Relation between meat consumption and mood: potential role of bacterial indole?

Catherine Philippe, Jean Marion Torres, Fabien Szabo de Edelenyi, Laurent Naudon, Paule Latino-Martel, Sylvie Rabot, Pilar Galan

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INTERNATIONAL SOCIETY OF MICROBIOTA

JISM

INSIDE

Abstracts of the Third World Congress on Targeting Microbiota

October 21-22-23, 2015
Institut Pasteur - Paris, France

October 2015 | JISM Vol. 1 | Pages 1 to 126

3rd World Congress on

Targeting Microbiota

October 21-23 2015

Institut Pasteur - Paris, France

Marvin Edeas – Chairman of the Scientific Committee

Yuri Ikeda – President of the International Society of Microbiota

International Society of Microbiota

Your abstract is referenced on the Journal of the International Society of Microbiota

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Welcome to 3rd World Congress on Targeting Microbiota

… Microbiota is facing important challenge… Towards Clinical Revolution…

Dear Colleagues,

It is a pleasure to welcome you for the **3rd World Congress on Targeting Microbiota** which is helding at **Institut Pasteur,** Paris, France on **October 21-23, 2015.**

During Pasteur Congress, many hot topics will be discussed:

- The Role of Microbiota in Health and Diseases: The Mechanistic Aspects
- Recent Advances on Microbiota Clinical Researches & Innovations in 2015
- Microbiota & Biomarkers: From Predictive to Personalized Medicine
- New Challenges to Prevent and Treat Metabolic Diseases, Cancer, Liver, Kidney, Lung and Inflammation-Related Diseases
- Impact of Environment, Food Conservators & Antibiotics on the quality and biodiversity of microbiota
- Can we modulate the diversity and quality of microbiota?
- Skin Microbiota: Recent advances on its characterization and manipulation in diseased conditions
- Fecal Microbiota Transplantation: Practical Issues & Regulation

Workshop: Exploration & Sequencing of Human Microbiota: The perfect signature

All you need to know about the recent methods, devices and platforms will be presented during this workshop, organized on October 21.

To conclude this congress, a general discussion will be held between speakers, scientists and industry sector about Microbiota and medicine of tomorrow. Among questions which will be discussed:

- Can one strain fit with all populations?
- To date, the most relevant challenge: can we modulate and manipulate the human gut microbiota?
- How diet, food, antibiotics, hormones, prebiotics and faecal microbiota can affect resident microbiota?
- How to avoid speculations and marketing on microbiota not supported by actual data?

We wish you an interesting congress.

Dr Yuri Ikeda – Dr Marvin Edeas

Chairman of the International Society of Microbiota

Chairman of the Scientific Committee

Targeting Microbiota 2015 thanks

On behalf of Yuri Ikeda, Chairman of ISM, Japan and **Marvin Edeas,** Chairman of Scientific Committee, France, we would to thank all speakers for their contribution:

Premysl Bercik, MacMaster University, Canada
Annamaria Bevivino, ENEA.Casaccia Research Centre, Italy
Hervé Blottière, MetaGenoPolis, France
Rémi Burcelin, INSERM, France
Moktar Chawki, Clinique du Parisis, France
Anat Eck, VU Medical Center, the Netherlands
Julien Diana, INSERM, France
Lorenzo Drago, University of Milano, Italy
Grégory Dubourg, Université de Marseille, France
Serguey Fetissov, Rouen University, France
Ernst Holler, University Hospital Regensburg, Germany
John-Olov Jansson, Gothenburg University, Sweden
Katri Korpela, Helsinki University, Finland
David MacIntyre, Imperial College of London, UK
Javier Pizarro-Cerda, Institut Pasteur, France
Jacques Ravel, University of Maryland School of Medicine, Baltimore, USA
Miria Ricchetti, Institut Pasteur, Paris
Knut Rudi, Norwegian University of Life Sciences, Norway
Filip Scheperjans, Helsinki University Central Hospital, Finland
Andreas Schwiertz, MVZ Institut für Mikroökologie, Germany
Iradj Sohbani, Hôpitaux Universitaires Henri Mondor, France
Harry Sokol, Hôpital Saint Antoine, France
Elena F. Verdu, McMaster University, Canada
Liping Zhao, Shanghai Jiao Tong University, China

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We would like to thank especially the media partners:
Microbiome, Beneficial Microbes, AllSeq

Welcome to Targeting Microbiota World Congress

On behalf of the Organizing Committee, welcome to the 3rd World Congress on Targeting Microbiota, which is being held at the Pasteur Institute in Paris, on October 21 to 23, 2015.
We would like to take the opportunity to give you some additional information about the meeting arrangements.

The Abstract book contains:

- Speakers' abstracts (the abstracts of the oral presentations follow the order of the program)
- The abstract of posters on display
- Top 5 Ideas from the conference
- Top 5 Contacts from the conference

Badges

Upon registration you have received your own personal badge. Please wear this badge during the entire meeting including the coffee breaks and lunch.

The conference secretariat will be located in the area in front of conference hall.

Instructions for participants

Chairpersons

The Chairpersons will be seated at the president's table.

Speakers

Speakers are invited to give their Power Point presentations for downloading on the computer to the technical team **OUTSIDE AND NOT INSIDE THE CONFERENCE HALL.**

As the schedule is rather tight and to allow sufficient time for discussions, we would be very much obliged if the timing requirements were respected.

Poster Contributors

Please ensure that your poster is displayed at the appropriate location, PLEASE RESPECT YOUR POSTER NUMBER. Poster contributors are invited to stand by their poster during the poster sessions.

Group Photo

- **A group photo will be realised at 12H30 on Thursday and at 12H30 on Friday with all participants and speakers.**

Speakers Dinner

A dinner is organized on October 22, between speakers and attendees.

If you are interested to register, please contact the staff on site.

Mobile Phones

As a courtesy to the speakers and other delegates, please turn off your mobile phones or to silent whilst in the conference room.

Personal Belongings

Please keep your valuables and working materials with you at all times. We would advise you to keep your name on the conference notes, as we may not be able to replace these if lost. **Neither Targeting Microbiota 2015 Conference nor the venue can be held responsible for any loss or damage to your property.**

Questions

Please state your name and institution or company before asking your question.

Conference Staff

Staff at the conference registration desk will be happy to deal with any queries you may have. If we receive any messages for you, they will be announced at the break in the session and can be collected from the desk.

➢ Top 5 Contacts of Targeting Microbiota 2015

Name : *Institution :* *Contact :*

Name : *Institution :* *Contact :*

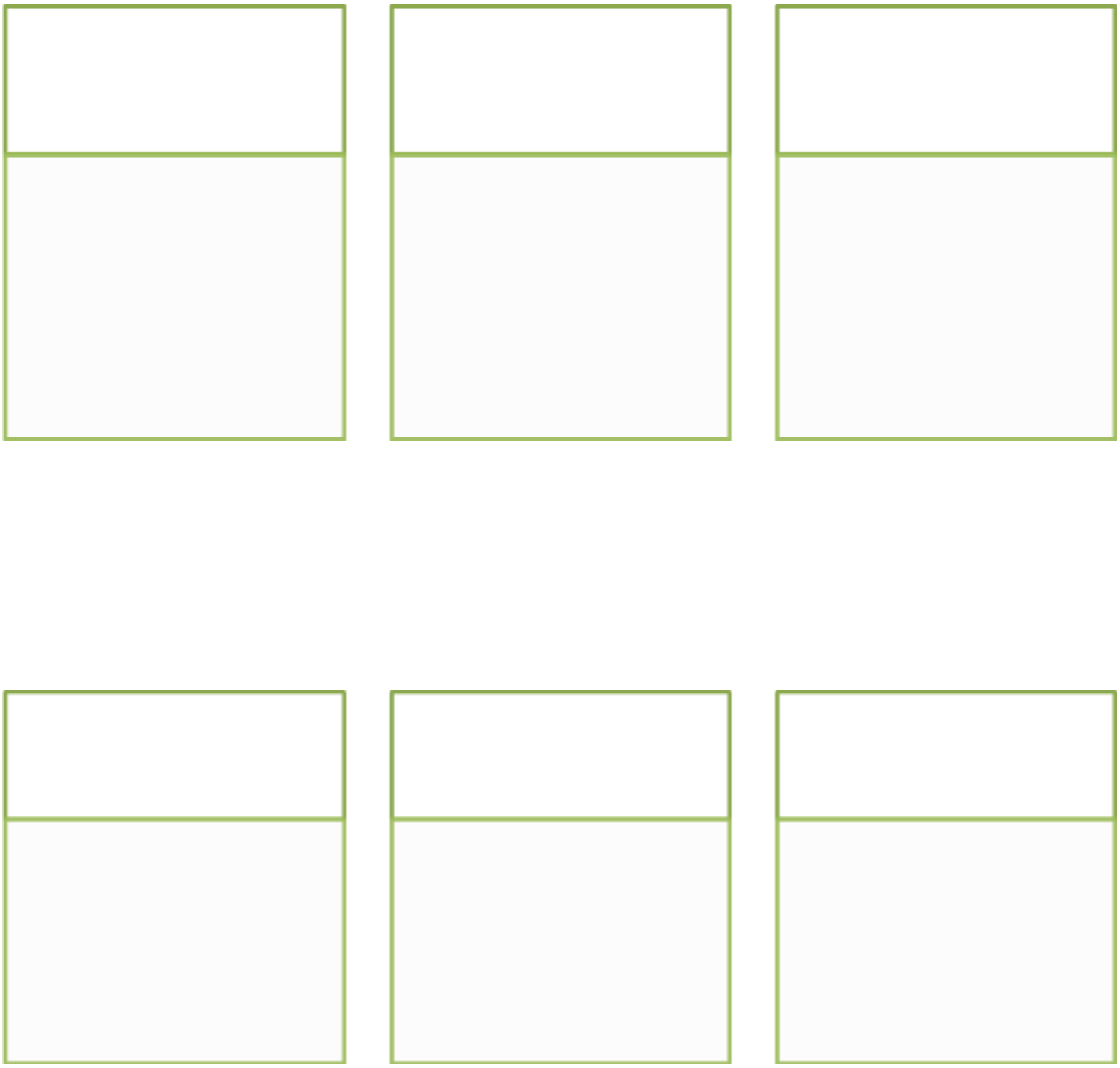
Name : *Institution :* *Contact :*

Name : *Institution :* *Contact :*

Name : *Institution :* *Contact :*

➢ **Strategic Contact :**

➢ Top Ideas of Targeting Microbiota 2015



➢ **Strategic Remarks :**

3rd World Congress on
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3rd World Congress on

Targeting Microbiota

Abstracts for the Workshop

Day 1 – October 21, 2015

INTRODUCTION OF ISM WORKSHOP: SCREENING METHOD TO STUDY HOST MICROBIOTA INTERACTIONS IN HUMAN INTESTINE

BLOTTIÈRE, Hervé

MetaGenoPolis, France

Dr Blottière proposes to use a functional metagenomic approach in order to model the microbiota's interaction with the host by combining the heterologous expression with intestinal reporter cell lines. He presents a methodology allowing a high throughput screening combining two biological systems. Therefore ideal conditions for homogeneity, sensitivity and reproducibility of both metagenomic clones as well as reporter cell lines have been identified and validated. We believe that this innovative method will allow the identification of new bioactive microbial molecules and, subsequently, will promote understanding of host-microbiota interactions.

CULTUROMICS AND PYROSEQUENCING: TWO COMPLEMENTARY TECHNIQUES TO STUDY THE GUT MICROBIOME

DUBOURG, Grégory

Université de Marseille, France

High-throughput sequencing techniques are considered since the last decade as the gold standard to characterize complex ecosystems, in particular the human gut microbiota. However, several biases and pitfalls inherent to the method had been reported as primer bias, extraction bias and depth bias.

The microbial culturomics, based on large scale culture conditions and MALDI-TOF identification of isolates, had been shown to be complementary with molecular techniques, offering a different perspective. Indeed only 15% of the bacterial species have been detected concomitantly by both culture and pyrosequencing performed on the same 3 stool samples in a pioneering study.

