emphysema evaluated by computer tomography (CT).

Methods: Bronchoalveolar lavage was sampled from the right middle lobe of 91 COPD patients with bronchoscopy. Emphysema was assessed by CT of the lungs, defined as having more than 10% of emphysematous lung tissue, specified as tissue density of less than -950 HU. Whole genome sequencing was performed on the Illumina NovaSeq platform. Identification of taxa was made by the GAIA 2.0-software. Statistical analyses were performed in R (Phyloseq-, DeSeq2- and AncomBC-package).

Results: Alpha diversity (Shannon and Simpson) and Beta diversity (Bray-Curtis dissimilarity) were not significantly different between the groups. With DeSeq2, 10 species were differentially abundant. 9 species were more prevalent in non-emphysema, top 3 were *Moraxella catarrhalis*, *Alloprevotella tanneriae* and *Prevotella veroralis*, whereas only *Streptococcus australis* was more prevalent in emphysema. With AncomBC, only *Moraxella catharrhalis* was differentially abundant and more prevalent in non-emphysema

COPD.

Volcano plot, by emphysema vs non-emphysema



Log_2 negative in non-emphysema, positive in COPD with emphysema; p-value cutoff, 0.05

Conclusions: Overall, the lung microbiome in COPD is similar in emphysema vs non-emphysema, but several clinically significant species are differentially abundant.

Disclosure: No significant relationships.

Keywords: COPD, Microbiome, emphysema

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

DIFFERENCES IN GUT MICROBIOTA PROFILES AND PREDICTED FUNCTIONS IN MEN WITH AND WITHOUT PROSTATE CANCER

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Background and Aims: Although prostate cancer is the most common cancer in men in Western countries, there is significant variability in geographical incidence. This might result from genetic factors, discrepancies in screening policies or differences in lifestyle. Gut microbiota has been recently associated with cancer progression in several cancer types, and there is accumulating evidence of its role in prostate cancer. In this study, gut microbiota compositions between prostate cancer and benign patients were compared.

Methods: In a prospective multicenter clinical trial (NCT02241122), the gut microbiota profiles of 181 men with a clinical suspicion of prostate cancer were assessed utilizing 16S rRNA gene sequencing. Sequences were assigned to operational taxonomic units, and differential abundance analysis, α - and β -diversities, and predictive functional (PICRUSt) analyses were performed. Additionally, plasma steroid hormone levels were measured and presented according to the predicted microbiota steroid hormone biosynthesis profile.

Results: Several differences in the abundances of the gut microbiota genera between the subjects with and without prostate cancer were found. However, no differences in community richness were discovered. Predictive functional analyses revealed higher 5- α -reductase, copper absorption, and retinol metabolism in the prostate cancer associated microbiome. Plasma testosterone associated negatively with the predicted microbial 5- α -reductase ($p=0.030$).

Conclusions: Gut microbiota of the prostate cancer patients differed significantly compared to benign subjects. Microbial 5- α -reductase, copper absorption and retinol metabolism are potential mechanisms of action. These findings could explain the observed association of lifestyle, geography, and prostate cancer incidence.

Disclosure: No significant relationships.

Keywords: copper, retinol, Prostate cancer, Steroid hormone biosynthesis, 5- α -reductase

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

REGULATION OF GENOTOXIN PRODUCTION IN GUT RESIDENT KLEBSIELLA SPECIES: IMPLICATIONS FOR INFANT HEALTH AND ANTIBIOTIC RESISTANCE EMERGENCE.

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Background and Aims: Bacteria belonging to the *Klebsiella oxytoca* species complex are early-life gut colonizers that frequently carry the *til* biosynthetic gene cluster for secretion of the cytotoxic pyrrolobenzodiazepines tilimycin and tilivalline. Tilimycin is a DNA alkylating genotoxin. Toxigenic *Klebsiella* species are associated with necrotizing enterocolitis in premature infants and cause antibiotic-associated hemorrhagic colitis in children and adults when *til+* *Klebsiella* attain high densities. In the mature microbiota these opportunistic pathogens are present in low abundance, yet carriers are at continuous risk of toxin exposure even in the absence of colitis. We study the consequences of genotoxin exposure on host cells and gut microbiota and aim to identify factors or bacteria that modulate *til*-gene expression.

Methods: Mouse infection models in conjunction with in vitro assays.

Results: We showed that tilimycin generates DNA lesions in enterocytes and increased the burden of colorectal epithelial stem cell mutations. We further showed that tilimycin secretion in the murine intestine has cross-phyla antibiotic activity that decreased microbial diversity. Surviving bacteria sustained mutations including de novo antibiotic resistance. Targeted suppression of toxin production is therefore desirable. We showed that *til*-gene expression responds to carbon sources and bacterial indole but mechanistic understanding is lacking. We asked whether the regulatory networks involve quorum-sensing transcriptional activator SdiA or post-transcriptional carbon storage regulator CsrA. Results thus far indicate a regulative role of SdiA as *til*-promoter activity is increased in SdiA-deficient *K. oxytoca* compared to the wildtype strain.

Conclusions: Mechanistic understanding of *til*-gene regulation will support efforts to identify factors or bacteria that modulate toxin gene expression.

Disclosure: No significant relationships.

Keywords: genotoxin, toxin gene regulation, mutations, resistance emergence, Microbiome

071 / #253

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

UNDERSTANDING THE RELATIONSHIP BETWEEN CHILDHOOD OBESITY AND BIFIDOBACTERIA - AN INNOVATIVE METHOD FOR TARGETED GUT MICROBIOTA ANALYSIS

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Background and Aims: REM Analytics' Advanced Testing for Genetic Composition (ATGC) is a novel method for targeted microbiota analysis. One main application is a gut microbiota assay, focusing on key *Bifidobacteria* thought to concede health benefits such as micronutrient synthesis or disease protection.

Methods: Research has shown that "smaller changes in the gut microbiota community," can be key in health outcomes. REM's quantitative, high-throughput assays with species/subspecies resolution in areas of interest offer insight into these relationships, with demonstrated precision of approximately 4%. It can accurately distinguish between closely related members of the *Bifidobacterium* genus, such as *B. longum subspecies infantis* and *longum*, or *B. lactis* and *B. adolescentis*.

Results: In the scope of the NUTRISHIELD project, the assay was applied to investigate the correlation between gut Bifidobacterium levels and two health conditions in adolescents: obesity and Type I Diabetes. A significant relationship was observed between levels of *B. longum subspecies longum* and *infantis* and obesity, as well as *B. adolescentis* levels and Type I Diabetes.

Conclusions: Work is ongoing to study the role of these microorganisms in infant development, particularly in light of their role in human milk oligosaccharide metabolism in newborns. Better understanding of these mechanisms, particularly in infancy, may have implications for modulation of the gut microbiota to improve health.

Disclosure: No significant relationships.

Keywords: bifidobacteria, Obesity, microbiota, test, diabetes

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

ALTERATIONS IN GUT MICROBIOME COMPOSITION ARE ASSOCIATED WITH THE ONSET AND COURSE OF MULTIPLE SCLEROSIS: AN ITALIAN COHORT STUDY

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Background and Aims: Which are the triggers that convert self-reactive lymphocytes into an autoaggressive phenotype facilitating the first episode of demyelization in multiple sclerosis (MS) are still poorly understood; actually alterations in the composition of the gut microbiota are suggested as contributors. Here, we investigated whether alteration in the composition of the gut microbiota, in terms of species richness, distribution and functional potential, could be associated with the onset of MS and its immune system alteration in an Italian cohort.

Methods: Stool and blood samples were collected from MS patients and Healthy Volunteers (HV) highly matched for age, sex, diet and lifestyle and prospectively followed up after therapy initiation. DNA isolated from stools were subjected to shotgun metagenomic sequencing strategy in order to correlate gut microbiome composition and functions with fecal metabolites, analyzed with Gas chromatography–mass spectrometry, and with Th17 and Treg cells, analyzed by FACS, in the peripheral blood (PB).

Results: At the onset of MS, gut microbiome structure of patients was clearly different from that of HV and displayed a lower species richness and lower number of taxa: a reduction in abundance of genera belonging to Butyrate-producing bacteria correlated with a lower butyrate amount in the feces and with the decrease of Treg cells producing IL-10 and in the PB of MS patients compared to HV.

Conclusions: Our data indicate that gut microbial dysbiosis exist at the onset of MS and could be associated with the autoimmune response in the periphery, highlighting the importance of gut microbiome in the etiology of MS.

Disclosure: No significant relationships.

Keywords: Multiple Sclerosis, Th17 cells, Treg cells, dysbiosis

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

ELUCIDATING THE GUT MICROBIOME PROFILE AS A DISCRIMINATORY TOOL FOR DISTINGUISHING BETWEEN VIRAL-INDUCED AND NON-VIRAL INDUCED HEPATOCELLULAR CARCINOMA.

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Background and Aims: Altered gut microbiota has been associated with the development of hepatocellular carcinoma (HCC). Our study was aimed at identifying gut microbiota signature in differentiating between viral-related HCC (Viral-HCC) and non-hepatitis B-, non-hepatitis C-related HCC (NBNC-HCC).

Methods: Fecal samples obtained from 16 healthy controls, 33 patients with Viral-HCC (17 and 16 patients with HBV and HCV infection, respectively) and 18 patients with NBNC-HCC were assessed by 16S rRNA sequencing. Bioinformatic analysis was performed with the DADA2 pipeline in R program. Significantly different genera from top 50 relative abundance were used to classify between Viral- and NBNC-HCC by Random Forest algorithm.

Results: Our results showed that the HCC group displayed reduced alpha-diversity and altered gut microbial composition compared with healthy controls. Within the top 50 relative abundance, there were 11 genera such as *Faecalibacterium*, *Agathobacter* and *Coprococcus* significantly enriched in Viral-HCC, while 5 genera including *Bacteroides*, *Streptococcus* and *Erysipelatoclostridium* were significantly increased in NBNC-HCC. Based on their distinct signatures, a high diagnostic accuracy to classify HCC subgroups was achieved with an area under the curve (AUC) of 0.94. Compared to Viral-HCC, significant reduction of fecal butyrate levels but increased plasma surrogate markers of microbial translocation were demonstrated in NBNC-HCC.

Conclusions: In summary, our data indicated that gut dysbiosis was distinct regarding different etiological factors of HCC. Moreover, NBNC-HCC appeared to have reduced fecal butyrate but increased microbial translocation compared with Viral-HCC. Thus, the gut microbiota signature might serve as a potential biomarker for the diagnosis and therapeutic options for HCC.

Disclosure: No significant relationships.

Keywords: Gut Microbiome, Viral-HCC, NBNC-HCC, 16s rRNA sequencing, fecal butyrate

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

WESTERNIZED DIET ALTERS COLONIC MICROBIAL COMMUNITY COMPOSITION AND METABOLITE PROFILE IN A PIG MODEL FOR ULCERATIVE COLITIS

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Background and Aims: Western diet and microbial dysbiosis may contribute to the development and severity of ulcerative colitis (UC), a chronic inflammation of the colonic epithelium. This study investigated how a westernized diet, high in fat and protein from ground beef, affected colonic microbiota composition in a pig model using dextran sulfate sodium (DexSS) to induce UC.

Methods: Twenty-four piglets received either a standard control diet (**CT**) or CT supplemented with 15% cooked ground beef, mimicking a westernized diet (**WD**) typically high in fat and protein. UC was induced in half of each group using DexSS (**DSS** and **WD+DSS**). On day 19, digesta samples from proximal and distal colon and feces were collected for microbial analysis.

Results: DexSS treatment (DSS and WD+DSS) reduced alpha-diversity and altered microbial composition and metabolic profiles, with the combined effect being most significant. Microbial composition was affected by a significant interaction between DexSS and westernized diet, with DexSS and diet explaining 22% and 5% of the total variance, respectively. Compared to CT, the WD, DSS, and WD+DSS groups showed increased abundances of proteolytic pathogens like *Clostridium perfringens* and *Helicobacter trogontum*, while carbohydrate-degrading SCFA-producing commensals, particularly Lachnospiracea species, were reduced. No significant differences in SCFA concentration were observed. Total biogenic amines were significantly higher in the distal colon of the WD+DSS group, mainly driven by putrescine across segments.

Conclusions: A westernized diet may increase UC risk and severity by reducing SCFA-producing commensal bacteria, increasing abundance of proteolytic pathogens, and elevating microbial proteolytic-derived metabolites in the colon.

Disclosure: No significant relationships.

Keywords: Inflammatory bowel disease, ulcerative colitis, meat consumption, porcine model, 16S rRNA gut metagenomics

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

DISEASE AS A CONSEQUENCE OF POOR GUT-MICROBIOME INTERACTION IN THE NEONATE

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Background and Aims: The mechanism by which a malfunctioning microbiome causes disease is currently unknown. However, epidemiological observations strongly suggest an infant origin. To show that non-communicable disease may be a function of an inherited maternal microbiome deficiency. Note our recent peer-reviewed publication of May 15, this year [1]: Smith, D.; Jheeta, S.; López-Cortés, G.; Street, B.; Fuentes, H.V.; Palacios-Pérez, M. On the inheritance of microbiome-deficiency: paediatric functional gastrointestinal disorders, the immune system and the gut-brain axis. *Gastrointest. Disord.* **2023**, *5*, 209-232.

Methods: As a hypothesis paper, this work relies only on review of the epidemiological literature.

Results: While it is well-known that gut microbial diversity and physical and mental health are positively correlated [2], it is not clear as to exactly why. For a number of years now, we have been developing the hypothesis that diversity supports microbiome function by the bacteriophage-induced flexible expression of mobile genetic elements [3,4]. It is important to note that the immune system works in conjunction with the development of the gut-brain axis as the neonate grows into adulthood. Accordingly, disorders of the immune system [5] co-exist with diseases of weight gain and poor mental health [6], as exemplified by coeliac disease [7].

Conclusions: Our work strongly suggests that a key role is played by significant microbes, probably eukaryotes, transferred during the process of natural birth. It is likely some such microbes will have a role within the immune system as *microbial sentinel cells*.

Disclosure: No significant relationships.

Keywords: Maternal Microbial Inheritance, Mobile genetic elements, Microbiota-gut-brain axis

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

IS THE LOWER AIRWAYS MICROBIOME IN HEALTHY SUBJECTS AND PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE ASSOCIATED WITH CORONARY HEART DISEASE?

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Background and Aims: Dysregulation of the lung microbiota may cause or contribute to disease development, including chronic obstructive pulmonary disease (COPD) and coronary heart disease (CHD).

Methods: 98 COPD patients and 98 non-COPD controls from Norway, aged 47-82 yrs, took coronary CT angiography (CCTA) estimating calcium score (CACS) and artery lumen reduction, and underwent bronchoscopy with lavage (BAL). Sequencing of the 16S rRNA gene of the BAL samples was performed with an Illumina MiSeq. Differential abundance (DA) between all four groups (controls and COPD patients with or without CHD) for phylae and genera were tested with ANCOM-BC2 and alpha diversity with Shannon index, significance level 0.05.

Results: Altogether we identified 9 Phylae and 74 genera for CACS > 100; and 9 and 85 for artery lumen reduction >50%. The most common phyla among all four groups were *Firmicutes*, for controls without high CACS 58.4%, controls with high CACS 62.4%, COPD without high CACS 63.5%, and COPD with high CACS 69.5%. DA analyses revealed zero significantly DA taxa between any of the four groups. Alpha diversity did not significantly differ according to calcium score or artery lumen reduction (Shannon mean for lumen reduction >50% 3.63), neither in the overall study population, nor within COPD patients (Shannon mean 3.67, median 3.83) or healthy controls (Shannon mean 4.08, median 4.14).

Conclusions: Coronary calcium score > 100 and artery lumen reduction > 50% were unassociated with the lower airways microbiome in both COPD patients and healthy controls.

Disclosure: 2017: 100 000 NOK Grant awarded by Norwegian Respiratory Society/AstraZeneca

Keywords: COPD, CHD, CACS, microbiota

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

FECAL MICROBIOTA TRANSPLANTATION FROM STUNTED CHILDREN IN MICE LEADS TO DIET-INDUCED OBESITY WHEN COMPARED TO FECAL TRANSPLANTATION FROM HEALTHY CHILDREN

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Background and Aims: Background: Childhood undernutrition leads to a higher risk of adult obesity. We hypothesized that the gut microbiota of stunted children confers adaptability to food deprivation but leads to obesity in adulthood if an obesogenic environment is favored. Aim: To determine whether the gut microbiota from stunted children contributes to the development of obesity in adulthood.

Methods: Fecal microbiota transplantation (FMT) from stunted (S) or healthy scholar children (H) was conducted into 8-week C57BL6 male mice, after bowel cleansing with polyethylene glycol. Recipient mice were fed with either control (CT) or high fat and fructose diet (HFFr) for 15 weeks. The metabolic phenotype of mice was evaluated through glucose and insulin tolerance test, magnetic resonance and indirect calorimetry. Fecal microbiota was investigated using 16S rRNA gene sequence analysis.

Results: Mice fed with HFFr diet that received FMT from stunted children (S-HFFr) developed obesity, insulin resistance and glucose intolerance, while, to our surprise, mice that received FMT from healthy children (H-HFFr) were protected from these effects. Moreover, FMT modified fecal microbiota and beta diversity of recipient mice according to fecal donor. *Odoribacter* genus was negatively correlated with body fat percentage and weight and positively correlated with body lean percentage, postprandial Respiratory Exchange Ratio, and fasting VO₂ uptake.

Conclusions: Conclusion: Humanization with fecal microbiota from healthy children had a protective effect against diet-induced obesity and insulin resistance. This suggest that the nutritional status experienced during childhood, potentially influences the intestinal microbiota, and this in turn promotes or prevents the onset of obesity in adulthood.

Disclosure: No significant relationships.

Keywords: Stunting, Obesity, Insulin resistance, Malnutrition, fecal microbiota transplantation

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

OPTIMIZING STOOL DNA EXTRACTION PROTOCOL TO EVALUATE THE DEVELOPMENT OF THE NEONATAL RESISTOME IN NORTHERN NIGERIA

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Background and Aims: Our understanding of the development of neonatal resistome relies on accurate and reproducible microbiome data collection. A standardized microbial DNA extraction protocol is crucial because bias introduction in the extraction step may result in an inaccurate representation of the microbiome. This work aimed to optimize the DNA extraction protocol for a neonatal gut microbiome project in Nigeria, where access to third-party consumables and equipment can be limited.

Methods: We evaluated the performance of four DNA extraction kits (DNeasy PowerSoil, QIAamp Fast DNA Stool Mini, Zymo Quick DNA Fecal/Soil Microbe, and Zymobiomics DNA) using neonatal stool samples stored at different conditions. Total DNA concentrations of the extracts were compared, and samples from the two best-performing kits were sequenced by shotgun metagenomics using the rapid sequencing kit from Oxford Nanopore Technology.

Results: We confirm that some of the evaluated kits differed in efficiency which affected the DNA yield and microbial composition. Our results indicated that freshly collected samples produced the highest yield. Importantly, a comparison of the bacterial diversity and abundance between the extracts of the two best-performing kits showed similar composition.

Conclusions: gDNA extraction from freshly collected stool samples is recommended. Extraction kits with mechanical lysis are recommended to help with homogenizing sticky neonatal stool samples. DNeasy Powersoil and ZymoBiomics kits gave the highest yields, similar species diversity and abundance, and antimicrobial resistance prediction. In addition to unit cost, a decision on kit usage for research studies should be made in consideration of ease of access to consumables and the need for third-party reagents.

Disclosure: No significant relationships.

Keywords: DNA Extraction, Neonatal Gut Microbiome, Resistome, metagenomics

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

FEEDING THE MIND: MODERATING THE RELATIONSHIP BETWEEN GUT MICROBIOTA AND PRESCHOOL CHILDREN'S STRENGTHS AND DIFFICULTIES BY DIET.

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Background and Aims: Emerging evidence of the relationship between the gut microbiota (GM) and human behaviour has to date only investigated dietary influence as a confounder rather than as a predictor of that relationship. This study aims to determine if the relationship between GM and child behaviour is moderated by diet during the first year of life.

Methods: 1074 infants were recruited into the Barwon Infant Study, Geelong Australia. Milk-based diet was recorded at 1-, 6-, and 12-months of age, as either formula fed, breast-fed or mixed feeding, and solid food introduction was recorded at 6-, and 12-months using a Food frequency questionnaire focusing on weekly consumption of food groups such as fruits and vegetables. Dietary clusters, established using principal component analysis and k-means clustering, were used to investigate the moderating effect that diet has upon the relationship between GM and SDQ outcome measured at 4-years.