To date, testing more than 700, 000 colonies, microbial culturomics enabled the discovery of more than 130 totally new bacterial species, whose genome had been sequenced, as well as 2more than 200 bacteria never isolated in human This contributes to extent the knowledge about human gut microbial repertoire.

RECTAL SWABS FOR ANALYSIS OF THE INTESTINAL MICROBIOTA

ECK, Anat

VU Medical Center, The Netherlands

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The composition of the gut microbiota is associated with various disease states, most notably inflammatory bowel disease, obesity and malnutrition. This underlines that analysis of intestinal microbiota is potentially an interesting target for clinical diagnostics. However, since sampling method, storage and processing of samples impact microbiota analysis, it is important to define standards for reproducible and accurate sampling of gut microbiota that can be implemented in clinical routine. An ideal sample type for use in routine diagnostics should be easy to obtain in a standardized fashion without perturbation of the microbiota. Rectal swabs, that may satisfy these criteria, can be performed on demand, whenever a patient presents and appeared to be a convenient means of sampling the human gut microbiota.

DYNAMIC INTERACTIONS OF THE EQUINE HINDGUT MICROBIOTA IN HEALTH AND DISEASE

RUDI, Knut

Department of Chemistry, Biotechnology and Food Science,
Norwegian University of Life Sciences, Ås, Norway

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Horses derive 60-70 % of their energy through microbial hindgut fermentation. The fermentation is a delicate dynamic process where energy overload and can lead to a severe diseased acidotic state. Based on microbiome data we have developed dynamic models to both understand the diseased - and non-diseased microbiota. These models revealed the importance of short-term (within hours) dynamic interactions. We believe that dynamic modelling also can be a valuable contribution for understanding the role of the microbiota in health and disease for other organisms.

WHY SHOULD WE CARE ABOUT PRIMERS AND PLATFORM IN MICROBIOTA ANALYSIS?

CODOÑER, Francisco

Scientific Director at Lifesequencing SL

The human body contains 10 times more microbial cells than human cells. This microbial community that we found in our body has been classified as human microbiome (1,2). Using the presence of specific organisms, when analysing human samples, the human adults can be even classified into different classes, in the case of Human feces the samples are classify in enterotypes, enterotype 1 when genus Bacteroides is the most abundant, enterotype 2 if Prevotella is the most abundant genus, and enterotype 3 when we found more Ruminococcus species (3,4) Alterations in the ecosystem can produce alterations in the presence of specific organisms (dysbiosis), that can drive to an illness or been the indicator of the alteration. Among the illnesses that have been associated with microbial dysbiosis we can enumerated: i) auto-immune disorders, diabetes (5), rheumatoid arthritis, muscular dystrophy or multiple sclerosis, ii) neurological disorders (depression, autism (6), among others), iii) intestinal (Crohn disease, obesity (7),…) and iv) some types of cancer. In the case of other samples like skin, hear, etc… the classification is scarce at this stage.

In order to perform this kind of analysis based on the capture of specific rRNA region, we have to take into account several points:
a) Sample taken and conservation. Some considerations have to be taken in order to avoid the increase of aerobic bacterial and decrease of anaerobic ones, that could affect to the bacterial detection and count and to the conclusions.
b) 16s rRNA region of study. Depending on the region we are going to detect different profiles, optimal V3-V5.
c) Set of primers used. This is another key point, since different set of primers will pick different groups of bacteria. We will provide a comparison between two sets of primers mostly used.
d) Massive sequencing platform. 454 has been describe as gold standard in this kind of studies, specially to reach a good species taxonomical identification, we will show how MiSeq can be used as a real alternative to the 454.
e) Databases. This is another key point, not only for the analysis, but for the primer design and improvement, there is no complete database that can make the thing easy to analyse and interpret.

We will focus our attention in those points in order to give some insights in how to perform a proper microbiome analysis.

In any case, through the study of the microbiome (mostly coming from faeces) we can be able to disentangle the alterations or illnesses that a patient is suffering and maybe change to normal conditions with the introduction of an antibiotic (8), prebiotic (9) or probiotic (4).

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EVOLUTION AND CHALLENGES OF NGS TECHNOLOGIES FOR MICROBIOTA ANALYSIS AND THEIR VALIDATION FOR DIAGNOSTIC

MASSART, Sébastien

DNA Vision, Belgium

sebastienmassart@dnavision.be

NGS technologies have revolutionized the landscape of microbiota studies. Through a critical overview, the talk will review the pros and cons of the 3 main protocols to study microbiota by NGS. The challenges and opportunities of these developments will be underlined as well as the road toward their use in diagnostics.

16S SEQUENCING ANALYSIS: COMPARISON BETWENN RSII PACBIO VERSUS ILLUMINA MISEQ

VILANOVA, David

Bioinformatics & Bioanalysis Director, Libragen, France

In order to compare the potential use of short reads (Miseq) and long reads (Pacbio) for taxonomy assignment we have decided to evaluate their impact for 16S rRNA studies.

Our first study is to run an in-silico taxonomic assignment using short or long amplicons of the 16S rRNA gene and look for their taxonomic diversities. Our second study is to perform an experimental evaluation of the Pacbio RSII platform for 16S rRNA studies using our MOCK community. Our data suggests that although long amplicons are more suitable for taxonomic assignment there is still place to improve the use of the Pacbio platform for its routine use in new 16S studies.

IMPORTANT CONSIDERATIONS FOR ROBUST AND UNBIASED DNA AND R NA SAMPLE PREP FOR MICROBIOME STUDIES

MILLER, Sandrine

MO BIO Laboratories, USA

The number of Microbiome projects is exploding worldwide thanks to the democratization of next generation sequencing, which is affordable more than ever.

While a lot of focus is now put on data analysis challenges, due to the enormous volume of data generated, it is essential to remember the importance of the first step of the study workflow: nucleic acid purification.

Starting sample complexity and diversity is greater than ever and challenging samples such as human feces, skin, body fluids, and environmental samples are being analyzed. Thus, it is crucial to ensure that this first step of sample preparation is as unbiased as possible, enabling the full representation of microbial diversity to be examined, and to remove inhibitors of downstream applications, increasing sensitivity.

We are proposing to review the main technologies available to ensure that scientists do not start their experiment with “just DNA or RNA” but with “clean and unbiased DNA or RNA.

PRACTICAL ASPECTS OF MICROBIOTA AND KIDNEY DISEASES: HOW TO EVALUATE THE IMPACT OF MICROBIOTA VARIATIONS ON HEMODIALYSIS PATIENTS?

CHAWKI, Mokthar

Clinique du Parisis, France

Dr Chawki is studying the impact of probiotics on hemodialysis patients. He will present the clinical protocol and discuss how to interpret the variability of microbiota.

3rd World Congress on

Targeting Microbiota

Abstracts for Oral Presentation

Day 2 – October 22, 2015

INTRODUCTION LECTURE - MICROBIOTA 2015: TOWARDS A REVOLUTION? TARGETING MICROBIOTA-MITOCHONDRIA INTER-TALK: MICROBIOTA CONTROL MITOCHONDRIA METABOLISM

EDEAS, Marvin

International Society of Microbiota, Japan

Our aim is to highlight the subtle relationship that exists between microbiota and mitochondria. Microbiota targets mitochondria by modulating the Reactive Oxygen Species (ROS) production and the mitochondrial activity through interactions with toxins, proteins or other metabolites released by gut microbiota. The intriguing relationship that exists between mitochondria and microbiota is strengthened by the probable prokaryotic origin of mitochondria. Emerging data implicates a role for ROS, nitric oxide, Short Chain Fatty Acids and hydrogen sulfide in the cross-talk between microbiota – mitochondria and REDOX signaling. Several studies have shown that microbiota act and modulate mitochondrial activity, and use it as a relay to strengthen host-microbiotal interaction. This modulation depends on the gut bacterial strain quality and diversity to increase its pathogenic versus beneficial effects. Furthermore, based on conclusions from new studies, it is possible that microbiota can directly interact with the host cell gene expression by favoring bacterial and mitochondrial DNA insertion in the nuclear genome. The emerging knowledge of mitochondria-microbiota interaction may be of great importance to better understand the mechanism of mitochondrial and metabolic diseases, and the syndromes associated with change in quality and quantity of microbiotal species. We suggest that microbiota *via* mitochondrial modulation influence cell homeostasis and metabolism. The challenge will be to find strategies to modulate the quality and diversity of microbiota rather than acting on microbiota metabolites and microbiota related factors. The medicine of tomorrow will be completely personalized. Firstly there will be a test to show the quality, quantity and diversity of microbiota, and secondly a preventive or therapeutic strategy will be administrated (probiotics, diet, prodrug or fecal transplantation). The era of digital medicine is here.

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Weissig, V. and Edeas, M., Mitochondrial medicine. Vol. II Manipulating mitochondrial function. Preface. Methods Mol Biol. 2015, 1265

THE GUT MICROBIOTA & OBESITY: FROM CORRELATION TO CAUSALITY

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Gut microbiota has been implicated as a pivotal contributing factor in diet-related obesity; however, its role in development of disease phenotypes in human genetic obesity such as Prader-Willi Syndrome (PWS) remains elusive. In this hospitalized intervention trial with PWS (n=17) and simple obesity (n=21) children, a diet rich in non-digestible carbohydrates induced significant weight loss and concomitant structural changes of the gut microbiota together with reduction of serum antigen load and alleviation of inflammation. Co-abundance network analysis of 161 prevalent bacterial draft genomes assembled directly from metagenomic datasets showed relative increase of functional genome groups for acetate production from carbohydrates fermentation. NMR-based metabolomic profiling of urine showed diet-induced overall changes of host metabotypes and identified significantly reduced trimethylamine N-oxide and indoxyl sulfate, host-bacteria co-metabolites known to induce metabolic deteriorations. Specific bacterial genomes that were correlated with urine levels of these detrimental co-metabolites were found to encode enzyme genes for production of their precursors by fermentation of choline or tryptophan in the gut. When transplanted into germ-free mice, the pre-intervention gut microbiota induced higher inflammation and larger adipocytes compared with the post-intervention microbiota from the same volunteer. Our multi-omics-based systems analysis indicates a significant etiological contribution of dysbiotic gut microbiota to both genetic and simple obesity in children, implicating a potentially effective target for alleviation.

ROLE OF GUT BACTERIA IN HOST CONTROL OF APPETITE

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Appetite is precisely regulated by hormonal and neuronal circuitries generating alternating sensations of hunger and satiety. When animals eat they also feed the numerous bacteria inhabiting their gastro-intestinal tract. Nutrient-induced bacterial growth produces biomass that contains molecules which may interfere with the host hormonal circuitries controlling appetite. In particular, some bacterial proteins may display molecular mimicry with the host peptide hormones and may act as their conformational mimetics in the gut and in the circulation. Such effects of bacterial proteins on appetite pathways can be direct, via peptide hormone receptors as well as indirect via production of autoantibodies cross-reactive with peptide hormones. Recent data suggest that such bacterial proteins may be involved in regulation of appetite in normal and pathological conditions including eating disorders.

MODULATION OF HOST MICROBIOTA BY LISTERIA MONOCYTOGENES

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Listeria monocytogenes is a foodborne bacterial pathogen which can induce gastroenteritis, abortions in pregnant women and meningitis in the new born. Epidemic Listeria monocytogenes strains are characterized by the presence of a pathogenicity island coding for the toxic peptide listeriolysin S (Lls). Lls has been previously described as a virulence factor required for bacterial survival within animal hosts; our recent work demonstrates that Lls is the first described Listeria monocytogenes bacteriocin which modulates the host intestinal microbiota, favoring bacterial colonization of the intestine and allowing bacterial dispersal to other host organs.

ROLE OF GUT MICROBIOTA IN PARKINSON'S DISEASE

SCHEPERJANS, Filip

Helsinki University Central Hospital, Finland

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting approximately 1% of the population over 60 years of age. In addition to the classical motor symptoms of the disease, patients suffer from a broad spectrum of non-motor symptoms. About 80% of patients have gastrointestinal dysfunction, most frequently constipation, and these frequently precede the onset of motor symptoms by years. This is associated with neurodegeneration in the enteric nervous system. Recent research suggests that the gastrointestinal tract is actually the first structure to show neurodegeneration in PD and that this degenerative process may subsequently spread to the brainstem via the vagal nerve. Alterations of gut physiology, mucosal permeability, inflammation, and most recently gut microbiome composition have been reported in PD patients and may be relevant for the pathogenesis of the disease. The talk will give an overview of gut pathophysiology in PD and address the possible role of gut microbiota in this context.

PRENATAL STRESS AND GUT MICROBIOTA DIVERSITY IN MIDDLE-PREGNANCY FUTURE TOOL FOR SCREENING PRENATAL STRESS EXPOSURE?

UUSITUPA, HM[1]; AATSINKI AK[1]; PESONEN H[1]; MUNUKKA E[2]; KARLSSON H[1].
Presented by UUSITUPA, HM

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[2]University of Turku, Department of Medical Microbiology and Immunology
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Objectives: Gut microbiome and the brain-gut signaling system have been suggested to function in early programming of the central nervous system and regulation of the intestinal innate immunity (1). The objective of the present study was to elaborate how maternal stress affects maternal microbiota development during pregnancy.

Methodology: Study subjects (n=51) were drawn from an ongoing large Finnish birth cohort study FinnBrain (n=4010). The prenatal stress was analyzed from the questionnaires (PRAQ, anxiety; EPDS, depression symptoms; ASS/SCL-90, anxiety) at each trimester. Fecal samples were collected to 50-ml plastic conical collection tubes in the gestational week 24 at the homes of the subjects and stored in -70 C until sequencing was conducted. Illumina MiSeq sequencing were performed as described by Caporaso (2). Shannon index to describe the diversity was calculated at class- order- and phylum level.

Results: There was negative correlation between the diversity in phylum- (r=-0.281, p=0.046) and order level (r=-0.249, p=0.078), and EPDS, as well as between diversity in phylum-level and PRAQf3 (concern about own appearance) sum points (r=-0.326, p=0.019).

Conclusion: In conclusion, based on the results of this study, it seems that depression and concerns about own appearances are correlated with lower bacterial diversity at pregnancy.

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BIFIDOBACTERIUM ANIMALIS SUBSP LACTIS CNCM-I2494 RESTORES GUT BARRIER PERMEABILITY IN CHRONICALLY LOW-GRADE INFLAMED MICE

MARTIN, Rebeca (1); LAVAL, Laure (2); CHAIN, Florian (1); MIQUEL, Sylvie (1); NATIVIDAD, M Jane (3); CHERBUY, Claire (1); SOKOL, Harry (4); VERDU, F Elena (5); VAN HYLCKAMA VLIEG, Johan (6); BERMUDEZ-HUMARAN, G Luis (1); TAMARA, Smokvina (6); PHILIPPE, La
Presented by MARTIN, Rebeca

1: Probihot Lab, MICALIS Institute, France 2: Danone Nutricia Research
Probihot Lab, MICALIS Institute, France 3: FarncombeFamily Digestive Health Research Institute
Probihot Lab, MICALIS Institute, Canada 4: Probihot Lab, MICALIS Institute, Faculté de Mécine, Paris, France
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Objectives: Growing evidence supports the efficacy of many probiotics in the management of deregulated gut barrier. Bifidobacteria have been studied for their efficacy to treat a broad spectrum of gut disorders. The aim of this work was to study the effects of Bifidobacterium animalis subsp lactis CNCM-I2494, a strain used in fermented dairy products for a long time, on intestinal barrier function.Methodology and results: We first induced gut dysfunction in mice by a chronic low-grade inflammation model. Markers of inflammation, gut permeability and immune function were studied pointing out the absence of an active inflammation process. Nevertheless, gut permeability, lymphocyte populations and colonic cytokines were found to be altered in challenged mice. CNCM-I2494 was able to restore the function of the intestinal barrier improving intestinal permeability and restoring colonic goblet cell populations and cytokine levels. Furthermore, tight junction (TJ) proteins levels were also measured by qRT-PCR showing the ability of this strain to specifically normalize the level of some TJ proteins, particularly claudin-4 protein. Finally, CNCM-I2494 counterbalanced CD4+ lymphocyte alterations in both spleen and mesenteric lymphoid nodes.Conclusion: Altogether, these data suggest that CNCM-I2494 may be an efficient prevention and therapeutic tool for disorders associated with increased barrier permeability.

RATIONAL SELECTION OF LACTOBACILLUS STRAINS BASED ON BILE SALT HYDROLASE ACTIVITY

LONG Sarah L. , SHANAHAN Fergus, GAHAN Cormac G. M., JOYCE Susan A.
Presented by LONG, SARAH L.

APC Microbiome Institute, University College Cork, Ireland

Lactobacillus species form an integral part of the human gut microbiome. They are frequently applied as probiotics with the prerequisite that they can metabolize bile acid (BA). Bile salt hydrolase (BSH) enzymes are the gateway reaction for BA metabolism by gut microbes (Jones, Begley et al. 2008). BSH removes a glycine or taurine moiety conjugated to a bile acid yielding free BAs, these can then be subsequently further microbially modified. These modifications can result in alterations in host physiology (Joyce, MacSharry et al. 2014). Within the L.salivarius species alone allelic variation of the BSH proteins have been divided into four major groups, all of which have distinct differences (Fang, Li et al. 2009), one of these has been shown to be a significant factor in the reduction of host weight gain (Joyce, MacSharry et al. 2014). This study applied rational selection and screening to isolate and identify new strains of Lactobacillus with BSH enzymes with specific activities. Six isolates, of either pig or human origin, were characterized genetically and phenotypically, for their ability to alter BAs and for their physiological effect on the murine host. Their potential in conferring health benefits is discussed.

MATERNAL GESTATIONAL STRESS-INDUCED CHANGES IN GUT MICROBIOTA CORRELATE WITH ALTERATIONS IN THE PHYSIOLOGY OF THE ADULT OFFSPRING

GOLUBEVA, V. Anna (1); CRAMPTON, S. (2); DESBONNET, L. (1); EDGE, D. (1); O'SULLIVAN, O. (3); LOMASNEY, W. K. (1); ZHDANOV, V. A. (1); CRISPIE, F. (3); MOLONEY, D. R. (1); BORRE, E. Y. (1); COTTER, D. P. (4,5); HYLAND, P. N. (1); O'HALLORAN, D. K. (1); DI
Presented by GOLUBEVA, V. Anna

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Growing attention has recently been focused on the impact of early-life adverse experiences on the brain-gut axis communication. Here we investigate the impact of gestational stress (GS) on intestinal microbiome and the offspring physiology in adulthood. Sprague Dawley pregnant rats were subjected to repeated restraint stress from embryonic day 14 to day 20, and their male offspring were assessed at 4 months of age. GS induced long-lasting alterations in intestinal microbiota composition: 16S rRNA gene 454 pyrosequencing revealed a strong trend towards decreased numbers of bacteria in the Lactobacillus genus, accompanied by elevated abundance of the Oscillibacter, Anaerotruncus and Peptococcus genera in GS animals. GS affected the offspring's hormonal stress response, inducing an elevation of stress-induced corticosterone release in the blood. GS also altered the plasticity of respiratory control, resulting in de-stabilized breathing at rest and abnormal breathing frequency responses to both hypoxic and hypercapnic challenges. Intriguingly, relative abundance of distinct bacteria genera were found to be significantly associated with certain respiratory parameters and corticosterone plasma levels. These data indicate that changes to gut microbial colonization may be involved in the development of maladaptive alterations in central control of breathing and stress resilience induced by GS.

DIET, GUT INFLAMMATION AND THE MICROBIOTA: PREVENTING AUTOIMMUNE DIABETES IN THE INTESTINAL MUCOSA

FALCONE, Marika; SORINI, Chiara; COSORICH, Ilaria; SAITA, Diego; CANDUCCI, Filippo
Presented by FALCONE, Marika

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Alterations of the gut microbiota (1-3) and intestinal immune dysfunctions (4-7) are found in patients with Type 1 Diabetes (T1D) even before the onset of clinical disease. Our data provide a direct link between chronic intestinal inflammation, the associated microbiota modifications and autoimmune T1D. We show that induction of chronic DSS-mediated colitis in BDC2.5 mice, that carry a transgenic TCR specific for an islet autoantigen, triggers the activation of islet-reactive T cells. Conversely, resolution of gluten-induced inflammation in the intestine of NOD mice prevents T1D. Importantly, we demonstrate that intestinal inflammation triggers autoimmune diabetes by altering the intestinal microbiota and mucosal immunity. The loss of barrier integrity in DSS-treated BDC2.5 mice leads to a significant reduction of mucin-degrading Prevotella spp while chronic intestinal inflammation in NOD mice reduced intestinal colonization with immunoregulatory Clostridia spp specialized in primary fiber degradation and butyrate production. These alterations of microbiota composition modify mucosal immunity and favor differentiation of effector Th17 cells. Our results suggest that mucosal immunity is not only calibrated to tolerate commensal bacteria and food components and prevent chronic inflammation in the intestine but is also instrumental in modulating autoimmune diseases at sites distal from the gut.

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COLONIZATION OF MICE WITH LACTOBACILLUS POSITIVE OR NEGATIVE HUMAN INFANT MICROBIOTA INFLUENCES IMMUNE MATURATION IN OFFSPRING

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Early-life microbiota is thought to be important in promoting immune maturation and we have previously shown that early-life colonization with lactobacilli is protective against a future development of allergy. In this study, we aimed at determining the effect of Lactobacillus colonization in the context of a normal early-life human microbiota on immune maturation in a murine model. Germ-free mice were colonized with microbiota from human infants that was either positive or negative for lactobacilli. The mice were then bred, and immune parameters evaluated in their offspring. Different immune cell types were determined by flow cytometric analysis of gut-associated immune compartments as well as in more central locations such as the spleen and liver.

Colonizing parents with microbiota with different levels of lactobacilli affected immune parameters, both in terms of proportion of different immune-cell phenotypes and functional responses. Activation-induced secretion of IFN-g and IL-17 was found to be lowest in offspring of mice that were colonized with microbiota containing high levels of lactobacilli, and these had also lower IgA in serum. This mouse-model indicates that normal human microflora associated with colonization by lactobacilli or not has the capacity to significantly affect immune- maturation and responses later in life.

MINIGUT – A SMALL VOLUME COLON MODEL WITH INCREAED THROUGHPUT

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The evaluation of pre-, pro- and synbiotics and their proposed beneficial effects is pivotal for their implementation as health promoting bioactives. While human intervention studies are the golden standard for such assessments, in vitro gastrointestinal tract models provide a useful tool for a fast and reproducible assessment of treatment-related changes. Most in vitro colon models which incorporate the complex gut microbiota are limited by high working volumes and low throughput. With the dual objective of facilitating the investigation of rare and expensive compounds, as well as an increase of throughput, we have developed a prototype in vitro colon model with a working volume of only 5 mL. The microbial community composition dynamics in the MiniGut reactors are monitored by MiSeq-based 16S rRNA gene amplicon high throughput sequencing. Changes in substrate and metabolite components are investigated by gas chromatography and mass spectrometry (GC-MS). Multiples of five reactors can be added to increase numbers of biological and technical replicates, allowing e.g. the investigation of intrapersonal variations in gut microbial dynamics and acquisition of data with decreased statistical interference.

CANCER & MICROBIOTA: POTENTIAL OF FECAL MICROBIOTA FOR EARLY-STAGE DETECTION OF COLORECTAL CANCER

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Colorectal cancer (CRC) is one of the main major problems in health care. The incidence is growing in western countries and no evident cause is found although germline DNA mutations in less than 5% of patients are identified and environment factors such as food, style of life, medicine, etc... are now suspected to induce majority of cancers. Exhaustive quantitative and qualitative evaluation of all factors from the environment is not possible. The microbiota may be considered as platform offering host and environment interactions for studying CRCs. And growing data show specific changes in microflora (dysbiosis) in colon cancer patients' stools or adherent to the colonic mucosa. The hypothesis that colon cancer might be a bacteria-related disease is suggested and perspectives discussed.