Results: The relationship between GM composition and increased risk of emotional problems at 4-years was found to be moderated by diet. *Collinsella*, at 1-month, is positively associated with emotional problems, in breastfed infants, and *Akkemansia* is positively associated with emotional problems in those with higher-than-average meat consumption at 6-months.

Conclusions: This study presents findings indicating that 6-months of age, a significant period of dietary change with solid food introduction, is also a critical period of development of the GM for influence upon both strengths and difficulties at 4-years.

Disclosure: No significant relationships.

Keywords: Behaviour, Gut microbiota, breastfeeding, Gut-brain axis, Solid Food Introduction

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

INFLUENCE OF LONG-TERM MACROLIDE THERAPY ON HOST METABOLISM VIA INTESTINAL MICROBIOTA AND METABOLOME ALTERATION

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Background and Aims: Long-term low-dose macrolide therapy is used widely in the management of common chronic respiratory diseases. While the associated clinical benefit is believed to result from an anti-inflammatory effect, the antimicrobial properties of macrolides can cause collateral disruption of commensal intestinal microbiology. We investigated the effect of macrolides on intestinal commensal microbiology and host physiology.

Methods: Healthy adults received 4-weeks of either low-dose erythromycin or low-dose azithromycin, as used clinically (n=10 per antibiotic group). Changes in gut microbiome characteristics were assessed by metagenomics sequencing, and markers of systemic immune and metabolic regulation were assessed in fasted sera. Parallel assessments were performed in murine models of long-term low-dose erythromycin exposure (3 months).

Results: We observed shifts in gut microbiome composition (p=0.0002), consistent with a reduction in microbial capacity relating to carbohydrate metabolism and short-chain fatty acid biosynthesis. These changes were accompanied by alterations in serum biomarkers relating to systemic immune and metabolic homeostasis. Murine models of erythromycin exposure demonstrated altered glucose tolerance and energy metabolism. Erythromycin-exposed mice showed reduced fasting blood glucose, while metabolic activity monitoring suggests that an increased utilization of protein and carbohydrate for energy. Transplantation of erythromycin-exposed murine microbiota into germ-free mice demonstrated that changes in intestinal microbiology caused by macrolide exposure resulted in alterations of host physiology and serum biomarkers related to metabolic homeostasis, but not systemic immune regulation.

Conclusions: Our findings highlight the potential of long-term low-dose macrolide therapy to influence recipient physiology via alteration of the gut microbiome, with the capacity to contribute to long-term extra-pulmonary health outcomes

Disclosure: No significant relationships.

Keywords: gut microbiome and metabolome, metabolic health, immunoregulation, murine and gnotobiotic antibiotic model, Macrolide antibiotics

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

POLYUNSATURATED FATTY ACIDS-RICH DIETARY LIPID PREVENTS HIGH FAT DIET-INDUCED OBESITY IN MICE

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Background and Aims: Diet is the primary factor affecting host nutrition and metabolism, with excess food intake, especially high-calorie diets, such as high-fat and high-sugar diets, causing an increased risk of obesity and related disorders. Obesity alters the gut microbial composition and reduces microbial diversity and causes changes in specific bacterial taxa. Dietary lipids can alter the gut microbial composition in obese mice. However, the regulation of gut microbiota and host energy homeostasis by different polyunsaturated fatty acids (PUFAs) in dietary lipids remains unknown.

Methods: We demonstrated that different PUFAs in dietary lipids improved host metabolism in STC-1 cell lines and high-fat diet (HFD)-induced obesity in mice.

Results: The intake of the different PUFA-enriched dietary lipids improved metabolism in HFD-induced obesity by regulating glucose tolerance and inhibiting colonic inflammation. Moreover, the gut microbial compositions were different among HFD and modified PUFA-enriched HFD-fed mice.

Conclusions: We have identified a new mechanism underlying the function of different PUFAs in dietary lipids in regulating host energy homeostasis in obese conditions. Our findings shed light on the prevention and treatment of metabolic disorders by targeting the gut microbiota (Haneishi et al. Sci Rep. 2023).

Disclosure: No significant relationships.

Keywords: polyunsaturated fatty acids, Gut microbiota, Obesity, dietary lipids

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

HIGH COLONIZATION BY PROBIOTIC ESCHERICHIA COLI A0 34/86 STRAIN IS ASSOCIATED WITH A LESS DIVERSE MICROBIOME RELATED TO CHILDREN'S AGE

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Background and Aims: Probiotic supplementation in childhood serves as an additional source of bacterial colonizers and represents an opportunity to beneficially manipulate the intestinal microbiome. Differences in the ability of probiotic strains to colonize the gut may be related to the variously diversified gut microbiome.

Methods: We report the results of the association between composition of the gut microbiome and the colonization capacity of the probiotic strain *Escherichia coli* A0 34/86 (CNB - Colinfant New Born supplement) in the cases of three healthy children in different development stages (infant, toddler, pre-school), as a preliminary insight to possible future prospective studies of this subject. Microbiome composition was estimated by 16S rRNA gene sequencing of 55 stool samples collected during approximately 3.5-13 months long periods. Detailed characterization of the *E. coli* population was performed using colony PCR.

Results: In all children, genetic determinants typical for the probiotic *E. coli* A0 34/86 strain were detected immediately after administration of the probiotics. Analysis of the initial sample composition (the last sample taken before the probiotic administration) showed that the gut microbiome of infant and toddler with lower bacterial diversity was more successfully colonized by the probiotic strain.

Conclusions: In our case report of three children, we showed for the first that supplementation with CNB probiotics in early infancy and toddlerhood was associated with high *E. coli* A0 34/86 colonization and a significant change in the composition of the gut microbiome. Our results indicate that administration of CNB for its recommended duration might be efficient only in very early childhood.

Disclosure: No significant relationships.

Keywords: Sequencing, *E. coli*, Probiotics, Microbiome, children

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

PROLONGED WATER-FASTING AND THE GUT MICROBIOTA: PRELIMINARY RESULTS OF AN OBSERVATIONAL STUDY

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Background and Aims: Water-only fasting (“WF”) is defined as a dietary intervention with absence of food intake and ad libitum water consumption. WF, complete or partial, has anti-inflammatory and anti-oxidative effects, as well as positive effects on lipid metabolism, the endocrine system and emotional well-being. WF alters the gut microbiota composition and functionality, which could be a plausible explanation for the observed effects.

Methods: Three healthy male volunteers participated in a medically supervised WF, consuming solely water for 7 consecutive days. Body composition, bowel movements and basal metabolic rate were monitored before, during and after WF. Prior and post intervention stool samples were obtained and 16s rDNA-Seq was conducted.

Results: Preliminary findings suggest that WF significantly impacts the gut microbiota by altering its composition on all taxonomic level and dominant pathways. The taxonomic changes were not consistent among subjects and depended on baseline gut microbiota composition. The resulting regulation of potentially pathogenic and pro-inflammatory Proteobacteria and increase in anti-inflammatory Lactobacillus-Group could potentially mediate the anti-inflammatory and anti-oxidative effects of WF. Function-wise, pathways involved in food degradation, especially sugar- and fiber-degrading pathways and fermentation were reduced, as well as pathways involved in the biosynthesis of purine and pyrimidine bases.

Conclusions: Although based on a small study sample, the results of this study suggest that prolonged WF may have a potential therapeutic effect on the gut microbiota.

Disclosure: No significant relationships.

Keywords: prolonged fasting, water-only fasting, Gut microbiota, pathways, anti-inflammatory

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

DIVERSITY AND COMPOSITION OF SALIVA MICROBIOTA IN CHILDREN WITH LOW AND HIGH PLANT CONSUMPTION

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Background and Aims: Nitrate supplementation influences the oral microbiota. Some plants are rich in nitrate, yet the role of plant consumption in oral microbiota is scarcely studied. Previously, we showed high sugar consumption to associate with a nitrate reduction pathway. Here, we aimed to explore whether microbial diversity and composition in saliva differ between children with low and high consumption frequencies of fruits, berries and vegetables (= plants).

Methods: Using 16S rRNA gene sequencing, we characterized the saliva microbiota of children (mean \pm SD age 11.7 \pm 0.4 years), randomly selected from the Finnish Health in Teens (Fin-HIT) cohort study. We excluded subjects with recent antimicrobials use (< 3 months) and low sequencing depths. We compared differences in alpha diversity (Shannon, inverse Simpson and Chao1 indexes) and beta diversity between children with a low ($9 \leq$ times a week, n=254, 1st tertile) and a high (≥ 16 , n=216, 3rd tertile) plant consumption.

Results: Alpha diversity and beta diversity did not differ between low and high plant consumption even after considering sugar and other possible confounders (p for all > 0.05). Similarly, when examining a subgroup with low sugar consumption only (n = 156), no differences emerged.

Conclusions: Consumption frequency of plants did not associate with the diversity and composition of the saliva microbiota. Next, we will examine the role of whole diet on the diversity, composition and functional capacities of the saliva microbiota. This will enable us to gain a deeper understanding of the role of the diet on the oral microbiota.

Disclosure: No significant relationships.

Keywords: oral microbiota, diet

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

A META-ANALYSIS ON THE EFFECTS OF LEGUME PROTEINS ON HUMAN HEALTH AND GUT MICROBIOME

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Background and Aims: Diets that prioritize fruits, vegetables, legumes, and whole grains while limiting meat intake, known as Plant-based diets (PBDs), have been associated with better health outcomes, lower mortality rates, and reduced environmental impact. Legumes are the primary source of protein in PBDs. Recent research has shown that the amount and type of dietary protein can significantly impact the composition and function of the gut microbiota.

Methods: Therefore, a comprehensive literature screening was conducted to assess the impact of legumes and their proteins on the human gut microbiome.

Results: Consuming legumes has been linked to several health benefits, such as improvements in cardiovascular risk factors, oxidative stress, and inflammation. Human trials have shown that legume consumption has the potential to positively affect the abundance of beneficial gut bacteria, as well as the diversity and richness of the microbiome. However, research on the effects of legume proteins on microbiota composition and function is currently limited to animal models, with only a few clinical trials available.

Conclusions: Hence, to fully understand how legumes may affect human health and regulate the composition and activity of the gut microbiome, ad hoc designed trials evaluating the addition of legumes and/or their proteins to diet are required. **Acknowledgements:** This work was partially funded by the project ON Foods - "Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods", funded by the European Union - NextGenerationEU, NRRP - Mission 4, Component 2, Investment 1.3 - PE00000003 (D.M. Prot. 1550 of 11/10/2022; CUP E63C22002030007).

Disclosure: No significant relationships.

Keywords: Plant-based diets, legumes, legume proteins, Gut Microbiome, human health

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

POSITIVE EFFECT OF CHLORELLA BIOMASS/EXTRACT ON VIABILITY OF PROBIOTIC AND YOGURT BACTERIA

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Background and Aims: *Chlorella vulgaris*, a blue-green microalgae, represents an important source of beneficial nutrients such as carotenoids, pigments, proteins with well-balanced amino acid profiles, polyunsaturated fatty acids, minerals, and vitamins. Moreover, it is among the few microalgae designated as Generally Recognized as Safe (GRAS) and is approved by the European Food Safety Authority. One of the most important carbohydrate with prebiotic effect present in *chlorella* cells, is β -1,3-glucan which is also easily fermentable in the colon. Apart from β -1,3-glucan, bacterial growth is supported by vitamins (A, B1, B2, B6, B12, C, E, biotin, pantothenate, etc.) produced by *chlorella*.

Methods: This study describes the prebiotic properties of *chlorella* biomass and its influence on adherence of selected lactobacilli and bifidobacteria to human adenocarcinoma cell lines, Caco2 and HT-29. Based on positive prebiotic properties of *chlorella*, yogurts with content of 1.0 % of *chlorella* biomass/extract were prepared. Bacterial viability and sensory properties were investigated during 40 days of storage in yogurts prepared.

Results: *Chlorella* biomass supported the adherence ability of selected bifidobacteria and promoted growth of six out of eight probiotics. The viability of yogurt bacteria reached about 10^7 cfu/mL until the end of storage at 6 °C. Used concentration of *chlorella* was acceptable for consumers while maintaining benefits.

Conclusions: *Chlorella* biomass has significant potential for the development of new fermented products with beneficial effects for consumers. This research was funded by the Ministry of Agriculture of The Czech Republic, Institutional support, No. MZE-RO1423 and project no. QK 1910300.

Disclosure: No significant relationships.

Keywords: *chlorella*, bifidobacteria, yogurt, prebiotic effect, bacterial viability

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

GUT MICROBIOTA MODULATION WITH ANTIBIOTICS FOR DYBIOSIS-ASSOCIATED COLON POLYPOSIS: A CASE REPORT

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Background and Aims: Dysbiosis is defined as a state of imbalance of the gut microbiota ("GM"). Dysbiosis has been associated with certain types of inflammatory colon polyposis, coining the term dysbiosis-associated colon polyposis. The objective of this study was to attempt a favorable GM modulation in a patient suffering from a rare type of dysbiosis-associated colon polyposis by antibiotics administration based on a similar case report.

Methods: A 28-year-old female patient diagnosed with a rare form of inflammatory colon polyposis was administered with a per os course of ampicillin (1500mg/daily) and metronidazole (1200mg/daily) for 10 days, after several unsuccessful treatment attempts. The gut microbiota composition was assessed the day before therapy commencement and the day after therapy termination. Dietary intake and other potential confounding lifestyle factors (sleep, physical exercise) were monitored during the intervention and the records did not differ significantly before and during the treatment.

Results: The results of the two analyses demonstrated that the antibiotic treatment significantly modulated the initial GM dysbiosis: increases in bacterial α -diversity indices and substantial changes of relative abundances of several anaerobic and facultative anaerobic taxa on phyla, genera and species level were observed.

Conclusions: These data suggest that GM modulation with antibiotic treatment can be achieved in this specific condition, similarly to previous reports. The described resulting changes in GM composition seem promising for relieving dysbiosis and, therefore, long-term beneficiary in the treatment of dysbiosis-associated colon polyposis.

Disclosure: No significant relationships.

Keywords: dysbiosis, antibiotic treatment, Gut microbiota, diversity

E-POSTER VIEWING - AS07. INFLUENCE OF DIET AND DRUGS ON THE HUMAN MICROBIOME

A MICROBIOME GUT ANALYSIS IN A COMPANY POPULATION

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Background and Aims: This study aimed to characterize the gut microbiome of a homogeneous population sampled in a company based in the urban area of Brescia (Italy). The analysis gave the opportunity to collect data in an environment mimicking a real-world scenario.

Methods: Stool microbiota samples from 192 workers were collected using Copan SMART-eNAT® and 16S rRNA gene sequencing was performed to determine their taxonomic composition. Raw data were analyzed through Atlasbiomed proprietary platform returning, for each sample, indicators on a [2;10] range over areas including microbial diversity, probiotics, anti-inflammatory potential, vitamins, fiber metabolism, disease protection, butyrate gluten and lactose metabolism. Consultancy was performed by ZadeiClinic to include lifestyle and nutritional suggestions in a report provided to each participant.

Results: Data showed an average low anti-inflammatory potential in association with a low diversity and a general lack of production of B group vitamins. Data globally reflects a population showing signs of general inflammation, probably because Brescia area has been one of the most affected by COVID-19 pandemics in the period before the immunization campaign of early 2021.

Conclusions: Data showed the possibility to suggest intervention guidelines to participants: 1. A nutritional program conveyed through the on-premises canteen, supporting microbiome diversity by embracing the “eat the rainbow” nutrition plan; 2. Education on healthy lifestyle and correction of bad habits; 3. Integration; 4. Checkups. We plan to reprocess the data with statistical software to obtain aggregate information related to the identification of clusters, using methods as principal component analysis and heatmaps.

Disclosure: No significant relationships.

Keywords: Gut Microbiome, Sequencing, diet, Collection

089 / #434

E-POSTER VIEWING - AS08. FROM BENCH TO BEDSIDE – USING THE MICROBIOME IN CLINICAL TRIALS

DYNAMAP ENABLES FAST AND SEQUENCING-FREE MICROBIOME ANALYSIS THROUGH OPTICAL MAPPING OF FLUOROCODED DNA

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Background and Aims: We present **DynaMAP** – a new microbiome analysis method for **D**ynamic **M**icrobiome **A**bundance **P**rofiling, based on optical mapping. Optical Mapping (OM) of DNA has been widely used for genome analysis and helped resolving the assembly of the human genome in 2022. DynaMAP uses sequence-specific fluorescent labeling of metagenomic DNA at all occurrences of one or more selected 4-mers, followed by molecular combing on a surface. Automated single-molecule microscopy captures the fluorescence patterns of hundred of thousands of DNA molecules. Fluorescence along individual DNA molecules form barcode-like taxonomic markers with a resolution down to strain level. The DynaMAP workflow delivers microbiome profiles in under 3 hours. This is at least one order of magnitude faster than sequencing by synthesis. Unlike other molecular approaches for microbiome profiling, DynaMAP does not require amplification or sequencing of the DNA. This comes with a major advantage, as the current go-to methods require bias-prone DNA amplification and synthesis, introducing sequence artifacts (point variations, chimeras, heteroduplexes) and over/underrepresentation due to amplification bias. Furthermore, current sequencing-based methods come down to choosing between amplicon sequencing and metagenomic shotgun sequencing, which have a strong trade-off between cost and performance.

Methods: We benchmarked DynaMAP performance both on mock and fecal microbiomes.

Results: We demonstrate DynaMAP's the precision, taxonomic resolution and reproducibility

Conclusions: Capturing unique taxonomic barcodes on high molecular weight DNA allows to resolve the composition of complex microbial communities with high precision and with a taxonomic resolution down to strain level. We propose DynaMAP as an unbiased, time- and cost-efficient enabling for microbiome applications.

Disclosure: The authors of this poster are full time employees of Perseus Biomics, the company developing the proprietary technology platform DynaMAP.

Keywords: metagenomics, microbiome profiling, (pre-) clinical trials, LBP development, microbiome diagnostic

E-POSTER VIEWING - AS08. FROM BENCH TO BEDSIDE – USING THE MICROBIOME IN CLINICAL TRIALS

MODULATION OF THE GUT-LUNG AXIS AFTER SEVERE COVID-19 INFECTION THROUGH PROBIOTICS: A PILOT STUDY

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Background and Aims: The gut-lung-axis could be a potential therapeutic target for improving post-acute COVID-19 symptoms, and probiotics have been proposed as possible modulators. We conducted a pilot study to understand alterations in the gut-lung axis and to explore the effects of a probiotic in post-acute COVID-19 disease.

Methods: We included patients after severe COVID-19 disease (sCOV, n=21) to a randomized, placebo-controlled trial to test the effect of a probiotic (Pro-Vi 5, Institute Allergosan, Graz, Austria) in a six-month intervention and patients after mild disease (mCOV, n=10) as controls, to compared the intestinal microbiome, metabolome as well as patient-reported outcomes and biomarkers along the gut-lung-axis at baseline and throughout probiotic intervention.

Results: Compared to mCOV, sCOV patients showed lower microbial richness, which was significantly improved by probiotic intervention. A reorganization of Ruminococcaceae and Lachnospiraceae taxa was observed in sCOV patients, but remained unaffected by the intervention. Serum metabolome showed a dysregulation of lipoproteins in accordance with higher BMI and comorbidities in sCOV patients. HDL and LDL fractions/components were temporarily decreased in the probiotic group. Stool metabolome was altered at baseline in sCOV patients and an increase in L-DOPA after 3 months and butyrate after 6 months of intervention could be observed. Probiotics partially improved reduced quality of life and modulated altered immune responses in sCOV patients. Increased intestinal permeability at baseline remained unaffected.

Conclusions: The study provides evidence of long-term alterations of the gut-lung-axis after severe COVID-19 infection and suggests that probiotics can modulate biomarkers of the gut-lung axis.

Disclosure: VS has received research funds from Institute Allergosan

Keywords: gut-lung axis, intestinal microbiome, Metabolome, post-acute COVID-19 disease, probiotic intervention

E-POSTER VIEWING - AS08. FROM BENCH TO BEDSIDE – USING THE MICROBIOME IN CLINICAL TRIALS

GUT MICROBIOME CHARACTERIZATION OF 113 DONATIONS FROM MEDITERRANIAN HEALTHY DONORS OF A STOOL BANK

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Background and Aims: Stool banks provide stools from healthy donors to be used in fecal microbiota transplantation(FMT). Donors are selected with strict criteria and rigorously screened for infectious diseases and disorders associated with gut microbiota alterations. Stool banks samples can be considered a gold standard for healthy gut microbiota composition.

Methods: We established the first stool bank in Catalonia (Spain) and potential donors were subjected to medical examination, blood and fecal tests according to national consensus recommendations (Table 1). Donors who completed the screening phase contributed with stool donations for 2 months after which a final screening was performed. Only stool samples with Bristol stool scale between 3-5 were included. Stool microbial composition was analyzed by amplifying the V3-V4 16S rRNA region and sequenced on an Illumina MiSeq. Sequence processing and statistical analyses were performed using QIIME2 and R.

Results: We analyzed 113 samples received between 07/2021-03/2022 from 21 healthy donors. Donors median age was 31 years (IQR 28-34), 66.7% were female and the median number of samples per donor was 3 (IQR 2-5), three donors providing 51.3% (58/113) of stool donations. We observed good clustering within samples from same donors (Figure 1), which indicate a stable community through time, and similarities between most abundant bacterial families including Bacteroidaceae, Lachnospiraceae and Ruminococcaceae (Figure 2). We did not observe differences in alpha diversity metrics in relation to donor gender or age.

Table 1. Screening tests performed in all potential stool donors From:(Aira et al., 2022)

Sample	Determination	Parameter
Blood	General testing	Glomerular filtration rate, liver panel, C-reactive protein, thyroid-stimulating hormone (TSH) and thyroxine (T4), anti-transglutaminase antibodies, and lipid panel
	Bacteria	Serology for <i>Treponema pallidum</i>
	Viruses	Serology for cytomegalovirus (IgG), Epstein–Barr virus (IgG) and herpes simplex types 1 and 2 (IgG)a
		Serology for hepatitis A (IgM), B (HBsAg, IgM and anti-HBc IgG), C (hepatitis C virus antibodies) and E (IgM and IgG) viruses (antibodies)
		Serology for human immunodeficiency virus (HIV) types 1 and 2 and human T-lymphotrophic virus (HTLV) types 1 and 2 (antibodies)
Parasites	Serology for <i>SARS-CoV-2</i> (IgM and IgG)	
Stool	General testing	Detection of faecal occult blood ₂ and calprotectin
	Bacteria	Testing for toxigenic <i>Clostridioides difficile</i>
		Detection of gastrointestinal pathogens: <i>Campylobacter</i> spp., <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Yersinia</i> spp. or <i>Vibrio cholerae</i> ; detection of pathotypes of <i>Escherichia coli</i> (enterotoxigenic, enteroaggregative, enterobacter hemorrhagic, enteropathogenic or enteroinvasive) or <i>Helicobacter pylori</i> , <i>Plesiomonas</i> or <i>Aeromonas</i>
		Detection of multidrug-resistant bacteria: ESBL–producing enterobacteria, vancomycin-resistant enterococci, carbapenem-resistant enterobacteria or methicillin resistant <i>Staphylococcus aureus</i> (MRSA) ^f
	Viruses	Noroviruses, rotaviruses, adenoviruses, enteroviruses and SARS CoV 2
	Parasites	<i>Giardia lamblia</i> , <i>Cryptosporidium</i> spp., <i>Eutamoeba histolytica</i> , <i>Blasocystis hominis</i> and <i>Dientamoeba fragilis</i>
Fungi	Microsporidia	
Nasopharyngeal swab	Bacteria	MRSA
	Viruses	SARS-CoV-2

ESBL: extended-spectrum beta-lactamase; MRSA: methicillin-resistant *Staphylococcus aureus*; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TSH: thyroid stimulating hormone.

Reference:

Aira, A., Arajol, C., Casals-Pascual, C., González-Suárez, B., Marrí, S., Domínguez, M. Á., Guardiola, J., & Soriano, Á. (2022). **Recommendations for stool donor selection for fecal microbiota transplant. Consensus document endorsed by the Catalan Society of Digestology, Catalan Society of Infectious diseases and Clinical Microbiology and the GEMBIOTA group from Spanish Society of Infectious Diseases and Clinical Microbiology.** *Enfermedades Infecciosas y Microbiología Clínica (English Ed.)*, 40(3), 142–146. <https://doi.org/10.1016/j.eimce.2021.12.001>

Figure 1. Beta diversity (Bray-Curtis dissimilarity index) of stool donations according to donor (NMDS: Non metric dimensional scaling)

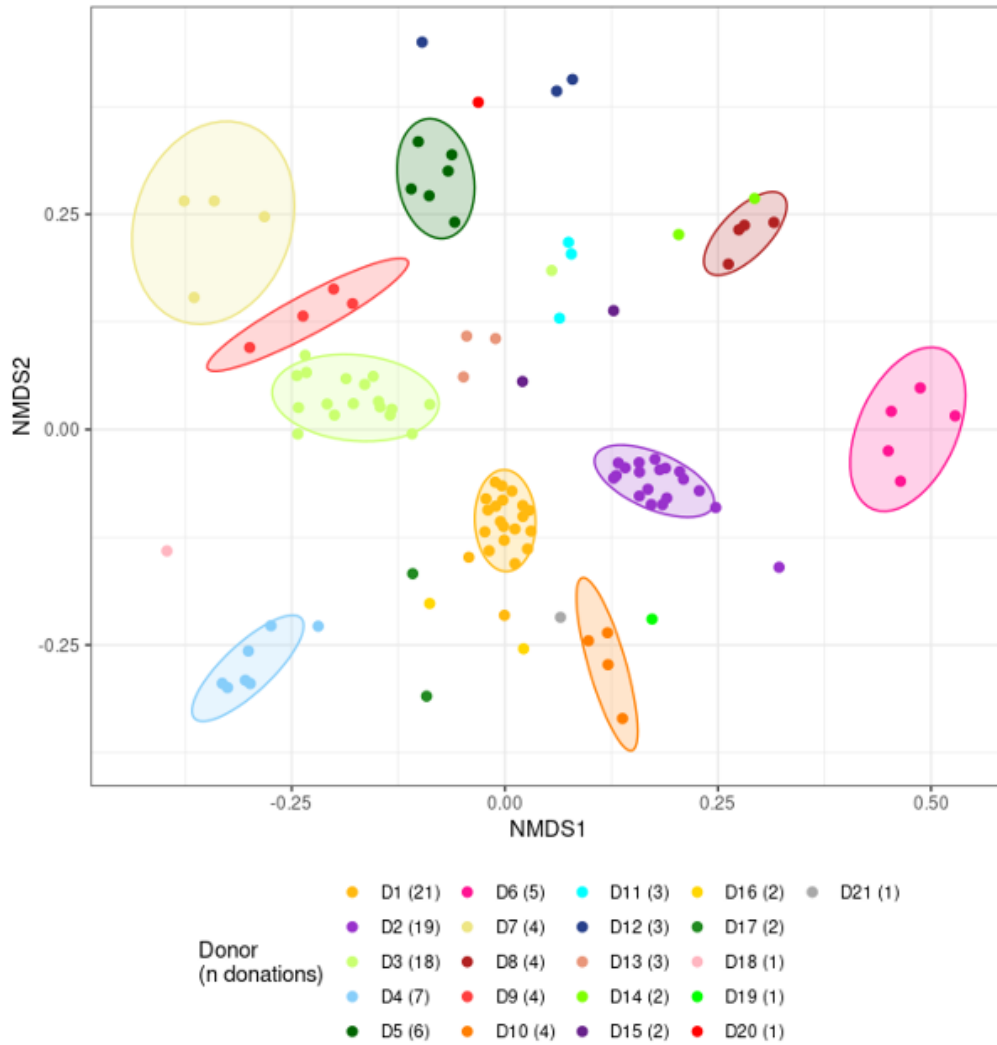
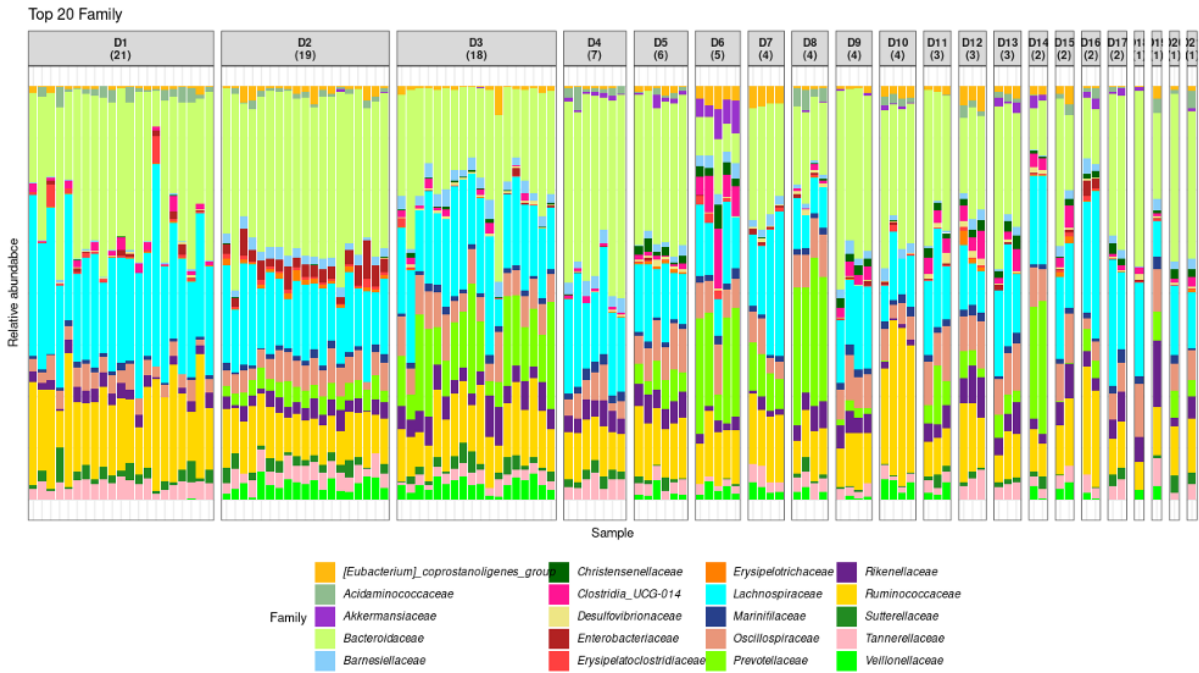


Figure 2. Bacterial taxonomic composition of stool donations showing the top 20 most abundant bacterial families. Stool donations are grouped by donors.



Conclusions: Gut microbial diversity and composition of individual healthy donors show consistency over time. This has important implications for future and more challenging indications of FMT.

Disclosure: No significant relationships.

Keywords: fecal microbiota transplantation, mediterranean healthy donors, stool donations microbiome characterization

E-POSTER VIEWING - AS08. FROM BENCH TO BEDSIDE – USING THE MICROBIOME IN CLINICAL TRIALS

SYMBIO-THERAPY TARGETING INFLAMMATION AND MICROBIOTA IN ULCERATIVE COLITIS: RATIONALE AND NON-INVASIVE DIAGNOSTICS AS AN AID IN MONITORING AND DECISION MAKING FOR PRECISION MEDICINE

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Background and Aims: Reciprocal microbiota-host influences constitute the basis for the establishment of a remarkably stable equilibrium in the intestinal ecosystem. We show that this equilibrium can take different forms, representing alternative stable states. A rapid, catastrophic, transition to a different state may be triggered when conditions change beyond a tipping point, and hard or impossible to reverse due to alternative stable state properties. This becomes important when different stable states condition health or chronic diseases like UC: a patient could become “locked” in a disease state.

Methods: Our analyses of pediatric UC data show that even after one year of conventional, anti-inflammatory, treatments only 36% of the patients reached a nonpathological host inflammation state, and only about half of these reached the least inflammation-prone microbiota state. The probability of clinical remission depended on the microbiota state before treatment. We postulate that remaining (low level) inflammation or unfavorable microbiota composition may lead to the re-activation of a vicious cycle of microbiota-host interactions and relapse.

Results: We integrated our observations in an innovative conceptual model, which indicates that combined action on inflammation and microbiota (symbio-therapy) should increase chances of restoration of a healthy symbiosis and remission.

Conclusions: We provide the first formal proof for the existence of disease-associated alternative stable states of the human intestinal ecosystem, taking host and microbiota into account. While affecting disease course, knowledge about their behavior opens conceptually new avenues for prevention and therapy. To this end, we propose a fecal sample based non-invasive diagnostic method as an aid in decision making.

Disclosure: No significant relationships.

Keywords: ulcerative colitis, intestinal ecosystem, alternative stable states, symbio-therapy, therapeutic innovation

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

BETA-RESORCYLIC ACID RELEASED BY LACTOBACILLUS REUTERI PROTECTS AGAINST CHEMOTHERAPY-INDUCED OVARIAN TOXICITY AND INFERTILITY

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Background and Aims: Chemotherapy-induced premature ovarian insufficiency (CPOI) triggers gonadotoxicity in females by reducing ovarian reserve, causing subfertility with no effective therapy available. Gut microbiota strongly associated with female reproductive disorders but the potentialities of gut bacteria and bacteria-derived metabolites to mitigate gonadotoxicity and infertility is yet to be elucidated.

Methods: We identified gut bacterial and metabolite biomarkers from both patients with POI after receiving cisplatin-based combination therapy and a mouse model of cisplatin-induced POI. Following this, the capability and the related mechanisms of both bacterium and metabolite in restoring ovarian function and fertility were examined. The mechanism of metabolite prevents cisplatin-induced ovarian damage were examined by in vitro on granulosa cells.

Results: We found that the dysbiotic gut microbiome in patients with CPOI exacerbates ovarian damage in mice. Multi-omics analysis showed decreased *Lactobacillus reuteri* and its catabolite, β -resorcylic acid (β -RA) in the CPOI group. Indeed, supplementation of *L. reuteri* or β -RA restored CPOI-induced disruptions to hormone levels, morphological damages, and decreases in follicular reserve. Most importantly, β -RA pre-treatment in CPOI mice effectively preserved oocyte function, embryonic development, and foetus health, thereby protecting against chemotherapy-induced subfertility. Mechanistically, β -RA suppressed the nuclear accumulation of SOX7 to prevent it from binding to the Bax promoter, thus inhibiting the priming of apoptosis in granulosa cells. Genetic knockdown of murine Sox7 by RNA interference protected against ovarian damage.

Conclusions: These findings highlight the significance of β -RA/SOX7/BAX axis mediated by *L. reuteri* and to protect ovarian toxicity and infertility induced by chemotherapy, shedding light on promising therapeutic strategies for CPOI treatment.

Disclosure: No significant relationships.

Keywords: chemotherapy-induced premature ovarian insufficiency (CPOI), Gut microbiota, β -resorcylic acid (β -RA), *Lactobacillus Reuteri*

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

COMPARING SELF-COLLECTED TO PHYSICIAN-COLLECTED SWABS FOR THE ANALYSIS OF THE VAGINAL MICROBIOTA IN WOMEN UNDERGOING IVF/IVF-ICSI TREATMENT: A PROSPECTIVE PILOT STUDY

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Background and Aims: The composition of the vaginal microbiota prior to an IVF/IVF-ICIS treatment can predict the reproductive outcome. The optimal determination of the vaginal microbiome is essential. Since self-collection is more convenient and patient-friendly, the current study aims to evaluate the reliability of self-collected swabs in comparison to physician-collected vaginal swabs.

Methods: The vaginal microbiota was investigated in patients before the start of IVF/IVF-ICSI treatment and immediately before a frozen-embryo transfer in a natural cycle. This prospective pilot study consisted of two cohorts. Cohort I involved patients self-sampling first, after oral instructions, followed by a physician-collected swab (n=115 patients, 230 swabs), and cohort II represents the reversed order of collection (n= 116 patients, 232 swabs). The interspace profiling (IS-Pro) technique² was used to analyze all collected samples.

Results: From May 2021 to March 2022, a total of 462 samples were collected from 231 patients (aged 21-44 years) who were primarily Caucasian (71%), with n=115 in cohort I and n=116 in cohort II. There were no significant differences in age, BMI, ethnicity, use of antibiotics or probiotics in the past three months, or use of vaginal soap between the cohorts. The vaginal microbiome profiles of both cohorts were found to be highly similar, regardless of the order of sampling, with a mean FatCos similarity of 0.93 (95% CI 0.91, 0.95) within cohort I and 0.94 (95% CI 0.92, 0.96) within cohort II.

Conclusions: Self-collection of vaginal swabs is considered reliable for the determination of the vaginal microbiome in a clinical setting.

Disclosure: X.G., Y.L., and S.S. have nothing to declare. A.B. is a shareholder of inbiome B.V. (NL). J.L. reports unrestricted research grants from Ferring, the Dutch Heart Association, ZonMw. He also received consultancy fees from Ferring, Gedeon Richter, and Titus

Keywords: Vaginal microbiota, In vitro fertilisation, reliability, vaginal swabs, pregnancy

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

THE ASSOCIATION BETWEEN RESPONSE TO IRON SUPPLEMENTATION AND GUT MICROBIOME AMONG HEALTHY NON-ANEMIC REPRODUCTIVE AGED WOMEN

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Background and Aims: Iron is an essential element for nearly all lifeforms, yet our understanding of iron hemostasis and absorption remains elusive. The aims of this study were to evaluate the composition of the host microbiome at baseline and following iron supplementation as well as the changes to anemia indices.

Methods: Pwm (n=26) w/o history of anemia were included in this study, consisting of 14 days of iron supplements and followup for 35 days, during which they provided 8 stool samples. Iron and anemia indices were collected at baseline, iron supplementation, and after washout. Participants were classified as fast, slow, or non-responders based on Δ ferritin in response to iron therapy. DNA extractions from stool samples underwent 16S amplicon sequencing and bioinformatics analyses of ASVs (DADA2), diversity metrics, taxonomic comparisons, and Dirichlet multinomial mixtures clustering.

Results: Participants were sorted into iron supplement based on the median and interquartile ranges for the rate of change in ferritin over time: fast (n=6, Δ ferritin \geq 75th IQR), slow (n=7, Δ ferritin > median & <75th IQR), and non-responders (n=13, Δ ferritin < median). DMM clustering revealed 3 distinct microbiome classes that approximated responder class. There was a sig. diff. in α diversity for DMM cluster but not clinician-defined classes. β diversity (Weighted UniFrac) revealed significance by responder class ($p=0.041$; posthoc tukey $p<0.0001$), and a multivariate linear model analysis identified 7 genera assoc. with Δ ferritin.

Conclusions: As anticipated by the principles of xenobiotics, gut microbes enable us to absorb iron and are differentially abundant in responders and non-responders.

Disclosure: No significant relationships.

Keywords: anemia, Microbiome

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

CHARACTERIZATION OF A PLACENTA RECONSTITUTION ASSAY TO FUNCTIONALLY STUDY BACTERIA MEDIATED MODULATION OF FETO-MATERNAL CELL COMMUNICATION.

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Background and Aims: Infertility is a complex medical condition influenced by various factors, including pathogens and the microbiota. The composition of the vaginal bacterial microbiota is highly dynamic and diverse between individuals. It is not clear how members of the microbiota affect fertility. We speculate that bacteria and their secreted molecules may affect early placentation by modulating feto-maternal cell communication. We aim to culture beneficial or harmful bacterial species of the vaginal-uterine cavity and screen them in a placenta reconstitution assay for capacity to modulate cells at the feto-maternal interface.

Methods: Endometrial and vaginal samples from 40 patients undergoing fertility treatment were collected for 16S rDNA sequencing and culturomics. To interrogating the potential of the reconstitution assay, we challenged villi and decidua explants of first trimester placentas with trophoblast-invading *Campylobacter fetus*.

Results: We identified patients with *Lactobacillus*-dominant and -submissiv microbiota and isolated 300 different colonies belonging to 75 bacterial species. *C. fetus* was used to start interrogating the potential of the functional assay. *Campylobacter* cause abortions (also in humans) and cytolethal-distending toxin release induces apoptosis in trophoblasts lining the placental villi. In good agreement with this and as previously demonstrated in first trimester trophoblast cell line ACH-3P, we found that *C. fetus* preferentially invades trophoblast columns. Moreover, we scored more apoptotic cells in infected tissue compared to non-infected controls, indicating correlation with an in vivo confirmed phenotype.

Conclusions: These results suggest that our model has the capacity to support mechanistic understanding how bacteria and their secreted effectors affect cells at the feto-maternal interface.

Disclosure: No significant relationships.

Keywords: functional assay, early placentation, bacteria, feto-maternal crosstalk

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E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

THE MENSTRUAL TAMPON AS A NOVEL SAMPLE COLLECTION TOOL FOR FOR VAGINAL MICROBIOME SCREENING.

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Background and Aims: Vaginal health screening has chronically low rates of uptake. This highlights the unmet need for a tool with comparable diagnostic accuracy, which is familiar, convenient and easy to use.