TAXONOMIC AND FUNCTIONAL METAGENOMIC ANALYSIS OF THE AIRWAY SAMPLES FROM CYSTIC FIBROSIS PATIENTS WITH LOWER AND HIGHER PULMONARY FUNCTION DECLINE

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Only recently scientists began to appreciate the complexity of polymicrobial infection such as cystic fibrosis (CF) and the implications it may have for disease prognosis and response to therapy. New insight into the impact of antibiotic treatment, patient age increasing, and periodic pulmonary exacerbation on CF microbiology has been obtained. However, it is still not clear how the airway microbiota composition changes during the severe lung disease progression. Understanding the role of the CF airway microbiota and detecting microbial species associated with the serious decline in lung function are key challenges for the delivery of new potential biomarkers for bacterial infections managements in CF patients and improving health care treatment. Dr Annamaria Bevivino from ENEA Casaccia Research Centre in Italy will present her work concerning the CF airway microbiota in patients with lower and higher pulmonary function decline, research activity funded by the Italian CF Research Foundation. Recently, her group showed that the airway microbial communities of CF patients change with a severe decline in lung function (PLoS ONE 2015;10:e0124348). In addition, they can make out that functional genes related to metabolism and antibiotic resistance changed along with a substantial decline in lung function. A relatively complex bacterial community is present only in stable patients with a normal/mild lung function whereas higher levels of lung decline can lead to a decrement of this bacterial complexity leaving the way clear for the proliferation of pathogens bacteria.

This work was supported by Grants from Italian Cystic Fibrosis Foundation (FFC#8/2012, FFC #10/2014 and FFC#14/2015).

METAGENOMIC ANALYSIS OF THE MICROBIOTA IN PATIENTS RECEIVING ALLOGENEIC SCT: ROLE OF HAMRFUL VERSUS PROTECTIVE MICROBIOTA AND THE IMPACT OF ANTIBIOTIC PROPHYLAXIS AND TREATMENT

Ernst Holler, Daniela Weber, Peter Oefner, Rainer Spang, Katja Dettmer, Frank Stämmler, Andreas Hiergeist, Josef Koestler, Wolfgang Herr, Andre Gessner
Presented by HOLLER, Ernst

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The role of the intestinal microbiota in allogeneic stem cell transplantation (SCT) has been well known for a long time, as van Bekkum reported almost absence of intestinal GvHD in germfree mice in 1974, and prophylactic decontamination has become standard of care for many years. More recently, serial metagenomic analysis of intestinal stool samples as well as metabolic approaches monitoring bacterial metabolites such as indoxylsulfate allowed a more detailed insight. Severe dysbiosis occcured early after transplantation (d0-10) and was characterized by an increase in enterococcal load and low indoxylsulfate levels indicating loss of protective clostridiales. Dysbiosis predicted severe intestinal GvHD and was associated with an increased long term treatment mortality underlining the central role of intestinal homeostasis in intestinal immune tolerance. Interestingly, type of antibiotic prophylaxis, early and prolonged use of systemic broad spectrum antibiotics and NOD2/CARD15 genotype were risk factors of early dysbiosis. Overall, these data suggest that modulation of microbiota by pro- and prebiotics or by altered strategies of antibiotic use might help to prevent intestinal inflammation and improve long term outcome in the setting of SCT.

LACTOBACILLUS SALIVARIUS LS01 AND BIFIDOBACTERIUM BREVE BR03 COMBINATION IN ATOPIC DERMATITIS: WHICH EVIDENCES?

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Atopic Dermatitis (AD) is a chronic, inflammatory skin disease that usually arises during early childhood and can last throughout adulthood.

Seasonal and perennial inhalant allergens, alimentary allergens, contact allergens, along with infections, contact irritants and emotional stress represent the common triggers for AD exacerbations. A deficiency in the barrier function of the gut is also involved in the pathogenesis of AD. The natural microflora gives its contribute to regulate mucosal barrier function and intestinal permeability.

Bacterial probiotics use against AD in pediatric population have been recently studied, with encouraging results. Probiotics have indeed showed a positive effect in the management of specific diseases, modulating mucosal immune responses and reducing gastrointestinal inflammation in children with alimentary allergies. These observations are probably due to the fact that natural intestinal microflora plays a crucial role in developing and maintaining normal immune functions and immunological tolerance.

Other immunomodulatory effects of probiotics are: the stimulation of intestinal immune system, the improvement of mucosal barrier functions, the induction of anti-inflammatory cytokines production and the help in maintaining immunological tolerance.

Recently, our group showed a good clinical efficacy and an improvement in the cytokine expression profile after treatment with L.salivarius LS01 in adult and pediatric patients suffering of moderate/severe AD.

Additional in vivo and in vitro studies have demonstrated that L.salivarius LS01 additioned to Bifidobacterium breve BR03 can have a positive clinical effects as well as an improvement of the intestinal balance with a decreasing of Staphylococci load and a reduced intestinal permeability.

In a very recent in vitro study this combination showed promising probiotic properties and beneficial immunomodulatory activity that are increased respect to the strains tested alone.

THE INTERACTIONS BETWEEN THE HOST, THE VAGINAL MICROBIOTA AND SEXUALLY TRANSMITTED INFECTIONS

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The vaginal microbiota forms the first line of defense against sexually transmitted infection (STIs). Population based surveys of the bacteria inhabiting the vagina have shown that several kinds of vaginal microbiota exist, that differs in bacterial composition and abundance. Further, in some women, these communities are dynamic and can change over short period of time, while in other, they are highly stable and do not change. The impact of both composition and dynamic of the vaginal microbiota on the susceptibility to diseases is still poorly understood. The application of modern genomic technologies, ecological principles and in vitro modeling affords a better understanding of the role of vaginal microbiome in health and diseases. Metagenomic sequencing provides a comprehensive view of the genetic make up of the bacterial species comprising the vaginal microbiome, and highlights associations between different genomic species of the same genus, or strains of the same species and their contribution to the protective properties of the vaginal microbiome. For example, the genomic make up of certain vaginal bacteria correlates with their ability to maintain a stable and protective vaginal microbiome. Further, the use of in vitro three-dimensional models of cervical epithelial cell lines is a good surrogate to evaluate the contribution of microbial products to the function of the vaginal microbiome. Using a 3D model of cervical epithelial cells, we have shown that the production of different isomers of lactic acid by Lactobacillus spp. is associated with protection against chlamydial infection in a pH dependent manner. Understanding the vaginal microbiome structure and functions is critical to devise novel and personalized strategies to maximize women's health.

THE IMPACT OF ERYTHROMYCIN ON THE VAGINAL MICROBIOME IN WOMEN WITH PRETERM PREMATURE RUPTURE OF THE FETAL MEMBRANES (PPROM)

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Disturbance of vaginal microbial communities during pregnancy is associated with increased risk of ascending infection, preterm premature rupture of the fetal membranes (PPROM) and preterm birth. Here we show that women presenting with PPROM have disparate vaginal microbiomes that are correlated with specific urinary metabolic profiles that may be useful for patient stratification. Erythromycin treatment for PPROM is associated with increased vaginal microbial diversity and fails to exhibit anti-inflammatory effects on vaginal epithelial cells in vitro. These results provide evidence that erythromycin treatment for PPROM does not resolve vaginal dysbiosis and alternative treatment strategies should be considered.

IDENTIFICATION OF BLOOD BACTERIAL PROFILES ASSOCIATED WITH LIVER FIBROSIS IN OBESE PATIENTS

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Although the modification of blood microbiota has been implicated in metabolic disease, metagenomic analysis of blood has remained largely unexplored. In view of the suggested role played by bacterial translocation in liver disease and obesity, we sought to investigate the relationship between blood microbiota and liver fibrosis in cohorts of patients with severe obesity.We carried out a cross-sectional study of obese patients, well-characterized with respect to the severity of the NAFLD, in a population comprising 50 patients of the Florinash cohort. Microbiota from patients was analyzed both quantitatively (blood) by 16S rDNA qPCR and qualitatively (blood and feces) by 16S rDNA targeted metagenomic sequencing.The 16S rDNA concentration was significantly higher in patients with liver fibrosis whereas the diversity indices were lower for all taxonomic levels considered. The assignation of 16S rDNA sequences showed specific differences in the proportion of specific bacterial taxa in both blood and feces that correlate with the presence of liver fibrosis thus defining a specific signature of the liver disease. We have shown that quantitative and qualitative changes in blood microbiota are associated with liver fibrosis in obese patients. Blood microbiota analysis provides potential biomarkers for the detection of liver fibrosis in this population.

ANAEROBICALLY CULTIVATED HUMAN INTESTINAL MICROBIOTA

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Presented by DAHLGREN, Atti-La

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For almost 20 years we have produced a human intestinal microbiota under strict anaerobic conditions, as an in vitro batch culture. This cultivated microbial ecosystem designated anaerobic cultivated human intestinal microbiota (ACHIM) has been reinoculated every second week during the years into new production batches. The cultivated microbiota has retained a microbial diversity comparable to that of feces from healthy donors, containing the major bacterial phyla Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria, as confirmed by gene sequencing.This batch-cultured microbial ecosystem has been tested for safety and clinical efficacy for many years in more than 400 patients, most of them suffering from recurrent Clostridium difficile-infection (RCDI). No adverse events and a cure-rate of more than 90 % have been reported in these patients, most of which received the cultivated microbiota administered into the upper duodenum by gastro scope.Results will be presented from an ongoing randomized controlled clinical study in Sweden, in which the efficacy of ACHIM is being compared to vancomycin in the treatment of RCDI. Clinical results from patients suffering from other diseases associated with gut dysbiosis will be reported briefly.

ANTIBIOTIC EFFECT MONITORING USING METAGENOMIC SIGNATURES

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We have collected a large collection (over 600 samples) of metagenomics samples from patients undergoing antibiotic therapy, Treatment of COPD, Helicobacter pylori, Otitis and Pyelonephritis by commonly used antibiotic schemes shows distinct patterns of microbiota composition shifts, which depends on dosage, initial conditions of the patients and specific antibiotic. Additionally demonstrated is the possibility to detect antibiotic resistance gene amplification in metagenome and possible genes associated with gene amplifications. Finally single nucleotide polymorphisms responsible for antibiotic resistance are analyzed, several canonical mutations (fluoroquinolones resistance in DNA Gyrase) are found as well as other mutational hot spots. Overall study demonstrates high potential of metagenomic analysis in the antimicrobial surveillance.

SYN-004, A NOVEL STRATEGY TO PROTECT THE GUT MICROBIOME FROM THE DELETERIOUS EFFECTS OF RESIDUAL IV β-LACTAM ANTIBIOTICS

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SYN-004 is a clinical stage oral β-lactamase therapy for use with intravenous (IV) β-lactam antibiotics to preserve the gut microbiome by degrading residual antibiotics excreted into the intestine. The intended indication for this enzyme is prevention of Clostridium difficile infection (CDI) and antibiotic associated diarrhea (AAD). SYN-004 was demonstrated to be safe in two preclinical studies in dogs and to not affect the systemic pharmacokinetics of the intravenous antibiotics. SYN-004 has now been studied in two phase 1 clinical trials, a single-ascending dose study and a multiple-ascending dose study, which demonstrated that oral SYN-004 was safe and well tolerated with negligible systemic availability. Two phase 1b/2a clinical trials have been initiated investigating oral SYN-004 in combination with IV ceftriaxone in healthy subjects with functional ileostomies to determine whether SYN-004, i) degrades intestinal ceftriaxone, ii) has any effect on systemic ceftriaxone, iii) is safe when delivered in combination with ceftriaxone and iv) is functional in the presence of proton pump inhibitors. A phase 2b double-blind, placebo-controlled study in patients receiving treatment with IV ceftriaxone for active infection is planned to start in 2015 to determine whether SYN-004 protects the gut microbiome and prevents opportunistic enteric infections like C. difficile.

DOES THE GUT MICROBIOME AFFECT MUCOSAL IMMUNE ACTIVATION AND INTRARECTAL SUSCEPTIBILITY TO SHIV ACQUISITION IN NAÏVE RHESUS MACAQUES?