Methods: A non-interventional study was conducted to evaluate using menstrual tampons for specimen collection for analysis of the vaginal microflora. 83 participants were included in the study. Samples were collected with cervical swabs by a physician, self-collected with a vaginal swab and menstrual tampons. A stability study with 21 women evaluated if samples can be frozen at -20°C before processing.

Results: There is no statistical difference in the results for each specific bacteria, proving that the tampon obtains a statistically equivalent sample to the standard method for each of the target bacteria. Moreover, on average, the tampon collected the highest total bacterial mass, *Lactobacillus* spp. and *Ureaplasma* spp. compared to the clinician-collected cervical swab. Analysis identified no statistically significant difference between corresponding fresh and frozen samples. Thus, it was concluded samples can be frozen before processing.

Conclusions: This non-invasively collected specimen may facilitate self-initiated testing and population-based studies as well as longitudinal vaginal microbiome data collection. Statistical analysis of DNA load between tampon and VS samples revealed no significant difference, suggesting tampons are just as effective for material collection as lower vaginal swabs. Further, the ability to freeze tampon samples will enable cost-efficient processing by laboratories.

Disclosure: We acknowledge that Daye is the inventor and manufacturer of diagnostic tampons.

Keywords: vaginal microbiome, screening, women's health, at-home sample collection, infections

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

EVALUATION OF THE DEGREE OF DYSBIOSIS IN HEALTHY YOUNG WOMEN.

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Background and Aims: The microbiota of a healthy adult is unique and relatively stable over time. It is known that antibiotic treatments and diet are among the important factors that can determine dysbiosis. Our goal is to highlight whether age can be considered an influencing factor in dysbiosis.

Methods: The selection of the lot was made through family medicine offices. Subjects' participation was voluntary. The inclusion criteria in the study were female gender, age between 18-45 years, without known pathologies. The young women appeared in the records of general practitioners and were invited to participate in the study. Dysbiosis analyzes were performed by a single local laboratory. The SPSS 22 program was used for statistical data processing.

Results: A total of 100 women agreed to participate in the study. The numerical representation of the subjects in the three established age groups (18-24; 25-34; 35-45 years) was approximately equal. 97% of the subjects presented dysbiosis of various degrees and 32% had pronounced dysbiosis. The age category with the most dibiosis pronounced was the 25-34 and 35-45 years old categories. Only 3% of women in the 18-24 age category had pronounced dysbiosis.

Conclusions: Age of subjects has the potential to influence dysbiosis in young women.

Acknowledgment.

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Disclosure: No significant relationships.

Keywords: young women, dysbiosis, age categories

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

THE DYNAMICS OF THE BREAST MILK MICROBIOME DURING THE FIRST MONTH AFTER BIRTH IN DEVELOPING COUNTRIES: THE CASE OF MOROCCAN WOMEN

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Background and Aims: At present, it is relatively difficult to gain a clear understanding of the process of breast milk maturation in developing countries. This study aims to investigate the dynamics of the breast milk microbiome of healthy Moroccan mothers.

Methods: Breast milk microbiome samples were analyzed from 10 healthy, breastfeeding, vaginally delivered, rural Moroccan mothers. Breast milk microbiome was assessed using 16S rRNA gene, data were denoised using DADA2 v1.22.0, differential abundance analysis was compared using DESeq2.

Results: 3455 ASVs were identified, of which 136 could be annotated as putative species. The Moroccan breast milk samples from three stages of lactation (LT0, LT1 & LT2) could be divided into two groups. The first group consists of the LT0 and LT1, dominated by *Unclassified Pseudomonas* species with low diversity. The second group with less species of the genus *Pseudomonas* and a high diversity, is related to LT2. DAA identified 20 ASV as significantly higher during the first month of lactation. 8 ASV were found in higher relative abundance in LT0 or LT1 and the remaining 13 in higher relative abundance in LT2. The DA species also included several species not previously reported in breast milk, such as *Chishuiella changwenlii*, and species of the bacterial genera *Hydrogenophilus*, *Atopostipes*, *Pantoea* and *Chryseobacterium*.

Conclusions: This study, the first of its kind in Morocco, showed that the composition, diversity and functional potential of the bacterial communities in breast milk vary according to the stage of lactation. The breast milk microbiota of healthy Moroccan women is personalized and dominated by species of the genus *Pseudomonas*.

Disclosure: No significant relationships.

Keywords: breast milk, Microbiome, 16S rRNA, Moroccan mothers, breastfeeding

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

DIFFERENTIAL EFFECTS OF CONTRACEPTIVE METHODS ON THE VAGINAL MICROBIOME AND HOST IMMUNE RESPONSE

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Background and Aims: Contraception and the vaginal microbiome play crucial roles in women's health. Non-*Lactobacillus*-dominated vaginal microbiome is associated with adverse health outcomes. However, the influence of contraceptive methods on the vaginal microbiome is not well understood. We examined the effect of three contraceptive methods on the vaginal microbiomes of South African women who participated in the Evidence for Contraceptive Options and HIV Outcomes (ECHO) trial.

Methods: Vaginal swabs from 162 women randomized to one of three contraceptive methods (intramuscular depot medroxyprogesterone acetate (DMPA)-implant, levonorgestrel (LNG)-implant, and copper IUD) at two ECHO sites (MatCH Research Unit and Setshaba Research Center) were collected at enrollment, 1 and 3 months after contraceptive initiation. Microbiome profiles were generated by targeting the V1-V3 of the bacterial 16S rRNA genes. Associations between microbiome profiles, clinical variables, cytokine expression and contraceptive method were evaluated.

Results: Longitudinal analysis revealed that women using LNG-implants tended to develop more optimal microbiome and low levels of pro-inflammatory immune markers, while women using copper IUDs tended to develop more complex microbiomes and increased local expression of immune markers. Women using DMPA-IM exhibited neither tendency. Analysis of the transition frequencies of the longitudinal data sets showed that women using copper IUD were more likely to transition to higher diversity microbiome profiles while users of LNG-implant showed greater probability of transition to an optimal microbiome dominated by *L. crispatus*.

Conclusions: Different contraceptives seem to differentially impact the vaginal microbiome. Copper IUDs may have negative implications (high-diversity microbiome) for women's gynecologic and reproductive health whereas LNG-implants may have positive health benefits.

Disclosure: G.A.B. is a member of the Scientific Advisory Board of Juno, LTD.

Keywords: LNG-implant, DMPA-IM, vaginal microbiome, immune markers, copper IUD

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

THE VAGINAL MICROBIOME CHANGES DURING VARIOUS FERTILITY TREATMENTS

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Background and Aims: Objective: Does hormonal treatment during fertility treatments influences the vaginal microbiome? Rationale: Bacterial vaginosis (BV) could affect fecundity, especially in the IVF population a negative effect on pregnancy results has been reported. It is hypothesized that the hormone treatment during fertility treatments could influence the number of Lactobacilli, and therefore the microbiome, with negative effects on the pregnancy results.

Methods: Study design Observational prospective study, 53 couples attending a fertility clinic in the Netherlands between July 2019 and August 2022. Study population: Vaginal samples were taken at start of treatment, oocyte retrieval or insemination from subjects undergoing intra uterine insemination (IUI) with mild ovarian stimulation, in vitro fertilisation (IVF) or intra cytoplasmic sperm injection (ICSI) with controlled ovarian hyperstimulation, respectively. AmpliSens® Florocenosis/Bacterial vaginosis-FRT qPCR and 16S rRNA gene-based amplicon sequencing were performed on all samples. Main study parameters/endpoints: Lactobacilli percentage, Shannon Diversity index

Results: Main results: Lactobacilli percentage decreases during IUI or IVF treatments (8.9%, 1.5-16.4). Shannon Diversity Index was not significantly different at intake compared to last sampled treatment (1.31 vs 1.39). Of the total of 53 persons, 9 switched from qPCR BV negative to qPCR BV positive during treatment. The persons switching to BV qPCR positive status had already a (not significant) higher Shannon Diversity Index at start of treatment.

Conclusions: the vaginal microbiome changes during fertility treatment Wider implications: If the vaginal microbiome of persons is deteriorating during fertility treatments, timing of following treatments, lifestyle changes or a freeze all strategy could be of possible benefit.

Disclosure: This study was funded by a grant from the Research Fund of the Haaglanden Medical Center (2021) and Stichting Researchfonds Bronovo. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

Keywords: vaginal microbiome, hormone treatment, ivf, iui, bacterial vaginosis

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

EFFECTIVENESS OF A MULTI-STRAIN PROBIOTIC COMPOSITION IN POST-MENOPAUSAL WOMEN AND ITS MECHANISM OF ACTION AGAINST UROGENITAL PATHOGENS

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Background and Aims: This study, following the collection of in vitro evidence, aims to evaluate the efficacy of a multi-strain probiotic composition in supporting post-menopausal women in an observational clinical trial.

Methods: The antimicrobial and antiadhesive properties of *L. plantarum* PBS067, *B. animalis* subsp. *lactis* BL050 and *L. rhamnosus* LRH020 were evaluated on vaginal and bladder epithelia infected with different urogenital pathogens (*Candida glabrata*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* and *Escherichia coli*). Moreover, co-aggregation between probiotics and *Gardnerella vaginalis*, *Escherichia coli*, and *Candida albicans* was also investigated to elucidate the mechanism of action. Following, 50 post-menopausal women were involved in a clinical trial (ISRCTN15737648) to evaluate the cytokines inflammatory pattern and vaginal microbiota fluctuation after 28-day of probiotic administration (3B CFU/day). Vaginal wellbeing has been also detected by Vaginal Health Index (VHI) questionnaire.

Results: In vitro results showed a very good inhibition of all urogenital pathogens tested. Moreover, probiotics demonstrated to co-aggregate with pathogens, reducing their growth and infective activity. Additionally, clinical results showed an interesting decrease of menopausal symptoms, the improvement of the VHI score as well as the reduction of inflammatory cytokines (i.e. IL-6). All participants reported improvement of overall wellbeing, including vaginal benefits, after probiotic supplementation.

Conclusions: This work widens the use of a combination of three probiotic strains, previously clinically tested in childbearing age women, to be effective also in post-menopausal women, elevating the multi-strain probiotic composition as an everyday-life sustainable ally.

Disclosure: Patrizia Malfa and Diletta Squarzanti are SynBalance employees

Keyword: probiotics, women's health, menopause

E-POSTER VIEWING - AS09. WOMEN'S HEALTH AND THE MICROBIOME

THE CORRELATION BETWEEN VAGINAL AND ENDOMETRIAL MICROBIOTA

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Background and Aims: For the uterus the absence of microorganisms or the presence of *Lactobacillus*-dominated microbiota is considered favorable. In contrast, opportunistic microorganisms (OM) are associated with poor pregnancy outcomes in women undergoing in vitro fertilization. The prediction of OM in the uterus through vaginal microbiota investigation would be advantageous because it allows avoiding invasive endometrial sampling.

Methods: 177 reproductive-age women (33.5±5.2 years) were included in the study. Endometrial and vaginal samples were obtained simultaneously and microbiota was quantitatively assessed by the real-time PCR kit "Androflor" (DNA-Technology, Russia). All patients were divided into 2 groups depending on the type of endometrial microbiota: Group 1 (OM proportion≥10%, n = 52) and Group 2 (proportion of OM<10% or no microbiota, n = 125).

Results: Vaginal microbiota of Group 1 (with OM) was characterized by higher alpha-diversity and higher quantities and proportions of 8 anaerobic bacteria groups (*Gardnerella vaginalis*, *Megasphaera spp./Veillonella spp./Dialister spp.*, *Sneathia spp./Leptotrichia spp./Fusobacterium spp.*, *Bacteroides spp./Porphyromonas spp./Prevotella spp.*, *Peptostreptococcus spp./Parvimonas spp.*, *Anaerococcus spp.*, *Eubacterium spp.*, *Atopobium cluster*) and *Streptococcus spp.* (p<0.05). Higher proportion of *Lactobacillus spp.* was detected in Group 2 (99.7% vs.86.2% by medians)(p<0.05). From all 10 parameters *Eubacterium spp.* proportion as a single marker demonstrated the best accuracy in predicting the OM-dominating endometrial microbiota (AUC — 0.713[CI 0.625-0.801]).

Conclusions: The non-*Lactobacillus*-dominated endometrial microbiota was associated with significant shifts in quantities and proportion of 9 groups of OM and lactobacilli in vaginal microbiota, therefore it seems promising to apply multivariate analysis to obtain more accurate model predicting the OM-dominating endometrial microbiota based on vaginal microbiota assessment.

Disclosure: No significant relationships.

Keywords: Androflor, real-time PCR, Vaginal microbiota, endometrial microbiota

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

THE ROLE OF THE GUT MICROBIOTA IN FOOD ALLERGIES

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Background and Aims: IgE-mediated food allergy (FA) is an unexcepted immune response occurring within 2 hours of exposure to specific foods. Oral food immunotherapy (OIT) is an active therapeutic strategy for FA and the gut microbiota is now becoming a research focus in the study of FA and treatment prognosis.

Methods: To characterize the gut microbiota, we collected 34 fecal samples from 17 walnut FA patients undergoing OIT, before and after treatment, and from age-matched controls (n=19). DNA was isolated and 16S rRNA gene sequences analyzed. To explain the gut microbiota's mechanistic contributions to the altered immune state in FA and to assess whether pre-and post-OIT microbiota differentially impact the immune system, we preformed fecal microbiota transplant (FMT) from allergic patients before (n=7) and after (n=7) treatment and from healthy controls (n=4) to germ-free mice and characterized their allergic response.

Results: The gut microbiota composition (β -diversity) between the pre-treatment group and the non-allergic control group was significantly different. The relative abundance of *P. copri* was also significantly different between the pre-treatment and control groups. In the mouse experiment, comparisons of β diversity demonstrated significant differences in the control group versus the pre-treatment group. Following FMT, the pre-treatment recipients had a greater drop in core body temperature, more movement, and piloerection when challenged with allergen, indicating transfer of human recovery from allergy to mice.

Conclusions: Our results demonstrate a link between FA, OIT and the gut microbiota. Because the microbiota is modifiable, this study can set the groundwork for discovery of additional therapeutic interventions to treat FA.

Disclosure: No significant relationships.

Keywords: prevotella copri, FMT, OIT, food allergy, walnut allergy

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

THE RESPIRATORY MICROBIOME ALPHA-DIVERSITY AND CHRONIC RESPIRATORY DISEASES IN CHILDREN: A SYSTEMATIC REVIEW

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Background and Aims: While there seems to be a consensus that a decrease in gut microbiome diversity is related to poorer health status, the associations between respiratory microbiome diversity and chronic respiratory disease remain a matter of debate, as highlighted by a recent review of the literature in adults. In industrialized countries, respiratory diseases and allergies are the most common chronic diseases in children. These diseases vary considerably between childhood and adulthood. We performed a systematic review of the alpha diversity of the respiratory microbiome in children with chronic respiratory diseases. We focused on studies in which a control allowed comparison.

Methods: We searched PubMed and Scopus. Four items were required: respiratory microbiome, diversity, chronic respiratory disease, and children younger than 18 years. Any articles that did not address these items, were not research articles, or did not have a case-control design were excluded.

Results: We reviewed 486 articles on the basis of title and abstract, of which 25 met our inclusion criteria. The diseases studied were mainly asthma, cystic fibrosis, wheezing, and respiratory allergies. The main sampling technique was the nasopharyngeal swab. All studies focused solely on bacteriome and measured the Shannon index. In more than half of the studies, the control group consisted of healthy subjects.

Conclusions: The majority of diseases were significantly associated with a decrease in diversity, with the exception of asthma, which, on the contrary, led to an increase. Only the studies on bronchiectasis, pulmonary hypertension and bronchitis showed no change or not concluding difference in the respiratory microbiome diversity.

Disclosure: No significant relationships.

Keywords: Respiratory microbiome, Chronic respiratory diseases, Alpha-diversity, children, dysbiosis

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

A NEGLECTED COMPONENT OF FECAL MICROBIOTA TRANSPLANTATION - VIRUSES

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Background and Aims: The prevalence of autoimmune diseases has been increasing in recent years. The gut microbiota is proposed to contribute to the development and progression of chronic gastrointestinal disorders, including autoimmune diseases by modulating immune responses. Fecal microbiota transplantation (FMT) in animal models represents an effective experimental method for investigating the role of microbial dysbiosis in the pathogenesis of autoimmune diseases. However, these studies are limited only to bacteria, while viral transport is unexplored.

Methods: We analyzed the transmission efficiency and persistence of FMT from human donors in the animal model with an emphasis on viruses, bacteriophages, and phage-bacterial interactions. Representative stool samples were shotgun sequenced. Bacterial MAGs were obtained by a hybrid co-assembly approach. Viral contigs were mined using Virsorter. The phage-bacterial links were obtained by comparing bacterial tRNA sequences with the viral contigs.

Results: We investigated viral genomes and their bacterial host coverage at different time points. The results indicated that 24.82% of identified donors' viruses were established in the recipients' gut 24 hours after FMT, but none of these persisted for three weeks. From all identified viruses, 4.47% showed tRNA hits with transplanted bacteria, which had lytic or lysogenic interactions. Increased coverage of some viruses after FMT was accompanied by bacterial vanishment.

Conclusions: Knowledge of phage-bacterial interactions in FMT could help to elucidate the role of gut microbiota in various autoimmune diseases, including autoimmune hepatitis, and potentially help design new FMT treatment strategies. However, it is vital to optimize transport from human donor to mouse recipient. Fundings: APVV-21-0370, VEGA 1/0649/21, and CDEIGENT/2021/008.

Disclosure: No significant relationships.

Keywords: fecal microbiota transplantation, Gut Microbiome, bacteriophages, autoimmune diseases, phage-bacterial interactions

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

RESEARCH OF THE ENDOMETRIAL IMMUNE CELLS IN WOMEN WITH REPRODUCTIVE FAILURE AND IMPAIRED ENDOMETRIAL MICROBIOTA

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Background and Aims: Increasing evidence suggests that the dysregulation of the endometrial immune cells is associated with implantation failure or recurrent pregnancy loss (RPL). These cells, such as macrophages, neutrophils, dendritic cells, T lymphocytes and endometrial NK cells are necessary for successful implantation and pregnancy and provide protection against pathogenic microorganisms. Therefore, our aim was to study endometrial immune cells in women with impaired endometrial microbiota and to compare their values after therapy.

Methods: Endometrial biopsies were performed during the mid-luteal phase of the monthly cycle for the period January 2022 to March 2023. Fifty-six women with reproductive failures with a mean age of 35.27 (± 4.11) years were studied. The microbial composition of the endometrial microbiota was examined by a molecular diagnostic method (REAL-TIME PCR, Femoflor 16®). Flow cytometry was used to detect endometrial immune cells (FACS Calibur, Becton Dickinson).

Results: After therapy in cases with disturbed microbiota, a statistically significant increase in endometrial NK cells was observed. There was no statistically significant difference in the percentage of other immune cells examined before and after therapy, but there was a decrease in neutrophils and plasma cells.

Conclusions: The detection of dysbiosis in the endometrial microbiota by molecular genetic methods and the study of endometrial immune cells are an important part of the diagnosis and treatment of women with reproductive failure. This facilitates assisted reproduction specialists in selecting appropriate antibiotic therapy and may help to develop new approaches to immunotherapy.

Disclosure: No significant relationships.

Keywords: uNK cells, endometrial microbiota, endometrial immune cells

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

EFFECT OF FOOD SUPPLEMENTS ON GUT MICROBIOTA IN OBESE CHILDREN: PRELIMINARY RESULTS OF ZIMBA TRIAL

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Background and Aims: The gut microbiota is composed by bacteria species that are in symbiotic relationship with human and involved in the production of dietary metabolites. Microbes can ferment the prebiotics influencing human health. Gut microbiota plays an important role in the regulation of metabolic, endocrine, and immune functions. Obesity is a serious concern worldwide and seems to be associated with alteration in gut bacterial flora. Recent studies reveals that the modulation of gut microbiota could affect positively the outcomes of several metabolic dysfunction linked to obesity, but it is still debated. In this study we want assess the efficacy of the association of Zinc, Myoinositol and GOS (galacto-oligosaccharides) vs administration only GOS (placebo) in pediatric obesity.

Methods: Zimba project is a placebo-controlled clinical trial. NGS analysis of 16S rDNA was performed according to Torre et al., 2022. Raw sequences were processed by MicrobAT software. Statistical analysis was performed using MicrobiomeAnalyst software.

Results: We analyzed 165 bacterial metagenomes in which alpha diversity showed not statistically significant differences but changes in beta diversity was observed. The prebiotic intake supplemented with Zn induced a significant increase in Firmicutes, *Bifidobacterium* sp., *Faecalibacterium prausnitzii*, unclassified *Lacnospiraceae*, *Ruminococcus* and *Dorea longicatena*, and decrease in *Bacteroides vulgatus* and *Parabacteroides distasonis* in respect to baseline.