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Presented by SUI, Yongjun

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The role of commensal bacteria in modulating immune activation in the gastrointestinal (GI) tract is largely unknown. As the risk of HIV-acquisition is increased by gut immune activation, we indeed observed that two cohorts of naïve Indian rhesus macaques with different rectal immune activation demonstrated significantly different challenge outcomes in the repeated low-dose intrarectal simian-human immunodeficiency virus (SHIV) challenge study. The rectal mucosal immune activation correlated inversely with the number of intrarectal exposures needed for viral infection. To assess whether microbial communities account for this non-pathogen-associated GI immune activation, we examined the gut microbiome by 16S rRNA MiSeq using the fecal samples collected one-week before the challenges. The preliminary principal component analysis of the gut taxa showed that these two cohorts had different microbial compositions. Down-regulation of the ratio of bacteroides/prevotella was observed in the more susceptible cohort, similar to that of HIV-infected patients. Furthermore, local immune activation might be influenced by firmicutes, which was more abundant in the resistant cohort and inversely correlated with rectal viral target cells. Detailed analysis and potential confirmatory studies are underway. The data provides novel insights into microbiome-host interactions, and paves the way for potential strategies to reduce the susceptibility to HIV-1 transmission.

ASSOCIATION OF GUT MICROBIOTA AND HOST SERUM CHOLESTEROL

LA-ONGKHAM, Orawan (1); KEAWSOMPONG, SUTTIPUN (1); NAKAYAMA, JIRO (2); NITISINPRASERT, SUNEE (1)
Presented by LA-ONGKHAM, Orawan

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The intestinal microbiota has a remarkable potential to influence host lipid metabolism via numerous activities. In this study, we characterized the gut microbiota of Thai adults aged 30-40 years divided into 3 groups based on their total serum cholesterol level (TC). Eight, nine and twelve subjects with high (HI), borderline high (BH) and normal (NM) TC level were analyzed by pyrosequencing technique with barcoded primers targeting the V6 to V8 region of the bacterial 16S rRNA gene. The relative abundance of Phascolartobacterium, Clostridium sensustricto and Alistips revealed a moderate negative correlation with TC level. Interestingly, the one species, Phascolartobacterium faecium displayed remarkable a strong negative correlation with TC level ($r = -0.507$, $P = 0.005$), and was significantly high abundance in NM subjects. In addition, Alistipes shahii and Gemmiger formicilis showed a moderate negative coefficient of -0.443 and -0.449 to TC level, and also exhibited significantly higher abundance in NM subjects than BH and HI subjects. Our results suggested that these gut microbiota may influence serum cholesterol. Here, principal component analysis based on food frequency questionnaire for estimating habitual dietary intake revealed that the dietary consumption and subjects with normal TC level were associated.

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MICROBIALLY MEDIATED BILE ALTERATIONS AFFECTS HOST METABOLISM

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SHANAHAN, Fergus; GAHAN, G Cormac
Presented by JOYCE, Anne Susan

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The gastro intestinal tract is accepted as a super organ where host factors along with the resident microbiota can dictate human health. The resident microbiota is influenced by many factors including disease, diet, medication as well as the host factors and also the ability to persist in the presence of bile. Bile is mainly composed of individual bile acid (BA) moieties and the ability to metabolise BA is a feature unique to some gut resident and associated microorganisms. Microbial BA modifying enzymes, bile salt hydrolases (BSHs), are distributed among the major phyla of bacteria in the gut as well as the gut Archaea and therefore the gut microbiota has a significant role in determining the complexity of bile acids and indeed their relative levels in BA profiles. A number of bile acids are recognized as natural or indeed competitive ligands for nuclear receptor signaling indicating their potential to influence host metabolism and impact upon host metabolic processes both locally and potentially systemically. Here, we show that bacterial bile salt hydrolase (BSH) mediates a microbe-host dialogue which functionally influences host lipid metabolism and plays a profound role in cholesterol metabolism and weight gain in the host.

ORAL SUPPLEMENTATION WITH L-GLUTAMINE ALTERS GUT MICROBIOTA AND PRO-INFLAMMATORY CYTOKINES OF OBESE AND OVERWEIGHT SUBJECTS

DE SOUZA, ZZ ALESANDRA; ZAMBOM, Z Adriano; ABBOUD, Y KAHLILE; REIS, K Sabrina; TANNIHÃO, FABIANA; GUADAGNINI, DIOZE; SAAD, JA MARIO; PRADA, O Patricia
Presented by PRADA, O Patricia

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Objective: The aim was to determine the effect of L-glutamine (GLN) on gut microbiota composition, waist circumference (WC), pro-inflammatory cytokines and hormones levels and insulin sensitivity in overweight and obese humans.

Methods: 67 overweight/obese adults with body mass index (BMI) between 25.03-47.12 kg/m2, were randomly assigned to either receive 30g of oral L-alanine (ALA) (control) or GLN daily for 14 days. Parameters were collected before and after supplementations.

Results: GLN altered Firmicutes and Actinobacteria phyla compared to ALA group. The F/B (Firmicutes/Bacteroidetes) ratio decreased after GLN. Dialister, Dorea, Pseudobutyrivibrio and Veillonella, belonging to the Firmicutes phylum, decreased after GLN. GLN did not change BW and BMI, but, reduced WC and serum leptin levels, suggesting a decrease in fat mass. GLN reduced insulin levels, HOMA index, serum TNF-α, IL-6 and lipopolysaccharides (LPS) levels. GLN did not alter serum adiponectin, GLP-1 and glycemia. ALA had no effects on these parameters.

Conclusion: GLN, for a short time, altered the composition of the gut microbiota in overweight and obese humans reducing the F/B ratio, which resembled weight loss programs. In addition, GLN reduced low-grade inflammation and improved insulin sensitivity, suggesting that may be an interesting therapeutic approach for individuals with overweight and obesity.

BACTERIAL COMMUNITY MODULATE BEHAVIORS OF EUKARYOTIC ORGANISMS: NEW PERSPECTIVES IN RESTORATION OF THREATENED CORAL REEFS

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Presented by AREVALO FERRO, Catalina

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Marine organisms have a complex life cycle that involves larval metamorphosis above surfaces, crucial to establish ecosystems as coral reefs. Nowadays corals face many threats and deterioration rates are alarming. Many projects try to find models that allow the recovery of coral reefs, focused in the interaction between bacteria and cnidarian larvae. Bacteria establish biofilms on surfaces producing, among others, Quorum Sensing (QS) molecules as N-acyl-L-homoserine lactones (AHLs). Here we evaluated the effect of AHLs on migration and metamorphosis of Hydractinia symbiolongicarpus, a hydrozoan that grows on shells of the crab Pagurus longicarpus. We first performed a characterization of the cultivable community of shells. Afterwards, signaling molecules extracts obtained from the community were analyzed by thin layer chromatography. We found that three bacterial species from shells synthetize four different AHLs or similar molecules. These extracts were tested in bioassays with H. symbiolongicarpus larvae, finding that QS molecules strongly induce the migration of larvae, a "pre-settlement" behavior in marine larvae. Induction of metamorphosis was more moderate; suggesting that other molecules might act synergistically with QS molecules to induce the whole metamorphosis process on surfaces. These molecules could be used in strategies directed to restore affected coral reefs.

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3rd World Congress on

Targeting Microbiota

Abstracts for Oral Presentation

Day 3 – October 23, 2015

FECAL MICROBIOTA TRANSPLANTATION: RECENT SCIENTIFIC DATA

SOKOL, Harry

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Dr Sokol will present the recent scientific advances of the fecal microbiota transplantation. He will highlight the recent clinical perspectives during ISM congress.

FECAL MICROBIOTA IN PEDIATRIC INFLAMMATORY BOWEL DISEASE AND ITS RELATION TO INFLAMMATION

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We investigated the intestinal microbiota in pediatric IBD especially related to the therapeutic response to anti-TNFα. The microbiota varied along a gradient of increasing intestinal inflammation. Baseline microbiota was predictive of anti-TNF-α responses. During the induction of anti-TNF-α, the microbial diversity and similarity to the microbiota of healthy controls increased in the responder group, but not in the non-responders.

JURISTICAL HURDLES AND POSSIBILITIES FOR COMPANIES PROVIDING FAECAL TRANSPLANT SERVICES

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Juristical hurdles and possibilities for companies providing faecal transplant services
According to definition in the broadest of terms, a drug is a chemical substance that has known biological effects on humans or other animals. However, what is a faecal transplantation in a legal context.

As a company with years of experience in the preparation of individual drugs based on microorganism we will present our experience with the German registration bodies and authorities on our application for faecal transplant preparations under GMP conditions.

BUSINESS, REGULATORY AND CLINICAL CHALLENGES IN COMMERICIALIZING A NEXT-GENERATION FMT DRUG FOR RECURRENT CLOSTRIDIUM DIFFICILE INFECTION: A U.S. PERSPECTIVE

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Background: There is a growing acceptance of fecal microbiota transplantation (FMT) for the treatment of recurrent Clostridium difficile infection (CDI) as well as interest in possible applications of the therapy for other diseases. Multiple companies are working on comercial applications of drugs based on human-derived microbes. However, the complex regulatory environment in the U.S. poses special challenges.

Objectives: Describe the current environment in the U.S. for developing FMT-based therapies.

Methodology: Case study: RBX2660 (microbiota suspension) for the treatment of recurrent CDI. RBX2660 is the first drug based on the human microbiota to begin moving through the U.S. Food and Drug Administration.

Results: RBX2660 has been demonstrated as safe for the treatment of recurrent CDI in a Phase 2 open label safety study. It is currently being investigated in a first-of-its kind randomized double-blind placebo-controlled Phase 2B study.

Conclusions: Advancing knowledge about FMT requires a structured program of clinical research best conducted under regulatory guidelines similar to those used for pharmaceuticals. Such an approach sets the stage for a sustainable business model that will further the dvelopment of microbiota-based drugs for other more complicated conditions.

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MAJOR SHIFTS IN THE GUT MICROBIOTA ASSOCIATED WITH HIV INFECTION

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Gut microbiota modifications occurring during HIV infection have recently been associated with inflammation and microbial translocation. However, discrepancies between studies justified a comprehensive analysis performed on a large cohort.

Next-generation sequencing of the 16S rRNA gene was applied to the faecal microbiota of 31 HIV-infected patients and compared to healthy controls. Sera were used to test the presence of 25 markers of inflammation and/or immune activation.

Diversity was significantly reduced in HIV individuals when compared to the controls and was not restored by ART. The relative abundance of several anaerobic species was critically less abundant in HIV-infected group and inversely correlated with inflammation/immune activation markers whereas aerobic pro-inflammatory species were found enriched.

Imbalance between aerobic and anaerobic flora raises the question of antioxidant supplementation as an adjuvant therapeutic in HIV patients.

GUT MICROBIOTA: NOVEL & STRATEGIC PLAYERS IN CELIAC DISEASE PATHOGENESIS

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Disruption to the microbiota in early-life has been implicated in celiac disease pathogenesis. Early antibiotic use has been associated with the emergence of celiac disease. Some studies indicate that delivery mode is also influential in the development of this disease, although not all studies support this link. Infants at high risk of developing celiac disease exhibit dysbiosis, where the Bacteroidetes:Firmicutes ratio is decreased, B. fragilis and Staphylococcus spp. are increased, and less B. longum and Bifidobacterium spp. are present. The studies indicate a potential association but there is currently no evidence or described mechanisms for alterations in the microbiota to modulate celiac disease risk. We found that HLA-DQ8 mice with microbiota lacking pathobionts and Proteobacteria (altered Schaedler flora, ASF) exhibit reduced sensitivity to gluten-induced immunopathology compared to germ-free and SPF mice harboring Proteobacteria (Galipeau, Am J Pathol in press, 2015). Moreover, both expansion of Proteobacteria in SPF mice using targeted antibiotic therapy, and supplementation of ASF mice with an enteroadherent E. coli led to gluten immunopathology in protected mice. The results support the hypothesis the microbiota has both positive and negative modulatory capacity on gluten-induced immunopathology and may open microbiota targeted therapies to prevent celiac disease in individuals with moderate genetic susceptibility.