Conclusions: Some of these bacterial species are essential for a healthy microbiota homeostasis but they could being modulate by Zn presence. Further analyses linked to the clinical and metabolic responses will be necessary to validate the proposed mechanisms at the base of the microbiota modulation.

Disclosure: No significant relationships.

Keywords: placebo-controlled clinical trial, Gut microbiota, human health, 16s rDNA, pediatric obesity

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

EXPLORING THE GUT MICROBIOME IN CHILDREN WITH ATOPIC DERMATITIS: INSIGHTS FROM REAL-WORLD DATA WITH IMPLICATIONS FOR INTERVENTIONS AND FURTHER RESEARCH

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Background and Aims: Atopic dermatitis is a skin condition that is characterized by chronic or recurrent dermatitis, pruritis, and lichenification with a high burden for those affected. Although the aetiology of the disease is not yet fully understood, studies suggest that dysregulation of the immune response, particularly the Th2 axis, is involved. Furthermore, studies have linked microbiota in host immune system regulation, though their mechanisms are not fully documented. Hence, studying the gut microbiome composition in atopic dermatitis could give insight into creating effective prevention and long-term treatments.

Methods: Our study examined the differences in the gut microbiome composition of 79 children aged 3 to 15 years from eight European countries, both with and without self-reported atopic dermatitis.

Results: Our findings revealed a significant enrichment of *Rikenella*, a genus known to be involved in inflammation regulation, and slight enrichment of potential pathobionts genera such as *Slackia*, *Ralstonia*, and *Catenibacterium* in atopic dermatitis group. In contrast, the healthy group had a higher abundance of prominent beneficial bacteria, known to be linked in mucosal immune homeostasis, such as *Christensenella*, *Butyricimonas*, *Odoribacter*, and *Ruminococcus*.

Conclusions: These results indicate that the compositional variation of the gut microbiome in atopic dermatitis and healthy children may play a critical role in regulating inflammation that suggests maintaining microbiota symbiosis could be an important strategy in managing this complex disease. More research is needed to comprehend the host-microbe interactions in atopic dermatitis, which could lead to the creation of preventative measures or interventions to improve the affected individuals' quality of life.

Disclosure: All authors work at BIOMES NGS GmbH, a service provider for NGS.

Keyword: gut microbiome, atopic dermatitis, children

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

**ROLE OF THE GUT MICROBIOME IN THE DEVELOPMENT OF IMMUNE CHECKPOINT INHIBITORS
MEDIATE ADVERSE EVENTS**

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Background and Aims: For cancer patients treated with immune-checkpoint inhibitor (ICI), immune-related adverse events (irAE) remain the major hurdle. Evidence suggest that the composition of gut microbiome correlates with ICI efficacy and potentially with irAE. We aimed to unravel the role of intestinal bacteria associated with irAE and immune colitis.

Methods: Metagenomics was performed on 81 NSCLC and 26 melanoma patients treated with ICI. In a sub-group of 23 patients including 11 colitis-free, and 12 paired samples before and during colitis, culturomics was performed. Next, in vitro LPS concentration was quantified using HEK cells. Finally, oral supplementation with colitis-associated isolated bacteria or FMT from colitis patients was performed to test anti-PD-1+anti-CLTA-4 response and the severity of inflammation in the DSS-induced colitis murine model.

Results: Combination of metagenomics and culturomics demonstrated that patients with colitis had a decrease in bacterial diversity and an enrichment of Dorea, Clostridium species, and Paraclostridium bifermentans compared to colitis-free patients. In matched patient samples, LPS level in feces was significantly increased at the time of colitis. In DSS colitis murine model, P. bifermentans and Bacteroides intestinalis supplementation was significantly associated with more severe colitis compared to DSS alone. Additionally, we showed that cell and cell-free supernatant of P. bifermentans synergized with combination ICI treatment to reduce tumor burden in mice. Lastly, FMT from patients with colitis increased anti-tumor response

Conclusions: We isolated bacteria that increased colitis and improved ICI response through mirroring patients' phenotype when they developed irAE. Overall, these results provide more insight to explain why irAE are associated with ICI efficacy.

Disclosure: No significant relationships.

Keywords: immune-checkpoint inhibitor, Gut microbiota, immune related adverse events, Colitis, Cancer

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

EVALUATION OF GUT AKKERMANSIA MUCINIPHILA, FAECALIBACTERIUM PRAUSNITZII AND BLOOD IMMUNE CELLS TH17, TREG IN PATIENTS AFTER LIVER TRANSPLANTATION

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Background and Aims: The gut microbiota has been demonstrated to interact with host immunity and to modulate both innate and adaptive immune processes. However, alterations in the gut microbiota and T-helper populations Th17 and T regulatory (Treg) in the late post-transplant period are not well understood. The aim of the study was to evaluate Akkermansia muciniphila (AKM) and Faecalibacterium prausnitzii (FAEP) levels in feces and in parallel the blood Th17 and Treg subsets in the late post-transplant period of patients after liver transplantation (LT). The obtained results were compared to appropriate control groups to evaluate a possible difference between transplanted patients on immunosuppressive therapy and healthy individuals.

Methods: Fecal samples from 23 LT patients and 9 controls were tested with commercially available qPCR kit for AKM and FAEP. 17 of them and 9 healthy controls were also examined for peripheral blood Th17 and Treg subsets, by flow cytometry.

Results: We found a statistically significant decrease in the abundance of both AKM and FAEP compared to the control group. LT patients showed significantly higher percentages of Th17 in peripheral blood than healthy controls and a reduction of Treg cells.

Conclusions: In this pilot study, we found a reduction in the quantity of the two studied beneficial bacteria, as well as reduced immune tolerance and increased activation of the immune system in LT patients. The observed changes may be due to the natural course of post transplantation liver condition and/or to immunosuppressive therapy. Additional investigations are needed to elucidate the underlying mechanisms.

Disclosure: No significant relationships.

Keyword: Akkermansia muciniphila, Faecalibacterium prausnitzii, Th17, T regulatory, liver transplantation

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

PREBIOTIC INTAKE ALLEVIATES ENDURING EFFECTS AND IMMUNE DYSFUNCTION DURING PUBERTY THROUGH GUT MICROBIOTA AND EPIGENETIC MODULATION

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Background and Aims: Puberty is a critical developmental period of life characterized by marked physiological changes, including distinct changes in the immune system and gut microbiota development. Exposure to immune stressors such as LPS during puberty has been found to lead to long-lasting negative immune disturbance by inducing pathogenic Th17 cell differentiation and related-dysbiosis. In this study, we hypothesized that pubertal exposure to LPS may cause long-term dysfunction in gut immunity by enduring dysregulation of Th17 inflammatory-related signaling pathways, and epigenetic changes. Prebiotic intake may mitigate gut immune dysfunction.

Methods: To this end, immune, signaling pathways, microbial and epigenetic studies were carried out to better decipher the immunoprotective effects of prebiotic intake. Techniques included multiplex immunoassay, Western blotting, and qPCR to assay cytokines, proteins, and miRNAs expression, and DNA methylation analysis. 16s rRNA sequencing was conducted for microbiome analysis.

Results: The results revealed a significant lasting dysregulation in selected cytokines, proteins, and miRNAs involved in key signaling pathways related to Th17 differentiation and function, in the small intestine of adult mice challenged with LPS during puberty. In contrast, dietary intervention was shown to mitigate the lasting adverse effects of LPS on gut immune function and reverse the effect of IL-6/STAT3 pathway expression through epigenetic mechanisms. DNA methylation analysis demonstrated that enduring changes in gut immunity in adult mice might be linked to differentially methylated genes involved in inflammatory response.

Conclusions: Prebiotic administration has shown to prevent LPS-induced inflammation and dysbiosis.

Disclosure: Funding for this project was provided by the AHCC Research Association as well as by a New Frontiers in Research Fund - Exploration NFRFE-(2019-01497) grant to C.M

Keyword: Prebiotic- Gut Microbiome- Puberty-microRNAs- Th17 signaling pathway

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

ASSOCIATION BETWEEN GUT MICROBIOME, INTESTINAL PERMEABILITY AND INFLAMMATORY LANDSCAPE IN HEALTHY INDIVIDUALS AND IRRITABLE BOWEL SYNDROME PATIENTS

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Background and Aims: Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder. IBS is associated with increased serum intestinal permeability (IP) marker zonulin and altered cytokine profile, but its relevance in context with gut microbiome composition is unclear. This study was conducted to investigate correlations between the gut microbiome, serum zonulin, and cytokine profiles.

Methods: Stool, serum samples and clinical data were collected from 34 controls and 25 IBS patients. Metagenomic shotgun sequencing of faecal samples was performed and a relative abundance of taxa was detected with *MetaPhlan*. Levels of serum IP (ELISA) and inflammatory markers (ProcartaPlex assay) were measured and related to the gut microbiota with Pearson correlation.

Results: In several control group samples, we observed a strong positive correlation* between *Lactococcus phage bIL67* and IL-2, IL-1 β , GM-CSF, TNF- α , as well as *Coprococcus eutactus* with IL-15. Strong negative correlation was detected between *Dorea longicatena* and *Blautia*, *Parabacteroides*, *Coprococcus*, *Ruminococcus*, and *Alistipes* genera with IL-1RA, IL-17A. In the IBS group, a strong positive correlation was observed between *Eubacterium rectale* with eotaxin and *Butyrivibrio crossotus* with IP-10, while a strong negative correlation was between *Dorea longicatena* and *Faelibacterium prausnitzii* with IL-1 β . Zonulin had a moderate and weak association with gut microbiome composition in both cohorts. * ≥ 0.7

Conclusions: Changes in the gut microbiome are linked with alterations in specific cytokine profiles. These findings will provide support for further studies to understand if IP and inflammatory markers correlate with LPS levels and bacterial taxa found in the blood. This study was supported by project No. 8.2.2.0/20/1/006.

Disclosure: No significant relationships.

Keywords: Gut Microbiome, irritable bowel syndrome, zonulin, cytokine

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

ASSOCIATION BETWEEN SMOKING AND ORAL MICROBIOTA

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Background and Aims: There has been no systematic research on the effects of cigarette smoking on the oral microbiota in the large Norwegian population. Therefore, we aimed to investigate the relationship between smoking and the oral microbiome in a general population.

Methods: Samples of gingival fluid were collected concurrently with spirometry in 447 adults (52% males) from the RHINESSA study, Bergen center. Bacterial DNA from the 16S rRNA gene was isolated from gingival fluid, sequenced by Illumina®, and assigned bacterial taxonomy by the Human Oral Microbiome Database. The relationship of microbial abundance with smoking by using the Analysis of Compositions of Microbiomes with Bias Correction 2 (ANCOM-BC2) with a 5% false discovery rate, adjusting for age, gender, BMI and education. The smoking status was assessed through a questionnaire.

Results: The mean age was 27 (range 18-47) years. The prevalence of previous and current smoking was 16% and 14 % respectively. There was significant lower shannon diversity and evenness among current smokers as compared to never smokers, but observed richness was higher among current smokers. There was significant difference in beta diversity between never smokers as compared to current and previous smokers. The abundance of the genera Bacteroidetes_[G-3] was significantly higher among those with previous smoker as compared to never smoker. Similarly, the abundance of genera Peptostreptococcaceae_[XI][G-4] and Cardiobacterium was significantly lower among previous smoker as compared to never smokers.

Conclusions: Our findings indicate that smoking cigarettes can potentially modify the oral microbiome, which could result in changes in functional and metabolic pathways relevant to smoking-related illnesses.

Disclosure: No significant relationships.

Keywords: oral microbiome, smoking, bacteria

E-POSTER VIEWING - AS10. THE HUMAN MICROBIOME AND THE IMMUNE SYSTEM

THE DERANGED MATERNAL GUT MICROBIOME IN OBESE PREGNANT MICE MAY AFFECT THE MATERNAL IMMUNE RESPONSE

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Background and Aims: Maternal obesity (MOB) is a significant risk factor for pregnancy complications in mother and child. Surprisingly, the mechanisms by which MOB negatively influences pregnancy are largely unexplored. We hypothesize that MOB changes the gut microbiome and induces immunological changes, which affect maternal and fetal health.

Methods: Female C57BL/6 mice (6 weeks) were randomly assigned to a high-fat or matched low-fat diet (8 weeks) to obtain obese and lean mice. Feces were collected before and during pregnancy (E7,14,18) for microbiome analysis using 16S rRNA sequencing. Obese and lean pregnant mice were sacrificed at day 18 (E18), fetuses were weighed, whereafter maternal spleen and Peyer's patches (PP) were collected, percentages of T cell subpopulations were measured by flow cytometry.

Results: Fetal weight was significantly decreased in obese (1.05gram±0.02) versus lean (1.16gram±0.03) mice (Mann-Whitney, p<0.001). Before and during pregnancy, the gut microbiome of obese pregnant mice significantly differed from lean pregnant mice (PERMANOVA, p<0.001), and various bacterial genera were either significantly increased (e.g., *Lactococcus*) or decreased (e.g., *Bifidobacterium*). At E18 of pregnancy, splenic Th1 cells were increased (Two-way-ANOVA, p<0.01), and Th17 cells were increased in the PP (Two-way-ANOVA, p<0.001). Correlation (Spearman's) of microbiome abundances with immune cells, showed that various genera that changed during MOB were positively or negatively correlated with immune cell populations.

Conclusions: This study shows that MOB affects the gut microbiome and immune response in pregnant mice, and that various bacterial genera in the gut correlated with immune cells. This suggests a role for the gut microbiome in inducing immunological changes during MOB.

Disclosure: No significant relationships.

Keywords: Obesity, Immune system, pregnancy, Microbiome, Mice

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

ASSOCIATION BETWEEN INDOOR BACTERIAL COMMUNITIES, LUNG FUNCTION AND AIRWAY INFLAMMATION

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Background and Aims: Indoor bacterial exposure has been linked to asthma, but little is known about the role of specific bacterial taxa in relation to lung function and airway inflammation. We aimed to study the association between indoor bacterial exposure and lung function and airway inflammation measured as Fractional exhaled Nitric Oxide (FeNO) in adults population.

Methods: The bacterial communities of settled airborne dust samples from the bedrooms of 1038 participants in the European Community Respiratory Health Survey (ECRHS) III were characterised by 16S rRNA amplicon sequencing, and bacterial load by qPCR. The samples were collected concurrently with spirometry and FeNO measurements (outcomes). Adjusted linear regression stratified by sex were used to model the association between bacterial profiles and outcomes.

Results: Higher bacterial diversity and richness were associated with an increase in FVC and FEV1 Z scores in males ($P < 0.05$), and with elevated FeNO in females only ($P < 0.05$). Most bacterial genera associated with higher lung function were from the Actinobacteriota phylum. Higher relative abundance of Bacteroidia, Myxococcota, and Clostridia was associated with lower lung function, as was true also for several bacterial genera that from the core of the oral microbiome, including Streptococcus. Higher FeNO levels were positively associated with the presence of Campylobacter and negatively with the presence of Cellulomonas.

Conclusions: We conclude that a higher microbial diversity is associated to higher lung function in males and increased inflammation and lower lung function in females. Further studies are needed to understand the relation between exposure to specific types of bacteria and lung conditions.

Disclosure: No significant relationships.

Keywords: Spirometry, diversity, Bacteroidia, FeNO, Bacterial communities

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

ASSOCIATION BETWEEN INDOOR ENVIRONMENTAL MICROBIOTA OF LIVING SPACES AND CHRONIC ASTHMA AND RESPIRATORY ALLERGIES IN EUROPE: A SYSTEMATIC REVIEW

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Background and Aims: Asthma is a common chronic disease characterized by narrowed airways due to inflammation and muscle constriction. The impact of microorganisms in the environment on asthma is an emerging research area. This literature review focuses on the associations between indoor environmental microbiota of living spaces and asthma/respiratory allergies in Europe. The focus was on studies conducted in the last ten years, utilizing techniques based on dust measurements and including a control group for comparison.

Methods: The databases PubMed, Scopus, and GreenFile were searched.

Results: We reviewed 490 articles on the basis of title and abstract, of which 12 met our inclusion criteria. Twelve studies met the inclusion criteria, conducted in Finland, France, Sweden, Germany, Austria, Greece, the UK, and one study included ten European countries. Eight studies investigated microbial diversity in children's homes, while four focused on adults. Various sampling techniques, including PCR and DNA sequencing, were used to analyze dust samples. Associations were found between fungal DNA levels in childcare center dust and respiratory symptoms. Higher fungal concentrations were observed in homes with asthmatic children. Indoor humidity and pet presence correlated with microbial agents in mattress dust and asthma outcomes. A total microbial exposure index was proposed as a better predictor of asthma. Asthmatic households had lower microbial richness/diversity. High fungal diversity exposure during infancy inversely related to sensitization to airborne allergens.

Conclusions: Further research is needed to establish clinical implications.

Disclosure: No significant relationships.

Keywords: Indoor microbiota, Fungi, Asthma, Dust, Respiratory allergies

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

PROTECTING THE PIGLET AGAINST ENTEROTOXIGENIC ESCHERICHIA COLI-MEDIATED POST WEANING DIARRHEA USING SPECIFIC BINDING PROTEINS

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Background and Aims: Antibiotic overuse increases the risk of antimicrobial resistance (AMR) development. The highest antibiotic usage in livestock is found in pig production, with piglet post-weaning diarrhea (PWD) as the main driver. Therefore, it is crucial to develop strategies that diminish piglet PWD without use of antibiotics. Piglet PWD is mainly caused by enterotoxigenic *E. coli* (ETEC), fimbriae-type F4 and F18. Thus, to reduce ETEC proliferation in piglets, we evaluated a novel feed additive comprising two bivalent heavy-chain variable domain (V_HH) constructs, specifically binding ETEC key virulence factors (F4 fimbriae and LT toxin).

Methods: We conducted *in vitro* studies, investigating the constructs' specificity and ability to bind F4 fimbriae and LT toxin, followed by an *in vivo* ETEC F4 challenge study with F4R⁺-piglets, investigating construct impact (separate or in combination) on fecal microbiota composition.

Results: The constructs specifically bound to F4 fimbriae (construct BL1.2) and LT toxin (construct BL2.2) without affecting ETEC strain growth. Oral administration of the constructs to ETEC F4 challenged piglets reduced ETEC F4 and LT toxin fecal shedding. Challenge-related microbiota perturbation was reduced in BL1.2 + BL2.2 and BL2.2 piglets compared to control piglets. The BL1.2 + BL2.2 piglets had higher *Prevotellaceae*, *Lactobacillaceae*, and *Ruminococcaceae* abundances compared to control piglets, largely attributable to the genera *Prevotella* and *Lactobacillus*. The BL2.2 piglets had higher *Lachnospiraceae* family abundance compared to control piglets.

Conclusions: The results indicate that the novel V_HH constructs stabilize the piglet gut microbiota during the critical weaning transition and stimulate potentially beneficial bacteria.

Disclosure: The results presented in this abstract have been obtained in a research collaboration project currently running. In this project, we aim at investigating the ability of bivalent heavy-chain variable domain (V_HH) constructs (binding proteins) to reduce the

Keywords: Gut microbiota, antimicrobial resistance, Pigs, Post-weaning diarrhoea, V_HH constructs

119 / #410

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

EFFECT OF MATERNAL DIET ON THE MICROBIAL COMMUNITY OF OFFSPRINGS

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Background and Aims: In this pilot study we have analyzed the microbiome profile of adult and newborn dogs in a half year period. The major aim of our study was to analyze the effect of maternal carbohydrate (CH) intake on the bacterial and viral community of puppies.

Methods: We have examined 19 healthy Pumi dogs (a Hungarian herding dog, about 200 samples, 10 samples per dog). Three adults and 16 puppies were involved. 16 puppies are from three litters. Two litters are from the same mother. DNA samples were isolated using the Macherey-Nagel Nucleospin DNA Stool Kit. High-quality DNAs were used for library preparation. We have carried out long-read sequencing with the aim to span the entire hypervariable region (V1-V9) of bacterial 16S rRNA gene for the better classification of the bacterial composition of samples. For this, we used the Oxford Nanopore Technologies MinION platform. Illumina DNA Prep libraries were also run on a MiSeq instrument for shallow shotgun sequencing (analysis of virome, bacteria and other pro-and eukaryotic cells within the samples).

Results: Our data shown that the maternal diet (commercially available food with higher CH content vs. raw meat diet) has an effect on the most abundant bacteria genera, especially on Blautia, Escherichia and Peptacetobater.

Conclusions: Maternal diet has an impact on the microbial composition of the newborn puppies.

Disclosure: No significant relationships.

Keywords: metagenomics, Canis gut, nanopore, 16S sequencing, Whole genome sequencing

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

MICROBIOME TRACKING ALONG THE PROCESS CHAIN OF FERMENTED SAUSAGES

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Background and Aims: The food microbiome derives from the interaction of microorganisms from primary production, raw materials, operators, environment, and production systems. Hence, the food chain may be considered an important way of antimicrobial resistance genes ARGs transmission from animal to humans. The processing systems of fermented meat are considered one of the main sources of ARGs. The aim of this work was to investigate the dynamics of the microbiome as well as the incidence of ARGs during the production chain of a spontaneous meat fermentation from farm to fork.

Methods: Shotgun metagenomic analysis was performed on samples collected from swine feces, feed, pork carcasses and fermented sausages. Thirty healthy omnivorous volunteers were then enrolled. The subjects were to consume about 40 g of fermented sausages daily for seven days. Stool samples were used for shotgun sequencing and for classical microbiological analysis.

Results: Metagenomic analysis showed a transfer of lactic acid bacteria from the chain through the gastrointestinal tract of volunteers. This result was confirmed by the culturomics approach. ARGs analysis showed the transfer of genes belonging to beta-lactam, tetracycline and phenicol/quinolone class carried by plasmids from meat to humans belonging to *Enterococcus faecium*, *Lactobacillus johnsonii* and *Limosilactobacillus reuteri*. In addition, flanking region analysis showed how the food chain and the process environment is responsible for the ARGs transfer.

Conclusions: The metagenomic sequence data generated in this study allowed to identify the food stages and sources with the greatest impact on the persistence and/or reduction of resistant populations in the production chain of fermented meat sausages.

Disclosure: No significant relationships.

Keywords: fermented food, human microbiome, Microbiome, ARGs, Resistome

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

ADHESION OF BACTEROIDES VULGATUS TO THE COLONIC MUCOSA OF HEALTHY BEAGLES- AN EX-VIVO STUDY

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Background and Aims: In colitis, obligate anaerobes are likely to disappear under an inflammatory environment of the colon due to increased oxidative stress. Adherence to the colonic mucosa is a crucial step for obligate anaerobe bacteria to colonize and interact with the host epithelium and the immune system. In dogs with steroid responsive enteropathies, *Bacteroidetes phylum* was reported to decrease and eventually to reach abundances comparable to the healthy controls after one year from treatment. There are no studies in dogs investigating adhesion of *Bacteriodes vulgatus* to paraffin-embedded canine colonic mucosa. Therefore, in this study we aimed to investigate the adhesion capacities of *B. vulgatus*, an anaerobic bacterial strain, and also to determine its hydrophobicity properties.

Methods: After isolation of canine *B. vulgatus* DF28 from feces of a healthy Beagle dog, the strain was cultured in fastidious anaerobe growth media, labeled with Alexa Fluor dye, and placed on slides of colonic mucosa from six healthy Beagle dogs.

Results: The results showed that *B. vulgatus* DF28 adhered well to the canine colonic mucosa at both bacterial concentrations of OD600 = 0.5 (899 [834–958] bacteria per field) and OD600 = 1 (1617 [1329–1805] bacteria per field). The hydrophobicity properties of *B. vulgatus* DF28 were at medium level (46%).

Conclusions: In this study, we successfully used paraffin-embedded dog colonic sections to investigate the adhesion properties of *B. vulgatus* DF28. Further studies are needed to determine the efficacy and safety of this strain to be used as a potential probiotic.

Disclosure: Mirja Huhtinen, Yannes S. Sclivagnotis, and Ulrike Lyhs are employed by Orion Corporation, Espoo, Finland. This research was funded by Orion Corporation, Espoo, Finland.

Keywords: dogs, Colonic mucosa, Adhesion, Bacteroides vulgatus

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

WILD-DERIVED MOUSE GUT MICROBIOME TRANSPLANTATION IN LABORATORY MICE ALLEVIATES ALLERGIC AIRWAY INFLAMMATION

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Background and Aims: Laboratory SPF mice are instrumental to model human diseases and find new treatments, but they may not always capture the grit of real life. There are serious concern that their clean standardized environment result in poor bench-to-bedside translation. Mouse models suffer from an immature immune system, possible due to a low-diversity gut microbiome. The aim of the study was therefore to test the importance of the gut microbiota in wild vs. SPF mice for host immune response in an allergic airway inflammation model.

Methods: Germ-free BALB/c were transplanted with fecal microbiota from either SPF or wild-captured mice, and their offspring were exposed nasally with house-dust mites to induce allergic airway inflammation. We examined effects on lung histopathology, immune responses in the gut, lung and serum, gut barrier integrity, and cecal metabolite profile.

Results: The wild microbiome reduced lung inflammation and decreased levels of pro-inflammatory cytokines in the recipient mice compared to the microbiome from SPF mice. Ileal expression of *gata3* was significantly lower and gut barrier-related genes higher in the wild-recipient mice. Intestinal microbiome and metabolomics analysis revealed distinct profiles associated with the wild-derived microbiome.

Conclusions: The wild mouse microbiome improved gut barrier integrity and reduced the sensitivity to house-dust mite-induced allergic airway inflammation compared to the microbiome from SPF mice. Preclinical studies using this model should, thus, consider using both dirty 're-wilded' and SPF mice for testing new therapeutic compounds, due to significant effects of their respective microbiomes and derived metabolites on host immune responses.

Disclosure: No significant relationships.

Keywords: Wild microbiome, animal models, dirty mice, allergic asthma, gut barrier

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

IDENTIFICATION OF ANTIBIOTIC RESISTANCE GENES IN SEWAGE OF IASI MUNICIPAL WASTEWATER TREATMENT PLANT USING BIOINFORMATIC TOOLS

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Background and Aims: Pharmaceuticals are being released into the environment predominantly within wastewater treatment plants (WWTPs), and their presence in sewage create conditions for selection of antibiotic resistance genes (ARGs) through selective pressure and horizontal gene transfer. This complex phenomenon represents a major concern due to public health risk, and there is limited knowledge about it in Romania. The present study aimed to investigate antibiotic resistance in the influent of Iasi municipal WWTP. Bioinformatic tools were used assess the incidence of ARGs and resistance bacteria (ARB).

Methods: DNeasy UltraClean Microbial kit (Qiagen) was used to extract metagenomic DNA. Using manufacturer's recommendations, TruSeq DNA PCR-Free kit was involved in creating the sequencing libraries. Paired-end sequencing was achieved with Illumina NovaSeq 6000 instrument. Metagenomic data were assessed with HOME-BIO tool. Analysis of antibacterial resistance genes was realized using the Resistance Gene Identifier software against the Comprehensive Antibiotic Resistance Database.

Results: The analyzed samples revealed the presence of bacteria as the dominant group, while archaea and viruses were less prevalent (< 2% abundance). The best represented phyla were Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria and Verrucomicrobia. *Pseudomonadaceae*, *Comamonadaceae*, *Moraxellaceae* and *Campylobacteraceae* appeared to be the prevalent families. *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecium*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* were found to be the most frequent human bacterial pathogens. Several ARGs were highlighted, but those associated with resistance to aminoglycosides, β -lactam antibiotics and tetracyclines were detected frequently.

Conclusions: Through metagenomic analysis we detected the presence of ARGs and pathogenic bacteria in WWTP, with important impact for public health.

Disclosure: No significant relationships.

Keywords: Antibiotic Resistance Genes, Antibiotic Resistant Bacteria, Pathogenic bacteria, Wastewater Treatment Plants

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

THE IMPACT OF DIETARY FACTORS ON THE FECAL MICROBIOTA OF CARNIVORES AND HERBIVORES

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Background and Aims: The composition and fluctuations of gut microbiota is crucial for animal health and welfare. Therefore, assessing the species-specific impact factors of microbial fluctuations such as diet may be a vital tool in successful animal husbandry.

Methods: 96 fecal samples of 12 individuals out of 4 species (*Connorchates taurinus* [herbivore, ruminant], *Equus quagga/zebra* [herbivore, hind-gut fermenter], *Ursus arctos* [omnivore], *Panthera tigris* [carnivore]) were assessed in this study. Following sample collection and DNA extraction, the analysis was performed using Illumina MiSeq 16 S rRNA sequencing and the microbiome research platform Qiime2. Here a multivariate response linear regression analysis was performed to reveal potential impacts of specific food items on the host animal's gut microbiota.

Results: The results demonstrated that specific food items were highly influential on the microbiome. In the case of the carnivorous species *Panthera tigris*, the most impactful food item was a bunny rabbit, with the bacterial taxa Lachnospiraceae and Clostridiaceae being the most affected. For the herbivorous species *Connorchates taurinus* and *Equus quagga/zebra* the presence of carrots in their diet showed the most impact, with Lachnospiraceae and Rikkenelaceae (*Connochaetes taurinus*) and Lachnospiraceae and Spirochaetaceae (*Equua quagga/zebra*) being most impacted.

Conclusions: The results suggest that species-specific food items impact and individuals gut microbiome and hence may impact their host's health.

Disclosure: No significant relationships.

Keywords: 16 S rRNA sequencing, diet, Zoobiology, Gut microbiota

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

ASSESSING THE BIOGAS POTENTIAL OF VARIOUS INDUSTRIAL WASTEWATERS: A STUDY ON CHARACTERISTICS AND MICROBIAL COMMUNITY STRUCTURE

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Background and Aims: Industrial wastewaters have great potential for biogas production due to their large generation/discharging amounts and low cost, making them an attractive resource for waste-to-energy conversion processes. However, the biodegradability of these wastewaters depends heavily on their characteristics, such as organic and inorganic composition.

Methods: To investigate the biogas potential of various industrial wastewaters, we collected 25 samples from 19 sites, including manufacturers of ramen, sauce, dairy products, soda, liquor, medicine, paper, silk, and a metal-cutting factory. The characteristics of each wastewater, such as solids, COD, pH, alkalinity, and elemental analysis, were analyzed, and biochemical methane potential (BMP) tests were performed. At the end of the BMP tests, DNA was extracted for 16S rRNA amplicon sequencing.

Results: The composition of the wastewater, BMP results, and microbial community structure analysis were then tested for correlations.

Conclusions: The results of this research will provide valuable insights into the biogas potential of different types of industrial wastewaters and the factors that influence their biodegradability.

Disclosure: No significant relationships.

Keywords: biochemical methane potential, industrial effluent, amplicon sequencing, anaerobic digestion

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

SHAPING THE SUBWAY MICROBIOME THROUGH PROBIOTIC-BASED SANITATION DURING THE COVID-19 EMERGENCY: A PRE-POST CASE-CONTROL STUDY

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Background and Aims: The COVID-19 pandemic has highlighted the impact of public transportation, such as subways, or the transmission of potential pathogenic microbes among humans. Consistent with this, massive chemical disinfection was mandatorily introduced during the emergency and remain in place. However, chemical disinfectants show temporary action, high environmental impact, and potentially enhancing action on antimicrobial resistance (AMR) of the treated microbes. Instead, eco-sustainable probiotic-based sanitation (PBS) was recently shown to stably shape the microbiome of treated environments, providing long-term reduction of pathogens (including SARS-CoV-2) and AMR. Our study aimed to assess the applicability and impact of PBS on the microbiome of the subway environment.

Methods: The PBS effect, compared to disinfectants, was evaluated in two trains, one receiving chemical disinfection and the other sanitized by PBS. The surface and air train microbiome and its resistome were characterized by culture-based methods, 16S rRNA NGS, and real-time qPCR microarray. SARS-CoV-2 presence was also assessed using digital droplet PCR.

Results: The results showed a clear and significant decrease in bacterial/fungal pathogens ($p < 0.001$) and of SARS-CoV-2 ($p < 0.01$), in the PBS-treated train compared with the chemically disinfected control train. NGS profiling evidenced diverse clusters in the population of air vs. surface and demonstrated the specific action of PBS against pathogens rather than the entire bacteriome.

Conclusions: Collected data provide the first direct assessment of the impact of different sanitation procedures on the subway microbiome, allowing a better understanding of its composition and dynamics and showing that the PBS approach may be highly effective in counteracting pathogens and AMR spread in our increasingly urbanized and interconnected environment.

Disclosure: No significant relationships.

Keywords: subway microbiome, probiotic sanitation, disinfection, COVID-19

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

IS FEEDING BEHAVIOR DETERMINE GUT BACTERIA IN DESERT INVERTEBRATES ?

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Background and Aims: Digestive system in last years had became the hart of many studies. The relationships between the host and its gut microbiota range across the entire spectrum of interactions, i.e., from pathogenic to obligate mutualism The gut environment is considered to be an unstable system due to its multifunctional goals and its response to changes in its surrounding niches, abiotic physical disturbance and other physiochemical conditions that are typically unfavorable for colonization. The objective of the present study was to identify the bacterial community in the digestive system of common desert invertebrates that share the same habitat. The present study elucidates the importance of feeding habitat and trophic structure to the microbiotic diversity of the digestive system

Methods: DNA extraction from the digestive systme of each organism was conducted followed by amplication of 16s rRNA gene of bacterial sequences.

Results: A total of 117,029 high-quality sequence reads were identified as belonging to the bacteria domain Each invertebrate sample had average reads as follows: (1) *S. palmatus* (1976); (2) *A. dilitata* (2060); (3) *S. zonata* (2880); and (4) *H. Reaumuri* (4355). The total number of OTUs was 643. The data was analysed on phylum level in each one of the invertebrates.

Conclusions: Nutrition affects the longevity and successful performance of all biological functions of these desert-xeric invertebrates, which face extreme and unpredictable abiotic conditions, such as food source heterogeneity. Our hope is that this study will serve as a basis for future studies on the gut microbiomes of desert-xeric invertebrates.

Disclosure: No conflict of interest by any author of this abstract

Keywords: invertebrates' 'desert' 'ecosystem' ' digestive system', invertebrates microbiome, 'invertebrate microbiome'

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

COMPARING INVASIVE AND NONINVASIVE FECAL SAMPLING IN WILDLIFE MICROBIOME STUDIES: A CASE STUDY ON WILD COMMON CRANES

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Background and Aims: In ecological and conservation studies, responsible researchers strive to obtain rich data while minimizing disturbance to wildlife and ecosystems.

Methods: We assessed if samples collected noninvasively, in this case, from common cranes (*Grus grus*), can be used for fecal microbiome research, comparing microbiota of noninvasively collected fecal samples to those collected from trapped common cranes at the same sites over the same period.

Results: We found significant differences in fecal microbial composition (alpha and beta diversity), which likely did not result from noninvasive samples' exposure to soil contaminants, as assessed by comparing bacterial oxygen use profiles. Differences might result from trapped birds' exposure to sedatives or stress. We conclude that if all samples are collected in the same manner, comparative analyses are valid, and noninvasive sampling may better represent host fecal microbiota because there are no trapping effects. To illustrate this point, combining movement data from GPS-tagged cranes with noninvasively sampled fecal microbiota samples of birds spatiotemporally overlapping with the tagged birds, we found evidence that supports the role of diet in structuring bacterial communities. We also showed that the wild bird gut microbiota undergoes not only seasonal shifts but is also affected by management schemes and local agricultural practices.

Conclusions: Experiments with fresh and delayed sample collection can elucidate effects of environmental exposures on microbiota. Further, controlled tests of stressing or sedation may unravel how trapping affects wildlife microbiota, but our application already shows how valuable this method can be in ecological and conservation studies.

Disclosure: No significant relationships.

Keywords: management, Microbiome, non-invasive sampling, wild animals, conservation

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

MICROBIAL ECOLOGY OF THE FELINE ORAL CAVITY CHANGES BY SEVERITY OF DISEASE

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Background and Aims: Aggressive periodontitis is difficult to treat and can be devastating, resulting in discomfort and early tooth loss. The etiology of this disease remains unknown and patient response to treatment is difficult to predict. Given the known importance of microorganisms in oral diseases, it is likely there is a complex microbiome component that is not understood.

Methods: We selected felines as a model system because human and feline genomes are >90% similar and dental structures as well as clinical manifestations of disease share similarity. To assess the impact of the microbiome, buccal mucosal swabs from 41 cats (n=9 healthy, n=17 aggressive periodontitis, n=7 chronic periodontitis, n=7 feline chronic gingivostomatitis) were collected. Total RNA was extracted, sequenced, and taxonomic distribution was evaluated using multivariate metatranscriptomic analysis.

Results: Microbiome diversity was increased in healthy patients compared to those with oral disease, which displayed similar diversity metrics across disease groups. Healthy and early gingivitis samples had higher relative abundances of *Moraxella* sp. and *Pyschrobacter* sp. compared to chronic gingivitis samples, which had higher relative abundance of *Ureaplasma* sp. Stratification of disease groups by microbial taxonomic clustering revealed oral diseases were not homogeneous and suggests oral microbial ecology may serve as a biomarker for diagnosis of progressive disease.

Conclusions: Treatment effectiveness via disease markers and causative organisms aligned with host response to treatment. Community structure and prevalence changed with treatment and ultimate outcome. This model aligns with what is known in human disease and can be used to understand oral diseases and the microbiome structure in multiple species.

Disclosure: No significant relationships.

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

EXPLORING THE GUT JUNGLE: ILLUMINATING MICROBIOME VARIABILITY AND IDENTIFYING INDIVIDUALS THROUGH TIME-SERIES CLUSTER ANALYSIS

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Background and Aims: In animal microbiome research, there is still uncertainty about the microbiota variability of different animal orders and the influence of diurnal variation. This is largely due to the fact that most large-scale comparative studies are based on only a few samples per species or per study site. We aim to address this knowledge gap by analysing multiple samples from carnivore and herbivore species. We also aim to develop a method to identify unknown individuals based on the microbiota composition to facilitate field studies.

Methods: We analysed 621 faecal samples from 31 species by sequencing the V3-V4 region of the 16S rRNA gene using Illumina MiSeq. For individual discrimination, we developed a pipeline including two agglomerative hierarchical clustering algorithms - a community detection algorithm and Ward's linkage with and without prior dynamic time warping.

Results: Our study shows that the microbiota of herbivore species is very similar even within individuals. In contrast, the microbial composition of carnivores is highly variable and inconsistent between species and within individuals. Nevertheless, individual-specific clustering is possible if dynamic time warping is used prior to clustering. Individuals could be distinguished by a combination of individual-dependent and core bacterial families, e.g. Acidaminococcaceae in wildebeest or Clostridiaceae in tigers.

Conclusions: The need for time series analysis has methodological implications and highlights the need to analyse at least 8 samples per individual to capture microbiome oscillations, particularly in carnivores. Individual microbiota profiles are vital to quickly detect deviations and establish microbiome analysis as a non-invasive tool in animal welfare.

Disclosure: No significant relationships.

Keywords: abundance pattern, herbivores, carnivores, 16S rRNA gene, cluster analysis

E-POSTER VIEWING - AS12. OTHER

THE KOMBUCHA SECRET - SCOPY'S MICROBIOME STUDY AND HEALTH PROMOTING BACTERIA IDENTIFICATION

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Background and Aims: Kombucha is a fermented tea-based drink produced by a Symbiotic Culture of Bacteria and Yeast (SCOPY). In recent years, it has gained popularity due to its nutritional and potential health benefits, pleasant taste and sparkling due to the release of carbon dioxide produced during fermentation. Despite some studies characterizing the microbial community present in kombucha, there are limited exploratory studies of the microbiota in the SCOPY.

Methods: To address this gap, DNA was extracted and the microbiota (Bacterial and Fungi) in scopy and kombucha was characterized. For this, a metabarcoding assay by long-read Next Generation Sequencing (Oxford nanopore Technologies) was performed in order to evaluate the community of microorganisms present in the starter culture (SCOPY) and the resulting kombucha.

Results: Our findings revealed the presence of bacteria that have previously been linked to positive health effects, particularly in alleviating alcohol-induced liver injury. Additionally, we found that most species in the SCOPY are involved in cellulose production, which may have potential applications in the development of bio-based packaging.

Conclusions: This is the first step to characterize, explore and to understand how microbes interact and evolve during the production of this beverage.

Disclosure: No significant relationships.