ENTERIC DIALYSIS—STABILIZATION OF THE GUT MICROBIOME USING PROBIOTICS AND PREBIOTICS (GUT-KIDNEY CONNECTION)

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Objectives:The use of beneficial microbes- probiotics is generally well recognized towards digestive, gut and immune health.Novel and niche application of probiotics and prebiotics as a dietary supplement in stabilization of Gut Microbiome towards Chronic Kidney Disease(CKD) is relatively new. Several observational small scale clinical studies from Kibow were carried out to help reduce some of the toxins and impart a better quality of life. Methodology: “Renadyl” a synbiotic dietary supplement was studied in randomized clinical trials in CKD 3 and 4 and ESRD patients. Results:Studies using Renadyl for “Enteric Dialysis” showed reduction in levels of various uremic toxins like urea and indoxyl glucuronide which is derived by the dysbiosed gut microbiota. Levels of C reactive protein also decreased with improved quality of life. This demonstrates the potential restoration of the gut microbiome dysbiosis with the use of specific strains of probiotics. Conclusion: The use of well researched clinically documented and safe probiotic/prebiotic dietary supplement has the potential to safely perform continuous 24h/7d uremic toxin removal and thus stabilize the gut microbiome and its dysbiosis.Hence the concept of “Enteric Dialysis” with continuous removal of uremic toxins may be the future key to providing an alternative HOPE for renal failure population.

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CONTRIBUTION OF HUMAN GUT MICROBIOTA METABOLISM IN ANTI-INFLAMMATORY ACTIVITIES OF ELLAGITANNIN-RICH MEDICINAL PLANTS

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Presented by PIWOWARSKI Jakub P.

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Ellagitannin-rich plant materials are used in phytotherapy as effective anti-inflammatory agents. Due to the not well-established bioavailability of ellagitannins, the mechanisms of observed therapeutic effects following oral administration still remain unclear.The aim of the study was to evaluate if selected ellagitannin-rich plant materials could be the source of bioavailable gut microbiota metabolites together with determination of produced metabolites' influence on innate immunity cells inflammatory response in vitro. The ex vivo formation of bioavailable urolithins A, B and C (UA, UB and UC) by human gut microbiota was established for aqueous extracts from Filipendula ulmaria, Geranium pratense, Geranium robertianum, Geum urbanum, Lythrum salicaria, Potentilla anserina, Potentilla erecta, Quercus robur, Rubus idaeus, Rubus fruticosus and pure ellagitannin-vescalagin. Urolithins were shown to modulate inflammatory response of human primary neutrophils, THP-1 cell line derived macrophages as well as RAW 264.7 murine macrophages (Fig. 1 and 2). Inhibition of NF-κB nuclear translocation was shown to be one of the potent mechanisms responsible for observed anti-inflammatory effects. Due to determined activity at the concentration range reachable in bloodstream, in the case of peroral use of the examined ellagitannin-rich plant materials the bioactivity of gut microbiota metabolites-urolithins has to be taken under consideration.

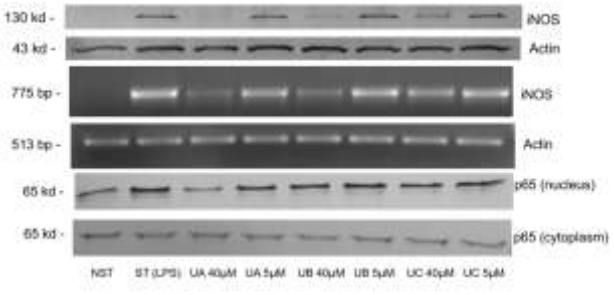

Fig. 1 Urolithins' impact on RAW 264.7 macrophages inflammatory response.

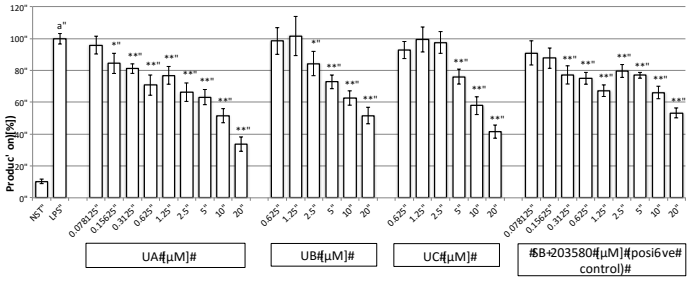

Fig. 2 Urolithins' impact on THP-1 cell line derived macrophages TNF-α production

MICROBIAL COMMUNITIES OF EARLY-STAGE CROHN'S DISEASE TISSUES

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Presented by O'BRIEN, Claire Louise

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Crohn's disease (CD) is characterized by bacterial imbalance and reduced intestinal microbial diversity. A decreased abundance of beneficial microbes, such as Faecalibacterium prausnitzii, is often observed. No study has determined if these changes are observed in the earliest Crohn's disease lesion: the aphthous ulcer, which overlie Peyer's patches and lymphoid follicles. The aim of this study was to describe the microbial communities of the earliest Crohn's disease lesion. Aphthous ulcers (n=12) and adjacent healthy mucosal biopsies (n=12) were obtained from CD patients, and age- and location-matched biopsies from healthy controls (n=12). DNA was extracted and amplified using primers targeting the V3-V4 regions of the 16S rRNA gene. The amplicon library was sequenced on a Roche 454 sequencer. Sequence analyses were done in Mothur, statistical analyses in JMP (v.9). Bacterial imbalance was not observed in the majority of aphthous ulcers and healthy CD mucosal biopsies. Faecalibacterium was not depleted in CD samples relative to controls, but bacterial diversity was reduced in CD tissues. Four bacterial taxa were common to all aphthous ulcers: Lachnospiracaeae, Unclassified Lachnospiraceae, Bacteroides, and an Unclassified bacterium.Changes in the gut microbiome associated with Crohn's disease may be a consequence, not cause, of the disease process.

COCOA BIOACTIVES, GUT MICROBIOTA AND MITOCHONDRIA

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Objectives. In this work we assess how cocoa flavanols and methylxanthines, two mayor Theobroma cacao bioactives, cooperate with intestinal microbiota in regulation of mitochondrial function.

Methods. Cocoa powder was fractionated by acetic acid/EtOH protocol. Residues were re-suspended in DMSO. Human colon specimens with mucus layer were obtained during diagnostic colonoscopy, homogenized in RPMI-1640 and incubated with various cocoa fractions in presence or absence of L. acidophilus or C. difficile. Short-chain fatty acids (SCFA) were measured in the incubation mixture by HPLC.

Results. Regardless of presence of cocoa flavanols, introduction of Clostridium difficile promotes appearance in the colonic homogenates of propionic acid which is a negative regulator of mitochondrial respiration, whereas Lactobacillus acidophilus promotes accumulation of butyrate, a well-known activator of mitochondrial oxidation. Butyrate production in the colonic homogenates was enhanced by co-inoculation of (-)-epicatechin, a predecessor of SCFA. Methylxanthines and products of their de-methylation formed by gut bacteria upregulate mitochondrial oxidation in seemingly independent manner.

Conclusion. Our preliminary results suggest that gut microbiota endorses biotransformation of cocoa flavanols and formation of short chain fatty acids - important messengers connecting gut bacteria and mitochondria.

TISSUE MICROBIOTA, THE NEXT GENERATION PARADIGM OF METABOLIC DISEASE

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The last decade demonstrated the crucial role of gut microbiota on metabolic diseases. We first identified that the low grade metabolic inflammatory process, which induces insulin resistance and obesity, was initiated by LPS absorbed from the gut microbiota. In addition, we now discovered that gut bacteria translocate towards adipose tissue and liver to establish a tissue microbiota responsible for metabolic phenotypes. The molecular crosstalk between the tissue microbiota and the host cells allows the identification of pharmacological targets and in the blood, the identification of predictive and stratifying biomarkers to ensure a personalized medicine and the validation of therapeutic and prevention programs. Our data demonstrate that NOD2, leptin and CD14 are molecular regulators of the bacterial translocation leading to the control of tissue inflammation, insulin resistance, and metabolic diseases. Upon an increased intestinal permeability due to an impaired intestinal immune defense the translocation of bacteria and LPS towards the adipose tissue favors the proliferation of preadipocytes and macrophages. A vicious proinflammatory circle takes place leading to the increase in adipose precursors. The latter will then overtime differentiate into adipocytes and contribute, not only to insulin resistance, but also to the increased fat mass. CD14 positive cells are responsible for this effect. We demonstrated that the increased bacterial translocation is due to an impaired intestinal defense where the dysbiotic microbiota from diabetic mice was responsible for the impairment of the intestinal macrophage to T lymphocyte crosstalk. This impairment was restricted to Th17 and Treg cells. The treatment of gut microbiota dysbiosis by prebiotics restored the intestinal defense and improved the metabolic phenotype. The adipose tissue microbiota was also dramatically impacted by the gut microbiota suggesting its important role on the onset of metabolic disease. Hence, a change in intestinal permeability and defense by the immune system is demonstrated on the top of the direct role played but a change in diet such as a fat-enriched diet on the adipose cells and insulin resistance.

REGULATION OF BODY FAT MASS BY THE GUT MICROBIOTA: POSSIBLE MEDIATION BY THE BRAIN

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Presented by JANSSON, John-Olov

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The gut microbiota is a complex community including the life forms bacteria and archaea, but dominated by bacteria mainly from the phyla Firmicutes and Bacteroidetes. The effect of gut microbiota on host physiology is not limited to the gastrointestinal tract. Emerging evidence suggest that gut microbiota could influence the central nervous system (CNS). New insight suggests that gut microbiota are an integral component regulation of energy balance, and that it can be regarded as an organ that contributes to efficient energy metabolism. The germ free mouse, that completely lacks gut microbiota, is a common model to study the interplay between gut microbiota and metabolism. Total lack of microbiota is a non-physiological condition, and it is nonspecific in its nature. However, it is has proven to be a good model for understanding the most constitutive functions of gut microbiota. In addition, data from alternative models such as probiotic- or antibiotic treatment could be problematic to interpret due to the immense complexity of gut microbiota, as well as our so far unsatisfactory knowledge of its constituents and their interrelations. Data from germfree mice are in line with interactions between gut microbiota and neuropeptides such as glucagon-like peptide (GLP-1) and brain derived growth factor (BDNF) in parts of the brain regulating body fat mass. Further studies are needed to evaluate the biological significance of these findings.

PANCREATIC BETA-CELLS LIMIT AUTOIMMUNE DIABETES: STRATEGIC ROLE OF GUT MICROBIOTA

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Antimicrobial peptides expressed by epithelial cells are largely described for the defense against pathogens but how they influence autoimmunity in peripheral tissues was unknown. We show that the cathelicidin related antimicrobial peptide CRAMP is expressed by the pancreatic endocrine cells.

Owing to its immunoregulatory capacities, CRAMP is protective against autoimmune diabetes in NOD mice, a model for type 1 diabetes.

We finally demonstrate that the gut microbiota via short-chain fatty acids directly governs the expression of CRAMP in the pancreas revealing the direct role of the gut microbiota in the maintenance of the immune homeostasis in peripheral tissues.

THE LINK BETWEEN DEPRESSION AND GUT MICROBIOTA

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Accumulating evidence suggests that the intestinal microbiota shapes immune responses of the host, affects its physiology by modulating gut motility and barrier homeostasis, and also influences host energy metabolism. Recent demonstrations of the ability of the intestinal microbiota to influence brain development, neurochemistry, and cognitive and emotive functions have prompted consideration of a microbiota-gut-brain axis. This axis has been implicated in the pathogenesis of a spectrum of disorders including intestinal and psychiatric diseases.

We have shown in mice that treatment with non-absorbable antibiotics alters behavior and brain chemistry, and that colonizing with specific microbiota can determine the behavioral phenotype of the host. To investigate the complex interactions between the microbiota and the host, we have investigated the role of bacteria on gut and brain function in a model of early life stress using maternal separation. We have found that in conventional mice, early life stress alters stress reactivity, induces changes in the gut cholinergic innervation and microbiota composition, and results in anxiety and depression. This abnormal behavior is absent in maternally separated germ-free mice but it is restored by bacterial colonization. This is associated with altered intestinal microbiota and metabolomic profiles. Our results indicate that early life stress induces changes in host physiology that lead to intestinal dysbiosis, which in turn triggers anxiety and depression.

USE OF MICROBIAL NETWORKS AS A STRATEGY TO RECOVER FROM GUT DYSBIOSIS

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Disruption of the normal composition and function of the gut microbiome (i.e. dysbiosis) is involved in a number of gastrointestinal pathologies such as chronic inflammation and metabolic syndrome. Attempts to treat these pathologies are frequently conducted with antibiotics or by means of functional ingredients (i.e. probiotics). Recent years have been revolutionized by the application of fecal microbial transplants, where the fecal microbiota from a healthy donor is transferred to a diseased individual.

We propose, as a possible alternative, the application of a well-characterized mixture of microorganisms that can guarantee the desired functionality and stability under fluctuating environmental conditions, thereby recovering the negative effects of dysbiosis. Specific microbial strains selected for their interdependence in metabolic networking capacities have been assembled in a bottom-up approach.

Different sets of mixes have been tested for their capacity to restore situations of dysbiosis (i.e. IBD, *Clostridium difficile* infection, antibiotics) in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). The best mix (i.e. PD-V4G-002) has been then tested in different dysbiosis-induced models such as TNBS-induced colitis and antibiotic-associated dysbiosis mouse models showing its *in vivo* efficacy as a strategy to support the recovery from gut dysbiosis.

Work supported by FWO.

IMPACT OF A DYSBIOSIS CORRECTOR ON GUT MICROBIOTA FROM ADULT BLOOD SIBLINGS

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HERNáNDEZ MOEDANO, Adan; GONZáLEZ AVILA, Marisela
Presented by GONZáLEZ AVILA, Marisela

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This study focused to evaluate the use of a “Dysbiosis corrector” (DC) on gut microbiota (GM) from partners of adult blood siblings (obese and eutrophic). Twenty partners of adult blood siblings (one obese and one eutrophic) were evaluated their nutritional status and GM status. Fecal samples from patients were processed separately to obtain GM from two groups (obese and eutrophic population). A pool of GM samples was inoculated in Automatic and Robotic Intestinal System (ARIS) 1,2 adapted separately to both physiological conditions. ARIS was fed with a formulated diet according to habitual feeding of each type population without modifications. Then, it was administrated a DC for single and continuous administration. Samples from ARIS were obtained at initial status, first and continuous administration. They were processed to determine GM changes and nutrient bioavailability (protein, lipids, carbohydrates, aminoacids and sugars). Microbiological analysis showed statistical differences between GM from every partner. Evaluation in ARIS showed GM modulation; higher amounts of proteins bioavailable, and lipids and sugars bioavailability diminished only for obese population. GM was different in the two study groups and it was different according to reports from other countries3. The use of a DC modifies GM and nutrient bioavailability.

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HUMAN MICROBIOME USING POLYPHENOL RICH FOODS, SUPPLEMENTS, AND PREBIOTICS, COUPLED WITH AVOIDANCE OF MAJOR DIETARY LECTINS REVERSES ENDOTHELIAL DYSFUNCTION

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Microbiome (MB) alterations promote inflammation and Endothelial Dysfunction (ED). A change in diet to alter MB, was studied for effects on ED, measured by Peripheral Arterial Tonometry (PAT) in 200 pts with vascular risk factors.Pts ate a diet of leafy greens, brassica vegetables, olive oil, nuts, radical reduction of grain, legumes, and fruits; and 4 oz amts of animal proteins and Casein A2 cheeses (The Matrix Protocol). Pts took 4,000 mg Fish Oil, 200mg Grape Seed Extract, 50 mg Pycnogenol, Grapefruit Seed Extract, 1 Tbs Balsamic Vinegar before meals, Glucomannan, and Inulin as prebiotics daily. Inflammatory markers were measured for 1 year. Endothelial Reactivity (ER) before and after a 5-minute arm occlusion using the EndoPAT 2000 (Itamar, Israel) was taken at baseline and 6 months.Results: TNF-alpha levels decreased from 4.5+/-1 to 2.5+/-0.4 (nl<2.5 pg/ml;) hs-CRP decreased from 4.5+/-2 to 0.9+/-0.8mg/L. Baseline ER was 1.88+/-0.7 (range 1.0-3.3), with 145/200 pts (72%)having ED (less than 1.60). At 6 months, ER increased to 2.25+/-0.5 (range 1.2-3.6) (p<0.01). We conclude that a limited grain, legume, fruit diet with emphasis on greens, olive oil, nuts, limited animal proteins; with fish oil, polyphenols and prebiotic fibers to manipulate the MB, normalizes ER and inflammation.

NEW INSIGHTS INTO POST-INFECTIOUS SEQUELAE: ENTEROPATHOGEN-INDUCED DISRUPTIONS OF HUMAN GUT MUCOSAL MICROBIOTA BIOFILMS CAUSE INTESTINAL INFLAMMATION

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Gut microbiota dysbiosis is associated with post-infectious intestinal and extra-intestinal inflammatory disorders via unknown mechanisms. Studies mostly investigate fecal microbiota, rather than mucosal microbiota biofilms. Campylobacter jejuni and Giardia duodenalis cause post-infectious sequelae. AIM: To uncover mechanisms causing post-infectious intestinal inflammation. METHODS / RESULTS: Giardia caused post-infectious intestinal hypersensitivity (balloon distension), after clearance of the parasite, in jejunum and rectum of neonatal rats. In Calgary Biofilm Device TM -enriched human biopsies, G. duodenalis or C. jejuni reduced microbiota biofilm exopolysaccharide (confocal/scanning, and biochemical analyses), promoted the release of planktonic bacteria (CFU), and favoured representation of Clostridiales bacteria (ILLUMINA, TRFLP). In germ-free mice, human microbiota exposed to Giardia increased intestinal lymphocyte aggregates, and augmented IL-1beta and other pro-inflammatory mediators. Planktonic bacteria from microbiota rendered dysbiotic by Giardia induced apoptosis, disrupted tight junctional ZO-1 and occludin, and translocated through human epithelial monolayers. Dysbiotic microbiota increased virulence genes and induced lethal toxicity in C. elegans. CONCLUSION: Enteropathogens disrupt human intestinal microbiota biofilms and release planktonic pathobionts, that cause post-infectious intestinal inflammation.

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ANTIBACTERIAL MOUTHWASH BLUNTS ORAL NITRATE REDUCTION AND INCREASES BLOOD PRESSURE IN TREATED HYPERTENSIVE MEN AND WOMEN.

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Background: Endothelial nitric oxide (NO) is fundamental to cardiovascular health. Nitrate provides a significant contribution to the circulating NO pool through the nitrate-nitrite-NO pathway. A critical step in this pathway is reduction of nitrate to nitrite by the oral microbiota. We aimed to assess the effects of antibacterial mouthwash on nitrate-nitrite-NO metabolism and blood pressure in treated hypertensive individuals.

Methods: Fifteen treated hypertensive individuals (mean age 65y) were recruited to a randomised controlled cross-over trial. Effects of 3 day use of antibacterial mouthwash on oral nitrate to nitrite reduction, salivary and plasma nitrate and nitrite, plasma cGMP and systolic and diastolic blood pressure were compared to control (water).

Results: Relative to control, three day antibacterial mouthwash use resulted in decreased oral nitrate to nitrite reduction (P=0.02), decreased salivary nitrite (P=0.01), increased salivary nitrate (P<0.001), and a trend toward a decrease in plasma nitrite concentration (P=0.09). Mouthwash use also resulted in higher systolic blood pressure (2.3 mm Hg; 95%CI: 0.5, 4.0; P=0.01), but not diastolic blood pressure (P=0.4) or plasma cGMP (P=0.7).

onclusion: Interruption of the nitrate-nitrite-NO pathway through the use of antibacterial mouthwash was paralleled by a small elevation of systolic blood pressure in treated hypertensive men and women.

THE EFFECTS OF CLOSTRIDIUM RAMOSUM ON ENTEROHAEMORRAGIC ESCHERICHIA COLI O157:H7

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Presented by KOYANAGI, Yukako

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Introduction: The intestinal flora plays a vital role in protecting host from enteric infections. We found that Clostridium ramosum, a member of the intestinal flora, protects mice from infection with enterohaemorrhagic Escherichia coli (EHEC). There are no reports about functional role of C. ramosum against enteric infections. In this study, we investigated the inhibitory effects of C. ramosum to clarify its mechanism against EHEC.

Methods: The inhibitory effect of C. ramosum on biofilm produced by EHEC O157:H7 was evaluated. The effect for adhesion of EHEC on the surface of intestinal epithelial cells was determined by using Caco-2 cells.

Results and Discussion: C. ramosum inhibited biofilm formation of EHEC. Culture supernatant of C. ramosum (CR-sup) and organic acids contained in CR-sup also showed similar effects. These results indicate that C. ramosum-secreted factors, in particular, organic acids play a vital role in inhibition of EHEC. In addition, adhesion of EHEC on the surface of Caco-2 cells was reduced in co-culture with C. ramosum.

These findings suggest that C. ramosum interferes with the survival and colonization of EHEC via a secretion of metabolites in gut. This study showed new perspectives of C. ramosum as effective bacteria on EHEC infection.

THE MICROBIOTA SHIFTS THE IRON SENSING OF INTESTINAL CELLS

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Objectives: The amount of iron in the diet directly influences the composition of the microbiota. We investigate whether the microbiota itself may alter host iron sensing.

Methodology : A serial of gnotobiotic rodents was compared with germ free (GF). We monitored how the systemic iron parameters and the intestinal iron-related proteins are varying with the complexity and the microbes considered.

Results: In intestinal cells, gut microbes induce a specific iron-related protein signature. In both the proximal and distal parts of the digestive tract, microbial colonization led to a decrease in the amounts of apical iron uptake-related proteins and an increase in the amounts of proteins involved in the intracellular storage and luminal export of iron. Our results suggest that the intestinal cells of GF rodents may be depleted of iron. We also show that representative adult commensals (Bacteroides thateiotaomicron VPI-5482 and Faecalibacterium prausnitzii A2-165) or probiotic (Streptococcus thermophilus LMD-9) reproduce some of the signatures driven by a complex microbiota.

Conclusion: The microbiota plays a key role in iron homeostasis, by strongly modifying the levels of iron-related proteins in the epithelium (Deschemin et al).

The microbiota shifts the iron sensing of intestinal cells deschemin et al, Faseb fj. 2015, fj.15-276840.
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METHODS FOR IMPROVING HUMAN GUT MICROBIOME DATA BY REDUCING VARIABILITY THROUGH SAMPLE PROCRESSING AND STORAGE OF STOOL

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Gut microbiome community analysis is used to understand many diseases like inflammatory bowel disease, obesity, and diabetes. Sampling methods are an important consideration for human microbiome research, yet are not emphasized in many studies. In this study, we demonstrate that the preparation, handling, and storage of human faeces are critical processes that alter the outcomes of downstream DNA-based bacterial community analyses via qPCR. We found that stool subsampling results in large variability of gut microbiome data due to different microenvironments harbouring various taxa within an individual stool. However, we reduced intra-sample variability by homogenizing the entire stool sample in liquid nitrogen and subsampling from the resulting crushed powder prior to DNA extraction. We experimentally determined that the bacterial taxa varied with room temperature storage beyond 15 minutes and beyond three days storage in a domestic frost-free freezer. While freeze thawing only had an effect on bacterial taxa abundance beyond four cycles, the use of samples stored in RNAlatershould be avoided as overall DNA yields were reduced as well as the detection of bacterial taxa. Overall we provide solutions for processing and storing human stool samples that reduce variability of microbiome data. We recommend that stool is frozen within 15 minutes of being defecated, stored in a domestic frost-free freezer for less than three days, and homogenized prior to DNA extraction. Adoption of these simple protocols will have a significant and positive impact on future human microbiome research.

PANCREAS INSUFFICIENCY IN CYSTIC FIBROSIS PATIENTS DRIVES A DIFFERENT FECAL MICROBIOTA AND METABOLIC PHENOTYPE

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Presented by IEBBA, Valerio

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Objectives Clinical evidence indicates a role for intestinal microbiota in Cystic fibrosis (CF) manifestations, but a report on how pancreas insufficiency could affect gut microbiota composition and function was never reported. We aimed at describing the fecal dominant microbiota and fecal/urinary metabolites in CF patients with (PS) and without (PI) pancreas sufficiency.