Keywords: Next Generation Sequencing, Oxford Nanopore Technologies, Microbiome, Kombucha, SCOPY

E-POSTER VIEWING - AS12. OTHER

DESIGN AND FABRICATION OF A NOVEL MICRO-DIALYSER FOR INVESTIGATING THE GUT MICROBIOTA-TISSUES CROSSTALK

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Background and Aims: The human gut microbiota (HGM) significantly impacts the host's physiopathology: their symbiotic interaction has considerable effects both locally and systemically, and abnormal HGM composition is associated with numerous disorders and diseases. *In vitro* culture models represent powerful tools for studying complex gut microbial communities and investigating their crosstalk with the host. However, the eukaryotic cells-HGM co-culture is still challenging. Indeed, direct exposure to microbes could cause cell infections or death, as microbial metabolites may have cytotoxic effects; moreover, cells and microbes usually require different culture conditions. Accordingly, this work presents a novel micro-dialysis system that can be used in co-culture microfluidic platforms for enabling the chemical crosstalk between eukaryotic cells and microbes and investigating host-HGM interactions without allowing their physical contact.

Methods: The device consists of an upper part, in which the supernatant from HGM *in vitro* cultures flows, a lower part, in which the dialysed fluid flows, and an interposed dialysing membrane. Different geometries of the micro-dialysis circuit were designed and tested.

Results: The device provided excellent hydraulic sealing for 2-5 ml/min fluid flows for at least 5 days, and both numerical simulations and dynamic experiments with fluorescent markers assessed the optimal dialysing ability of the system.

Conclusions: This device represents a promising tool to evaluate the indirect effects of the HGM on eukaryotic cells, by exposing them to the dialysed supernatants from HGM *in vitro* cultures. Moreover, the system could integrate a specifically designed culture chamber to perform the dynamic *in vitro* culture of the HGM and the dialysis process simultaneously.

Disclosure: No significant relationships.

Keywords: Human gut microbiota, Mathematical models, Microfluidics, Micro-dialysis

E-POSTER VIEWING - AS12. OTHER

DNA BARCODING CONFIRMED PRESENCE OF CELL WALL DEFICIENT VARIANTS OF CANDIDA PARAPSILOSIS IN BLOOD OF AUTISTIC CHILDREN AND THEIR MOTHERS

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Background and Aims: In our previous article, we showed that microorganisms can persist as cell wall deficient (CWD) variants in the blood of children with autism spectrum disorder (ASD) and their mothers (Markova, Sci Rep 2019; 9:13401). In this study, the majority of isolated yeast strains were identified as *Candida parapsilosis* using MALDI-TOF MS. The latter has serious limitations. First, it cannot discriminate reliably cryptic yeast species. Second, it is critical in MALDI-TOF MS to include a sufficient number of yeast strains grown under various conditions so that the database is robust enough to account for the species phenotypic variability. This is, particularly, important for the *C. parapsilosis* complex that has limited protein sequence variability, and at the same time, they express a broad spectrum of phenotypes at different growth conditions. Therefore, in the present study identity of isolated yeast was confirmed using DNA barcoding – sequence analysis of the D1/D2 region of ribosomal 26S rRNA gene (LSU) and internal transcribed spacer region (ITS).

Methods: DNA barcoding analysis was done as described previously (Gouliamova et al. Fungal Biology 2016; 120:179-190)

Results: DNA barcoding analysis confirmed the presence of *C. parapsilosis* in six blood samples and one urine sample of children with ASD and in the blood samples of three mothers.

Conclusions: Our study proved that yeast *C. parapsilosis* can persist in human blood as a CWD variants.

Disclosure: The authors have no conflict of interests to declare

Keywords: Yeast pathogens, Autism spectrum disorder, Cell wall deficient variants, DNA barcoding

E-POSTER VIEWING - AS12. OTHER

OPTIMIZING CONDITIONS FOR IN VITRO MODELING OF THE HUMAN GUT MICROBIOME TO STUDY INTESTINAL HEALTH AND DISEASE

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Background and Aims: In-vitro models of complex microbiomes could advance clinical microbiome research, but such models are sparsely described and challenging to apply to date. Here we present potential solutions to crucial challenges (sample storage and feeding strategies) implemented in our in-vitro microbiome model based on fresh human stool samples.

Methods: Stool samples were collected using an anaerobic microbiome collection kit (GutAlive®, MicroViable therapeutics, Spain) and stored for up to 48 hours. Faecal slurries were cultured in a DASbox® mini bioreactor system (Eppendorf, Germany) for five days with either no-feeding, batch, or continuous feeding. Microbial communities were analysed via 16S rRNA gene sequencing.

Results: Sample storage for up to two days did not significantly alter the microbial community structure. Although significant decline in alpha diversity and changes in beta diversity throughout the experiment were evident, storage time did not influence these developments. Interestingly, the initial composition of the stool sample could be rescued by reducing the feeding frequency. “Fasted” cultures retained most of their alpha diversity as well as community structure throughout the experiment, followed by batch feeding once per day and continuous feeding, which was most detrimental to microbial diversity.

Conclusions: Stool samples for in-vitro microbiome models can be anaerobically stored for up to two days. The amount of nutrients supplied to the cultures modulates the microbiome in culture, whereby nutrient restriction preserved diversity. This finding indicates that our platform might be a potential model for intermittent fasting or obesity settings.

Disclosure: No significant relationships.

Keywords: Gut Microbiome, in vitro model, DASbox, bioreactors

E-POSTER VIEWING - AS12. OTHER

ISOLATION AND IDENTIFICATION OF THREE STRAINS OF LIMOSILACTOBACILLUS REUTERI ORIGINATED FROM HUMAN FAECES AND THEIR POTENTIAL AS PROBIOTICS

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Background and Aims: In the present study, three strains of *Limosilactobacillus reuteri* isolated from infant faeces were identified based on their 16S rDNA sequences and further characterized.

Methods: Firstly, the safety aspects, such as antibiotic susceptibility, haemolytic and enzymatic activities were determined. Subsequently, their antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Salmonella enterica* Typhimurium, hydrophobicity, antioxidant activity, aggregation ability, and adhesion to human adenocarcinoma cell lines Caco-2 and HT-29 were evaluated.

Results: Isolates were sensitive to the most of the clinically important antibiotics and none of them showed haemolytic activity and production of harmful enzymes. Acidic supernatants of the tested *Limosilactobacillus reuteri* 21S2A and 21S2C inhibited the growth of all the pathogens tested, reaching about 80-90 % in contrast to the strain *Limosilactobacillus reuteri* 21S8A, which was only approx. half as efficient (40-50%) in inhibiting the pathogens tested. The isolates 21S2A and 2C did not show strong hydrophobic properties, as the adhesion values to the solvent n-hexadecane, did not exceed 20 %. Based on this, their surface was rather hydrophilic, on the contrary isolate 21S8A was hydrophobic (67 %). The ability to adhere to the Caco2 and HT29 cells made 68-78 % and aggregation ability made about 45 % after 24 h of cultivation.

Conclusions: According to the presented results, the *Limosilactobacillus reuteri* strains isolated from infant faeces possess interesting probiotic properties that make them potential candidates for probiotics. This research was funded by the Ministry of Agriculture of the Czech Republic, Institutional support, No. MZE-RO1423 and project no. QK 1910024.

Disclosure: No significant relationships.

Keywords: *Limosilactobacillus reuteri*, Probiotics, human faeces, antimicrobial activity, adherence

E-POSTER VIEWING - AS12. OTHER

A COMPREHENSIVE COMPARISON OF TECHNICAL METHODS FOR GUT MICROBIOME ANALYSIS

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Background and Aims: The background of our research highlights the importance of the gut microbiome and the need for careful consideration of methods for accurate metagenomic analysis. The aims of the study were to compare commonly used methods for microbiome analysis, validate results, and present an optimized methodology for accurate analysis of the gut microbiome.

Methods: The study compared commonly used methods for DNA isolation, sequencing, and bioinformatics of faecal metagenomic DNA from the gut microbiome. The methods compared included DNA isolation kits, sequencing platforms, library preparation methods, the variable region of the 16S rRNA gene, and the data analysis software and database used for taxonomic annotation. A commercially available microbial community mixture was also used to validate the results obtained.

Results: Our results present an optimized experimental methodology, as well as a thorough wet-lab and bioinformatics pipeline for the accurate analysis of the gut microbiome.

Conclusions: Multiple protocols and methods exist for the analysis of microbial DNA, careful considerations regarding the methods at each step of the workflow is necessary to obtain accurate results. These considerations include selecting an appropriate DNA isolation kit, sequencing platform (long- or short-read), library preparation method (amplicon or shotgun), the variable region of the 16S rRNA gene (in case of 16S amplicon-sequencing), and the utilized data analysis software and database for taxonomic annotation. Overall, the methods chosen for the microbiome analysis can significantly influence the results obtained, and thus inappropriate methods can give unreliable results.

Disclosure: No significant relationships.

Keywords: Sequencing, Gut Microbiome, 16s rRNA sequencing, bioinformatics

E-POSTER VIEWING - AS12. OTHER

ALTERATIONS IN THE GUT MICROBIOTA IN OVERWEIGHT SCHOOL-AGED CHILDREN IN BULGARIA

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Background and Aims: Nowadays, obesity is one of the most prominent and widespread human health problems. Recently, it has been shown that gut microbiota is involved in the regulation of multiple host metabolic pathways, which might play an important role in obesity development, but its significance in children is not clearly elucidated. The goal of our study was to quantify the bacterial groups: Eubacterium rectale group (EU), Bifidobacterium spp (BIF) and Bacteroides spp (BAC), also to determine calprotectin and leptin levels in overweight and normal-weight children aged 4-17 years.

Methods: Groups of 47 overweight children and 29 controls were tested with qPCR kit for EU, BIF and BAC. Fecal calprotectin and serum leptin levels were analyzed by ELISA. Additionally, in a define subgroup of obese children (BMI>30), fecal Akkermansia muciniphila (AKM) and Faecalibacterium prausnitzii (FAEP), were quantified.

Results: We observed significantly lower amount of BAC and FAEP in obese children vs. control group. Moreover, AKM was not detected in 53% of obese children. Calprotectin and leptin had significantly higher levels in the obese group vs. controls. There was no significant difference in the relative abundance of gut bacteria (EU, BAC, BIF) in the target group, compared to controls. Serum leptin levels were significantly higher in overweight and obese children vs. controls.

Conclusions: Our study, shows a distinct profile of gut dysbiosis, moderate intestinal inflammation and increased production of leptin in obese children. Further investigations are needed to identify the precise role of gut microbiota in the childhood obesity in order to define effective prevention.

Disclosure: No significant relationships.

Keyword: gut microbiota, obesity, overweight, children, leptin

E-POSTER VIEWING - AS12. OTHER

THE GUT MICROBIOME-FIRST STEPS IN PERSONALIZED MEDICINE

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Background and Aims: Individual differences in each person's microbiome make its examination a new and promising criterion for assessing patients' condition in the context of their comorbidities and lifestyle. The aim of our study was to investigate the gut microbiome of patients referred for obesity by an endocrinologist and patients referred for gastrointestinal and immunological disorders by other medical professionals.

Methods: Correlations were determined between the obtained amounts and ratios of the studied bacterial species and genera with body mass index, chronic diseases, medication intake, diet and intestinal status of the individuals. Multiplex Real Time PCR was used to simultaneously quantify *Bacteroides* spp., *Eubacterium rectale*, *Bifidobacterium* spp., *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, *Ruminococcus* spp., *Prevotella* spp. and *Lachnospiraceae* spp. to study the gut microbiome.

Results: When a disturbance in the ratio and dominance of some of the bacterial species and genera in the microbiome was detected, the patients were given recommendations for the intake of certain foods, supplements, probiotics and/or antibiotics, and a specialist - endocrinologist, nutritionist and/or gastroenterologist - was consulted.

Conclusions: Thus, the gut microbiome analysis was included as a personalized study to assist in the diagnosis and therapy of patients with obesity, food intolerances and bowel diseases. In this study, the first steps were taken towards introducing into routine clinical practice the currently known scientific information on the microbiome, the factors that influence its formation and its relationship to a range of diseases.

Disclosure: No significant relationships.

Keywords: Obesity, Mutaplex PCR, Personalized medicine, Gut Microbiome

E-POSTER VIEWING - AS12. OTHER

A 9-STRAIN BACTERIAL CONSORTIUM IMPROVES PORTAL HYPERTENSION AND INSULIN SIGNALING AND DELAYS NAFLD PROGRESSION IN VIVO

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Background and Aims: The gut microbiome has a recognized role in NAFLD and associated comorbidities such as Type-2 diabetes and obesity. Fecal transfer of intestinal microbes has been shown to improve disease by restoring endothelial function and insulin signaling. However, more patient-friendly treatments are required. The present study aimed testing the effect of a defined bacterial consortium of nine gut commensal strains on two in vivo rodent models of NASH: a rat model of NASH and portal hypertension (PHT), and the STAM™ mouse model.

Methods: In both studies the consortium was administered orally qd after disease induction. In the NASH rats, the consortium was administered for 2 weeks and compared to stool transplant. In the STAM™ study administration was performed for 4 weeks, and effects compared to vehicle or Telmisartan at the stage of NASH/early fibrosis. A second group of animals was followed for another 3 weeks to assess later-stage fibrosis.

Results: In the NASH rats, an improvement in PHT and endothelial function was observed. Gut microbial compositional changes also revealed that the consortium achieved a more defined and richer replacement of the gut microbiome than stool transplantation. Moreover, liver transcriptomics suggested a beneficial modulation of pro-fibrogenic pathways. An improvement in liver fibrosis was then confirmed in the STAM™ study. In this study, the bacterial consortium improved the NAFLD activity score, consistent with a decrease in steatosis and ballooning. Serum cytokeratin-18 levels were also reduced.

Conclusions: Administration of a specific bacterial consortium of defined composition can ameliorate NASH, PHT and fibrosis and delay disease progression.

Disclosure: I.P., M.L., S.B., and S.P. are employees of MRM Health, which produces and develops the nine-strain bacterial consortium discussed in this study. D.G. and N.H. were employees of MRM Health at the time this project initiated.

Keywords: Fibrosis, NAFLD, Gut Microbiome, Bacterial consortium, Portal hypertension

E-POSTER VIEWING - AS12. OTHER

SAUERKRAUT SUPPLEMENTATION AND THE ATHLETE GUT MICROBIOTA: PRELIMINARY RESULTS OF A PROSPECTIVE COHORT STUDY

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Background and Aims: The optimization of athlete's gut microbiota is an emerging field of research. This is one of the first studies conducted using sauerkraut as a probiotic-rich fermented food, in healthy elite athletes.

Methods: Participants (n=3, males=2, age range 25-35 years) were provided with sauerkraut (250 g/day) for 10 days and were instructed not to modify their usual diet and training regime. Compliance was monitored using dietary and lifestyle records. On Days 0, 5 and 11, stool samples were obtained using the Intest.pro test kit (BIOMES NGS GmbH, Germany) and 16s rDNA Seq was performed.

Results: Pre-intervention average daily fiber intake was equal to $20,1 \pm 7,0$ g and increased by 3,2 g (a total of $23,3 \pm 12,3$ g) during intervention, which can be attributed to the sauerkraut intake. The overall balance of the gut microbiota improved in all three subjects. Significant changes were seen as early as Day 5: an increase in the relative abundances of butyrate-producing genus Lachnospiraceae UCG-008 and decrease of unspecific Bacteroidales and Clostridia UCG-014. Additionally, an increase in the reductive TCA cycle I pathway was noted.

Conclusions: Preliminary results indicate that sauerkraut supplementation in athletes' diet can positively impact their gut microbiota composition and functionality. Due to study limitations, it cannot be concluded whether these effects can be attributed to the increased intake of pro-/prebiotics or other phenomena within the microbial community. However, these results highlight the importance of the "food first" concept in sports nutrition and the need for further research on probiotic and prebiotic administration in athletes.

Disclosure: No significant relationships.

Keywords: Gut microbiota, Probiotics, Prebiotics, athletes, sauerkraut

E-POSTER VIEWING - AS12. OTHER

GUT RESISTOME IN CHRONIC PULMONARY OBSTRUCTIVE PULMONARY DISEASE

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Background and Aims: Antimicrobial resistance (AMR) is a global health threat, driven partly by suboptimal use of antibiotics. Chronic obstructive pulmonary disease (COPD) patients are often treated with antibiotics during exacerbations. We aimed to compare the gut resistome of COPD patients to that of controls, and to see if previous exacerbations influence the gut resistome.

Methods: Single-center case-control study was conducted using fecal samples from COPD patients (n=67) and controls (n=63). Whole genome sequencing was performed on an Illumina platform, followed by bioinformatic processing. Host contamination was removed. Counts normalized by effective gene length, and relative to the total number of high-quality non-host counts in the sample. The AMR encoding genes (ARGs) were annotated using the Comprehensive Antibiotic Resistance Database. The relative abundance of ARGs was compared between COPD and controls, as well as between COPD patients with and without exacerbations in the previous 12 months.

Results: On average 16.2 AMR classes were found per sample, with ARGs constituting a mean relative abundance of 0.17%. There was no statistically significant difference in the number of AMR classes or relative abundance of ARGs between COPD and controls. Among AMR classes, tetracycline resistance was increased in COPD (chi-squared 4.93, p=0.03). For COPD patients with exacerbations during the previous 12 months, we found higher mean relative abundance of ARGs (chi-squared 6.20, p=0.01), with significant differences in tetracycline (chi-squared 14.1, p<0.001) and multidrug efflux pumps (chi-squared 5.8, p=0.016).

Conclusions: Relative abundance of AMR counts for tetracycline was increased in COPD patients. Total AMR relative abundance was increased among exacerbators.

Disclosure: Unrestricted grants and fellowships from Helse Vest, Bergen Medical Research Foundation, the Endowment of Timber Merchant A. Delphin and Wife through the Norwegian Medical Association, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline and Novartis.

Keywords: Whole genome sequencing, Resistome, COPD, gut

E-POSTER VIEWING - AS12. OTHER

THE GUT MICROBIOME IN COPD VARIES BY EMPHYSEMA STATUS

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Background and Aims: The association of the gut microbiota to chronic obstructive pulmonary disease (COPD) phenotypes is underexplored. We aimed to compare stool samples from COPD patients and controls and relate findings to common COPD phenotypes.

Methods: Single-centre case-control study with current and former smoking COPD patients (n=64) and controls (n=49). Bacterial 16S-rRNA gene, V3V4-region, was extracted from stool samples and sequenced. Emphysema was defined based on thoracic computed tomography (CT thorax) low attenuating areas \geq / $<$ 10% at threshold -950 and -910 Hounsfield units respectively. Microbial data was analysed with QIIME 2 and R.

Results: The genus Veillonella was decreased and a genus belonging to class Clostridia was increased in COPD compared to healthy controls. Beta diversity measure Bray Curtis differed in emphysema compared to controls, and 27 genera were differentially abundant in emphysema vs. controls. Nine of these genera belonged to the family Lachnospiraceae. Lung function, blood counts and COPD assessment test score were correlated with the relative abundance of several genera. Some of the genera showing the strongest correlation to lung function belonged to the family Lachnospiraceae.

Conclusions: We found small but statistically significant differences in the gut microbiota of COPD versus controls. Larger differences were seen in the gut microbiota according to CT-verified emphysema status. Correlations between the gut microbiota and lung function, blood cell counts, and CAT score were found.

Disclosure: Funded by unrestricted grants and fellowships from Helse Vest, Bergen Medical Research Foundation, the Endowment of Timber Merchant A. Delphin and Wife through the Norwegian Medical Association, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline and Novar

Keywords: Lachnospiraceae, Microbiome, gut, COPD, emphysema

E-POSTER VIEWING - AS12. OTHER

IRON SUPPLEMENTATION SHAPES POST-ANTIBIOTIC RECOVERY OF GUT MICROBIOTA AND PROMOTES CARCINOGENESIS IN THE APCMIN/- MOUSE MODEL

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Background and Aims: Colorectal cancer (CRC) induces anemia in a large proportion of patients and is usually treated with oral iron supplementation. Surgery, the main treatment for CRC, is routinely accompanied by prophylactic antibiotics to avoid infection. However, the combined effect of antibiotics and iron supplementation on gut microbiota and cancer progression remains unknown.

Methods: To assess the effects of iron supplementation on gut microbiota and CRC promotion and progression, *Apc*^{Min/+} mice were subjected to antibiotics prior to receiving a fecal microbiota transplant (FMT) from anemic CRC patients or healthy volunteers and were then fed different concentrations of dietary iron. Gut microbiota was assessed using 16S rRNA sequencing and short chain fatty acids (SCFAs) levels by liquid-chromatography mass spectrometry. Tumor counts and grades were recorded.

Results: An increase in CRC burden and progression were found in *Apc*^{Min/+} mice receiving FMT from CRC patients and oral iron supplementation compared to mice receiving FMT from healthy volunteers. Analysis of the gut microbiota composition revealed a decrease of SCFAs producer bacteria induced by oral iron supplementation only in mice that received FMT from CRC patients. In line with these results, butyrate levels were significantly decreased.

Conclusions: This study identifies possible deleterious effects of iron supplementation after antibiotic-induced dysbiosis on gut microbiota and may lead to modifications in the management of anemia in patients with CRC when antibiotics treatments are necessary.

Disclosure: No significant relationships.

Keywords: microbiota, dysbiosis, iron, ANTIBIOTICS, colorectal cancer

E-POSTER VIEWING - AS12. OTHER

SEASONAL VARIATION AND INDOOR PARTICULATE MATTER EXPOSURE: EFFECTS ON THE ANTERIOR NARES MICROBIOTA IN HEALTHY SUBJECTS

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Background and Aims: The microbial communities inhabiting our nares are continuously affected by the external environment. Indeed, an adult breathes on average 6-7 liters of air per minute and the upper respiratory tract is an interface between the external environment and the rest of the human body. Thus, understanding the effects of air pollutants, such as particulate matter, on the respiratory microbiome is challenging.

Methods: We analyzed the effects of indoor particulate matter exposures on the nasal microbiota of a population of 34 healthy office workers at the University of Milan, Italy, during the winter and summer seasons. The total suspended particulate (TSP, aerodynamic diameter < 100 µm) exposure was monitored within the offices. The taxonomic analysis was performed by 16S rRNA gene sequencing and downstream analyses were carried out using QIIME2 v2022.8.

Results: The alpha diversity estimated with the Shannon Index was higher (p-value = 0.03) in the summer compared to the winter. The beta diversity was also different between the two seasons (PERMANOVA, Bray-Curtis p-value=0.001, UniFrac p-value=0.002), and the indoor TSP exposure explained the 2% of the observed variance (PERMANOVA, Bray-Curtis p-value=0.02). In both seasons, we identified a core of 27 genera, and the TSP exposure was negatively associated with *Neisseria* abundance (coef=-0.32; p-value=0.02; q-value=0.16) and positively associated with *Pseudomonas* abundance (coef=0.31; p-value= 0.02; q-value= 0.16).

Conclusions: The results obtained indicate that the nasal microbiome changes between summer and winter and particulate matter influence these modifications.

Disclosure: No significant relationships.

Keywords: airway microbiota, indoor particulate matter, office workers, seasonal variation

E-POSTER VIEWING - AS12. OTHER

RESUSCITATION OF BLOOD MICROBIOTA IN A SARCOIDOSIS PATIENT CONFIRMED BY TRANSMISSION ELECTRON MICROSCOPY AND 16S RDNA SEQUENCING

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Background and Aims: Sarcoidosis is a multisystem inflammatory disease characterized by the formation of non-caseating, epithelioid-cell granulomas in the affected organs. Despite numerous studies, the etiology of sarcoidosis remains a mystery, but microbial involvement is among the strongest hypotheses.

Methods: Blood, lung biopsy, and bronchoalveolar lavage (BAL) samples from a patient with lung sarcoidosis were analyzed. The blood sample was divided into two parts - for culturing (0.2 mL) and DNA isolation (3 mL). Blood microbiota was resuscitated by cultivation in BHI broth supplemented with vitamin K for 24 hours at 43°C (1). Cultivated blood microbiota was fixed in 3% glutaraldehyde and processed for TEM (2). DNA was isolated from biopsy, BAL, non-cultured, and cultured blood samples. 16S DNA targeted sequencing was performed.

Results: TEM micrographs showed electron-dense and electron-transparent bodies scattered in and between blood cells. We observed structures resembling L-forms of bacteria. Sequencing analysis showed differences in bacterial abundance in native and cultured blood. The bacterial composition of the biopsy lung material was similar to BAL but differed from the blood samples.

Conclusions: Our method for blood microbiome resuscitation can be applied for further understanding of the possible microbial contribution in sarcoidosis pathophysiology.

Reference

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Disclosure: No significant relationships.

Keyword: blood microbiome, TEM, 16S rRNA sequencing

E-POSTER VIEWING - AS12. OTHER

EFFICACY AND SAFETY OF A SINGLE STRAIN PROBIOTIC AND SINGLE STRAIN POSTBIOTIC IN DIARRHOEA-PREDOMINANT IRRITABLE BOWEL SYNDROME (IBS-D)

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Background and Aims: This 3 arm study evaluated the effect of a single strain probiotic, a single strain postbiotic or placebo on participants with IBS-D.

Methods: In this randomized, double-blind, placebo controlled, three arm trial, 200 participants with moderate to severe IBS-D received either probiotic, postbiotic, or placebo for 12 weeks. Outcomes measured were: IBS symptom severity score (IBS-SSS), Bristol Stool Form Scale, Abdominal Pain Severity-Numeric Rating Scale (APS-NRS), IBS Quality of Life questionnaire (IBS-QoL), and the State-Trait Anxiety Inventory for Adults. The statistical analysis presented are for between group comparisons (probiotic compared to placebo) of the change from baseline to week 12.

Results: After 12 weeks of intervention, a statistically significant improvement in IBS-SSS was observed in both intervention arms compared to placebo (-173.30 (probiotic) and -177.60(postbiotic) vs -60.44(placebo), $p < 0.001$). The number of days per week with diarrhoea was significantly reduced by -3.52 days for probiotic, -3.81 for postbiotic as compared to -1.46 for placebo ($p < 0.001$). Greater reductions in APS-NRS scores were observed in the pro- and postbiotics groups (-2.20 (probiotic) or -2.06(postbiotic) vs -0.82(placebo), $p < 0.001$). The IBS-QoL scores had improved in the probiotic(+19.54) and postbiotic(+24.80) arms but worsened in the placebo(-5.74) arm ($p < 0.001$). Significant reduction was observed for anxiety scores as well.

Conclusions: Single strain probiotic and postbiotic intake for 12 weeks was associated with statistically significant improvements across outcomes measured in this trial. The changes observed in total IBS-SSS scores in both intervention groups are larger than any clinical trials included in the most recent McFarland 2022 meta-analysis assessing effect of probiotics on IBS-SSS.

Disclosure: All authors belong to ADM who provided the product and commissioned this clinical trial

Keywords: Microbiome, diarrhoea, abdominal pain, Quality of life, IBS-D

E-POSTER VIEWING - AS12. OTHER

THE IMPACT OF SEMEN MICROBIOTA ON THE EMBRYO QUALITY

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Background and Aims: Detecting the risk of poor-quality embryos based on semen microbiota assessment is necessary to adjust patient preparation in fertility treatment programs. This work was aimed at developing a mathematical prognostic model for predicting good and excellent quality embryos based on semen microbiota assessment in normozoospermia.

Methods: The study included 127 men whose semen was used for in vitro fertilization (IVF). Patients were divided into 2 groups depending on the proportion of good-quality blastocyst developed on the 5th day of culturing (good-quality blastocyst development rate, GBDR). The 1st group included 57 patients with GBDR \geq 40%, the 2nd group included 70 patients with GBDR $<$ 40%. All patients' semen was assessed at the day of fertilization. Spermogram parameters were evaluated in accordance with the WHO standards and semen microbiota composition was determined by means of real-time PCR using the "Androflor" kit. Discriminant analysis was used for development of the prognostic model.

Results: We developed a method for predicting efficiency of the embryological IVF stage in normozoospermia: EGO-Pro-N prognostic index (Embryos of GOod and Excellent quality Prognosis in Normozoospermia). If EGO-Pro-N value is higher than 0.212, the probability of receiving more than 40% of good and excellent quality blastocysts is low. Conversely, if EGO-Pro-N values is equal to or lower than 0.212, the probability is high. Sensitivity and specificity of the method are 71.9% and 70.0% respectively, effectiveness is 70.9%.

Conclusions: The developed model allows us to predict good and excellent quality embryos based on semen microbiota assessment in normozoospermia before IVF.

Disclosure: No significant relationships.

Keywords: semen microbiota composition, prognosis of ART effectiveness, real-time PCR, discriminant analysis

E-POSTER VIEWING - AS12. OTHER

BACTERIOME OF BRONCHOALVEOLAR LAVAGE IN PATIENTS WITH INHALATION INJURY DURING THEIR HOSPITALIZATION – A PILOT STUDY

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Background and Aims: Inhalation injury (INHI) is defined as acute airway injury caused by inhalation of hot steam and/or products of combustion. During the hospitalization of these patients, commensal bacterial populations colonizing the lungs, such as *Prevotella* spp. and *Veillonella* spp., are displaced by bacteria with pathogenic potential, such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In our pilot study, we aimed to determine the dynamic changes of the bacteriome in 10 patients with INHI during their hospitalization.

Methods: We characterized the bacteriome from oral and oropharyngeal swabs, bronchoalveolar lavage (BAL), catheter urine, and blood using 16S rDNA sequencing at 6 time-points of hospitalization (Days 1 - 28).

Results: Oral and oropharyngeal swabs revealed high abundance of bacterial DNA. The bacterial composition was quite stable among the studied time-points, and both their alpha- and beta-diversities were similar in similar matrices. The BAL bacteriome composition was related to the oral bacteriome of respective patients; high abundances of *Klebsiella* sp., *Enterobacter* sp., *Haemophilus* sp., *Escherichia* sp., *Staphylococcus* sp., *Pseudomonas* sp., and *Neisseria* sp. were found in BAL. The results showed that the urine and blood samples were almost sterile at the beginning of the hospitalization; however, in some cases, low amounts of *Klebsiella* sp. and high abundance of *Proteus* sp. or *Escherichia* sp. were found in the blood and urine samples, respectively.

Conclusions: To conclude, the oral bacteriome seems to be a source of bacterial lung infection in patients with INHI during their hospitalization. Therefore, the oral microbiota could serve as a potential screening marker for the lung microbiome.

Disclosure: No significant relationships.

Keywords: 16S rDNA sequencing, inhalation injury, airways, respiratory tract, bacteriome

E-POSTER VIEWING - AS12. OTHER

EFFECTS OF BILE ACID COMPOSITION ON BILIARY MICROBIOME

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Background and Aims: Bile acids are the major regulators of microbes. Since most of the bile acids are reused through the enterohepatic circulation, each person has a difference in composition. We wanted to know the composition and amount of bile acids correlated with the biliary tract infectious organisms.

Methods: We prospectively evaluated 204 patients who underwent biliary system drainage for a year. The bile acid composition was analyzed using HPLC. In order to analyze the direct effect of each bile acid on the microorganisms, the disk diffusion method was performed.

Results: The mean age of the patients was 74 years. The patients without bacteria in the bile juice were significantly higher than those detected, in the absolute amount of total bile acids(1.649ppm vs. 0.798ppm, $p=0.021$). In the composition of bile acid, the relative percentage of CDCA was significantly high (28.4% vs. 23.4%, $p=0.008$) and UDCA was low (20.2% vs. 14.8, $p=0.023$) in the group where bacteria did not grow. In the patient with Escherichia coli, the most common bacterial strain in the biliary system infection, the CA and CDCA ratios were significantly low and the UDCA ratio was high.

Conclusions: Bile acid has a notable inhibitory effect on the biliary microorganisms, while the effect varies according to the individual strain and composition of bile acid. The antimicrobial effects of bile acids in the biliary tract were consistent with the in-vitro susceptibility test result. Bile acid composition varied in each person, and the artificial regulation of bile acids composition may cause changes to the microbiome in the biliary system.

Disclosure: No significant relationships.

Keywords: Bile acids, Microbiome, Biliary tract

Online Only / #114

E-POSTER VIEWING - AS06. TEASING OUT A MECHANISM: HOW DOES THE MICROBIOME CONTRIBUTE TO DISEASE

QUANTITATIVE GUT MUCOSAL MICROBIOTA COMPOSITION IN INFLAMMATORY BOWEL DISEASE INTRODUCED TO INFLIXIMAB

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Background and Aims: Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastro-intestinal tract with the main subtypes Crohn's disease (CD) and ulcerative colitis (UC). IBD is growing rapidly in incidence and prevalence throughout the world and is strongly associated with an imbalance of the gut microbiota, although the pathogenesis is unknown. There are multiple conventional treatments, but moderate to severe IBD is treated successfully with anti-tumor necrosis factor alpha (TNF- α) infliximab (IFX). However, the problem is that up to half of the patients receiving IFX do not have a good long-term response. There are no methods available to predict the response to IFX, which would be crucial to save from both high costs and possible side-effects. Here the aim was therefore to investigate the mucosal microbiota composition of the gut and to determine whether it changes in responders over time.

Methods: This was investigated in a cohort including 63 IBD patients from whom biopsy samples from both small- and large intestine and fecal samples were collected before, during and after infliximab treatment. The microbiota composition was determined by MiSeq sequencing targeting the 16S conserved rRNA regions of DNA extracted from the biopsies, and the absolute abundance was determined by 16S qPCR. Additionally, the already published fecal fungal and bacterial microbiota composition was also included to get further comparison between these.

Results: The result show a significant increase in SCFA-producing bacteria, particularly belonging to the Clostridia class in the mucosal microbiota of responders over time.

Conclusions: The changes highlighted mucosal healing in responders to IFX.

Disclosure: No significant relationships.

Keywords: IBD, Infliximab, Biopsy, microbiota

Online Only / #388

E-POSTER VIEWING - AS03. GUT BRAIN AXIS

GUT MICROBIOME BALANCES AT EARLY LIFE ASSOCIATES WITH CHILDHOOD BEHAVIORAL SCALES CONSISTENT WITH THE DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS (DSM): A PILOT STUDY.

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Background and Aims: Behavioral effects, while related to neurodevelopment, are often more subtle and difficult to measure. The Childhood Behavioral Checklist (CBCL) is a standardized tool with six scales linked to Diagnostic and Statistical Manual of Mental Disorders (DSM) diagnostic categories. This study explored associations between the VLBW infant's gut microbiome and scales related to the Diagnostic and Statistical Manual of Mental Disorders (DSM) from the Childhood Behavioral Checklist (CBCL) at 4 years old

Methods: Biobehavioral measures were corrected for gender, delivery method, gestational age, infant birth weight, sepsis occurrence, and days on antibiotics. We identified the microbial signatures as microbiome balance (ratio of two bacterial abundances) which were observed to have the largest impact on the biobehavioral measures. For each of the CBCL scores as outcome, the balances were performed separately applying the selbal package implemented in R software. Two NIH grants supported this study (R01NR013094 and R01NR015446).

Results: The ratio of Enterobacteriaceae to *Clostridium perfringens* showed a positive association with the CBCL adjusted scales related to depression and anxiety. Similarly, the ratio of *Veillonella dispar* to *Clostridium perfringens* displayed positive association with adjusted CBCL scores for oppositional behavior.

Conclusions: Although limited by a nominal sample size, our pilot study indicates that the early-life gut microbiota is associated with childhood behavior in VLBW infants. In addition, this study finds the association of microbiome as balances, rather than individual entities, towards its impact on childhood behavior in VLBW infants.

Disclosure: No significant relationships.

Keywords: behavior, children, Diagnostic and Statistical Manual of Mental Disorders DSM, microbial signature, Gut Microbiome

Online Only / #204

E-POSTER VIEWING - AS02. THE FIRST 1000 DAYS OF LIFE: FROM PREGNANCY TO INFANCY IN HEALTH AND DISEASE

EARLY-LIFE SKIN MICROBIAL BIOMARKERS FOR ECZEMA PHENOTYPES IN CHINESE TODDLERS

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Background and Aims: Eczema is a common inflammatory skin disorder during infancy. Evidence has shown that skin microbiome fluctuations may precede eczema development, but their predictive values on eczema phenotypes remain unknown. We aimed to characterize the early-life evolution of skin microbiome and delineate its temporal associations with different eczema phenotypes (transient versus persistent; atopic versus non-atopic) in Chinese children.

Methods: We followed 119 term Chinese infants in a Hong Kong birth cohort from birth to 24 months old. These babies were recruited within 48 hours after birth regardless of any family history of allergic diseases. Eczema was diagnosed based on Hanifin and Rajka criteria, and its severity assessed by SCORAD score. Skin microbes at the left antecubital fossa were serially sampled by flocked swabs at 1, 6 and 12 months for bacterial 16S rRNA gene sequencing. These infants underwent skin prick tests with locally important allergens at 12 months.

Results: Atopic sensitization at 12 months was strongly associated with eczema persisting to 24 months (OR 4.95, 95% CI 1.29-19.01). Compared with non-atopic eczema, children with atopic eczema had reduced alpha diversity at 12 months ($p < 0.001$) and transiently higher abundance of the genus *Janibacter* at 6 months ($p < 0.001$). No microbe was associated with any eczema phenotype by 12 months.

Conclusions: Our findings suggest that atopic sensitization at 12 months may predict persistent eczema by 24 months, and atopic eczema at 12 months is associated with unique skin microbiome profiles at 6 and 12 months. Non-invasive skin microbiome profiling may have a predictive value for early-onset atopic eczema.

Disclosure: No significant relationships.

Keywords: biomarker, birth cohort, Eczema, Microbiome, skin

**E-POSTER VIEWING - AS01. META-OMICS TECHNIQUES AND INTEGRATIVE APPLICATIONS
COMPUTATIONAL AND STATISTICAL METHODS FOR MICROBIOME RESEARCH**

**AN IN SILICO METHOD TO MAXIMISE THE BIOLOGICAL POTENTIAL OF UNDERSTUDIED
METABOLOMIC BIOMARKERS: A STUDY IN PREECLAMPSIA**

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Background and Aims: Given that only a small proportion of the annotated metabolites are well-studied, a common dilemma in metabolomics is that the most distinguishable biomarker in a particular disease setting often has scarce information.

Methods: We developed a two-arms strategy, which combined data-driven and knowledge-driven methods, to estimate the potential relationship of understudied metabolites with a certain disease by connecting the related information of specific metabolites and disease. The first arm is based on the proteins regulated by metabolite and the downstream proteins by using the STRING database. The second arm assumed that metabolites correlated with specific metabolite abundance or shared structural similarity might be involved in similar biological functions and then predicted their interaction proteins by the STITCH database. Using metabolomics data in pre-eclampsia(PE), the potential links between PE and ononetin, the most significant but scarcely studied metabolite, were predicted by our analytic method and validated in subsequent mice studies.

Results: In the first and second-arm analyses, ononetin was predicted to regulate 33, 22 and 29 genes that were enriched in PE-related pathways, including regulation of systemic arterial blood pressure, inflammation, VEGF signalling, and angiogenesis (essential in placenta development). In the animal study, ononetin prevented PE-related symptoms in mice and restored the status of offspring, indicating its capability to alleviate PE. Biomarkers of anti-angiogenesis and the predicted genes altered by ononetin were also validated.

Conclusions: We provide a bioinformatics pipeline and validation process in evaluating the biological potential of understudied biomarkers to test the therapeutic capabilities of identified biomarkers.

Disclosure: No significant relationships.

Keywords: Gut-placenta axis, Preeclampsia, Metabolomics, Understudied biomarker

E-POSTER VIEWING - AS11. ANIMAL AND ENVIRONMENTAL MICROBIOMES

DISRUPTION OF THE SKIN, GILL AND GUT MUCOSAE MICROBIOME OF GILT HEAD SEABREAM FINGERLINGS AFTER BACTERIAL INFECTION AND ANTIBIOTIC TREATMENT

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Background and Aims: The activity of the microbiome of fish mucosae provides functions related to immune response, digestion, or metabolism. Several biotic and abiotic factors help maintain microbial homeostasis, with disruptions leading to dysbiosis. Diseases and chemical treatments, such as antibiotic administration, are known to cause dysbiosis in farmed fish, although the effects are dependent on the target species and studied tissues, as well as dose-dependent in the case of antimicrobials. The gilthead seabream is one of the most important reared species in the Mediterranean and Atlantic regions. Its production is greatly affected by pathogen infections and disease control strategies resorting to antibiotics are still frequently required. The goal of the present work was to characterize the effects of a disease outbreak and subsequent antibiotic treatment on the microbial dynamics of the skin, gill and gut microbiome of farmed gilthead seabream fingerlings.

Methods: The present work employed a 16S rRNA high-throughput metataxonomics approach.

Results: Although microbiota response differed between studied tissues, overall changes in composition, diversity, structure and predicted function were observed in all mucosae. The skin and gill microbiomes of diseased fish became largely dominated by taxa that have been frequently linked to secondary infections, whereas in the gut the genus *Vibrio*, known to include pathogenic bacteria, increased with OTC treatment.

Conclusions: The study highlights the negative impacts of disease and antibiotic treatment on the microbiome of farmed fish. Our results also suggest that fish transportation operations may have profound effects on the fish microbiome, but further studies are needed to accurately evaluate their impact.

Disclosure: No significant relationships.

Keywords: aquaculture, antibiotic, bacterial disease, dysbiosis