Methodology Two cohorts of CF patients underwent 16S MiSeq NGS sequencing (region V3-V4) to have a 'snapshot' of predominant microbial community in fecal samples (metagenomics), and Solid Phase Micro Extraction-Gas Chromatography/Mass Spectrometry (SPME-GC/MS) was used to characterize fecal/urinary metabolites (metabolomics).

Results Fecal microbiota composition significantly differed among PS and PI patients ($\chi2=6.107$, $P=0.0135$), with PI patients showing a peculiar fecal and urinary metabolites profiles ($\chi2=11.827$, $P=0.0006$). Multivariate analysis gave a model predictability of 100% for NGS profiles (Fig. 1), and 91% for SPME-GC/MS ones. PI patients showed a marked prevalence of Gammaproteobacteria ($P=0.0143$), Veillonella ($P=0.0019$) and Haemophilus ($P=0.0113$) (Fig. 2), along with toxic metabolites (cadaverine and putrescine) in both feces and urines.

Conclusion Preliminary results showed a predominant fecal microbiota associated with PI status, along with a peculiar fecal/urinary metabolite profile. These results could have a pitfall in diagnosis and CF patients' care having a pancreas insufficiency.

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THE GUT MICROBIOTA REGULATES MURINE CARDIAC TRANSPLANT OUTCOME

HITTLE, Elizabeth Lauren (1); BRINKMAN, Colin C. (2); FRICKE, Florian W. (1); BROMBERG, Jonathan (2); MONGODIN, F. Emmanuel (1)
Presented by HITTLE, Elizabeth Lauren

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The gut microbiota regulates systemic immunity, and microbiota characteristics can be defined that have pro- or anti-inflammatory effects. We hypothesized that the gut microbiota influences the survival of completely MHC mismatched murine cardiac transplants. Transplants were performed using BALB/c donor hearts grafted to C57BL/6 recipients. Gut microbiota manipulation was performed using an antibiotic cocktail on d -6 to -1, before fecal microbiota transplant (FMT) using fecal pellets from three groups: healthy C57BL/6, mice on d11 of pregnancy, and T-cell receptor transgenic colitic mice. Other groups received immunosuppression with anti-CD40L or tacrolimus plus FMT. 16S rRNA gene sequencing from fecal pellets was used to identify bacteria associated with transplant outcome. Cardiac allografts were harvested at d40 or rejection, and assessed for survival. Higher survival rates and lower fibrosis scores were observed with FMT performed using pregnant mouse feces/antibiotics/anti-CD40L. FMT performed with colitic mouse feces/antibiotics/tacrolimus resulted in higher fibrosis scores. Bifidobacterium pseudolongum was present in pregnant and normal mouse feces, but not colitic mice, and significantly associated with positive transplant outcome. In conclusion, the microbiota has a profound systemic effect on immunity with consequences for graft outcome. Microbiota manipulation could be beneficial in reducing inflammation, subsequent tissue damage, and graft rejection.

YOGURT DE PAJARITOS, A CHILEAN KEFIR WITH PROPERTIES OF INTEREST FOR INDIVIDUALS LACTOSE INTOLERANCE AND OSTEOPOROSIS, AND ITS POTENTIAL APPLICATIONS ON HUMAN HEALTH

DINAMARCA, Alejandro Miguel (1); IBACACHE-QUIROGA, Claudia (2); ASCENCIO, Estefany (1); RIQUELME, Valeria (1); DOBERTI, tamara (1); LEIVA, Gilda (1)
Presented by DINAMARCA, Alejandro Miguel

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Kefir is an ancient dairy fermented food produced by a microbial consortium formed by bacteria and yeasts. Its origin goes back until Russian Caucasus and, its consumption is of great interest for human health. During milk metabolization, there occur different microbe-microbe and microbe-substrates interactions that generate a stable ecological community with an eco-metabolism that removes undesirable compounds from milk, like lactose, whereas enzymes and molecules important for human health are synthetized. In this work we studied a custom made traditional kefir produced in Chile named Yogurt de Pajaritos in order to evaluate its potential to be used in different healthy and non-healthy individuals for the improvement of their life quality. Lactose degradation and the presence and activity of genes that encode enzymes with lactase activity were evaluated under different fermentation conditions. Fatty acids and inorganic compounds was also evaluated. Results indicate that Yogurt de Pajaritos includes a bacterium identified as Lactobacillus spp, witch contains a lactosidase gene that can be induced at different fermentation conditions. This kefir has low lactose concentration and a pH value of 4.5, and, with further studies, it could be recommended for individuals suffering from lactose intolerance and osteoporosis, under specific preparations protocols.

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3rd World Congress on

Targeting Microbiota

Abstracts for Poster Presentation by alphabetical order

OPIMIZED AND STANDARDIZED TECHNICAL AND BIOINFORMATIC SOLUTION FOR HIGH-THROUGHPUT TARGETED MICROBIOTA ANALYSIS

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Human microbiota is now considered as a real organ by scientific community that possesses his own physiology and pathology like others. Since few years, the revolution of Next-generation sequencing (NGS) platforms via 16S rDNA sequencing makes analysis of microbiota composition reachable, with data information never stop increasing. These technologies are now used routinely to assess bacterial community composition in complex samples. Molecular tool standardization has become one of the main challenging issue to allow high-throughput targeted sequencing approaches routine analysis allowing comparison between worldwide studies. Extraction and library preparation are critical steps that can induce biases between samples and have impact on taxonomical definition and the observed relative abundances of taxa. Here we present a fully high-throughput sequencing integrated solution successfully tested on several types of human microbiota comparison (faeces, biopsy, skin, sweat, saliva, and sputum samples…) Our high-multiplexing sequencing solution validated within complex metagenomics samples, provided high sequencing accuracy and technical reproducibility with no index biases based on taxonomic distribution analysis. Technical improvements are associated with a fully automated pipeline for raw data analysis and taxonomic affiliation. This solution is accessible through our services platform on both 454-Roche (GS Junior, GS FLX) and Illumina (Miseq 2*250pb, MiSeq2*300pb)

PROFILE OF TERM PLACENTAL MICROBIAL TRANSCRIPTOME

ANDRESON, Helena (1,2); ANDRESON, Reidar (3); SõBER, Siim (4); RULL, Kristiina (1,5,6); REIMAN, Mario (4); VAAS, Pille (7,8); TEESALU, Pille (7,8); REMM, Maido (3); LAAN, Maris (4)
Presented by ANDRESON, Helena

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The existence of various microbial DNA in human placental samples was described by Aagaard et al. (2014) using 16S rDNA and whole genome analyses. However, the DNA-based approach does not distinguish between living and dead bacteria. We aimed to characterize the abundance of living bacteria hosting the human placenta by utilizing the analysis of unmapped reads of placental RNA-Seq data from clinically well described cases over a range of term pregnancy outcomes. Methodology. Microbial profile of previously established RNA-Seq data of 40 placental samples taken at delivery (Sõber et al. 2015) was identified using MEGABLAST. Matches with highest bitscore for each read were recorded. Results. Subsequently, over 7.3 Gbp filtered non-human reads were analysed. Placental microbial transcriptome revealed three major bacterial phyla: Proteobacteria (60%), Actinobacteria (24%) and Firmicutes (16%). Consistent with the study based on 16S rDNA (Aagaard et al. 2014), the most prevalent single species present in every placental sample was Escherichia coli. Altogether 48 strains were identified. Conclusion. In conclusion, our data robustly demonstrates that placenta is a natural habitat for living bacteria (mainly for Proteobacteria). Although the prevalence of other bacterial phyla in term placentas appears to be moderate, their role is still to be investigated.

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A CROSS-SECTIONAL SURVEY TO DETERMINE THE KNOWLEDGE LEVELS REGARDING HYDATID CYST AMONG NURSES, DIETITIANS AND FOOD TECHNICIANS IN TURKEY

AYDIN, Mehmet Fatih (1); ADIGUZEL, Emre (1); KOC, Serife (1); COPLU, Mehtap (1); GOKMEN, Suleyman (2)
Presented by AYDIN, Mehmet Fatih

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Hydatid cyst (HC) is an important public health problem and has economical impact. We aimed to evaluate knowledge levels of nurses, dietitians and food technicians regarding HC in Turkey. A survey was performed with a questionnaire for HC. Results were evaluated by percentage and analyzed by chi-square test. Nurses, dietitians and food technicians have information about HC with percentages 89.13%, 53.06% and 40.00% ($p<0.05$). Food technicians knew what they must do infected organs (80.00%), nurses knew where cysts in the intermediate host seen mostly (86.95%), food technicians knew if cysts on internal organs can generate new cysts (85.00%), nurses knew if it play role in transmission that not washing the vegetables (92.39%) ($p<0.05$), dietitians knew if dogs are responsible for transmitting the HC (95.91%) with highest rates. Nurses, dietitians and food technicians said that they have ever participated to informative course about HC with percentages of 47.82%, 18.36% and 25.00% (p<0.05) and they said that there are some person who treated for HC around them with percentages of 17.39%, 2.04% and 10.00% ($p<0.05$). Nurses were found well-informed compared to others. Since some professionals have insufficient information it is recommended that information studies about HC should be performed in Turkey urgently.

PREVALENCE AND PHYLOGENIQUE IDENTIFICATION OF STAPHYLOCOCCUS AUREUS INVOLVED IN MASTITIS CASES IN ORAN, ALGÉRIA

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Presented by BENHAMED, Nadjia

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S. aureus mastitis are considered one of the major diseases in dairy cattle.This study determines phylogenetic profile of S. aureus isolated from Bovines mastitis inOran. MALDI-TOF,spa, MLST have identified.The molecular profile toxin was demonstrated byPCR. Antibiotic resistance of S. aureus and confirmed by amplification of the mecA gene. Results of spa typing, variety (T267, T021 and t007).MLST; reveals different(ST39, ST2598 and ST97).Toxin research shows that only some strains proved carriers of different virulence genes; pvlgene.Other strains were positive for tst gene (TSST-1), 100% of the S aureus isolates identified were SASM.this work have determined the phylogenetic profile, toxic and sensitivity profile to méticiline strains. Strains found produisantes as PVLare sensitive to methicillin,don't belong to ST30 commonly found in humans inAlgeria,relation to the type of stem ST97 are of bovine origin.study is the first molecular study of animal strains inOran.

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CARBAPENEM RESISTANT ENTEROBACTERIACEAE IN RECTAL MICROBIOTA OF HOSPITALIZED PATIENTS

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Presented by BORSA, Ata Baris

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Objectives: The aim of our study is to determine extremely antibiotic-resistant bacteria, carbapenem-resistant Enterobacteriaceae (CRE), in rectal microbiota of hospitalized patients.

Methodology: We investigated the presence of CRE's in rectal microbiota of hospitalized patients between October 2014–July 2015. Rectal swabs were collected from patients once a week. MacConkey agar containing 2mg/L Meropenem were used for screening. Suspected lactose-positive colonies identified by conventional methods. Carbepenem resistance confirmed with Etest; resistance mechanisms were investigated with Mastdiscs ID carbapenemase detection set.

Results: Out of 3323 samples, 10 (%0,3) *E.coli* and 74 (%2,2) *K.pneumoniae* were isolated. Interestingly, 6 *E.coli* and 20 *K.pneumoniae* were found susceptible to meropenem by Etest. 18 and 3 of susceptible *K.pneumoniae* and *E.coli* were positive for OXA-48. This were connected with weak-hydrolytic activity of OXA enzymes. Out of 54 meropenem-non-susceptible *K.pneumoniae*, 11 were found positive for OXA-48 and metallo-beta-lactamases together; 1 and 41 positive for metallo-beta-lactamases and OXA-48 alone respectively. Only 1 was positive for AmpC+porin loss. All meropenem-resistant *E.coli* were also positive for OXA-48.

Table 1. Resistance rates and mechanisms of the bacteria isolated from rectal swabs in MacConkey agar containing Meropenem. (MIC≤2 was accepted as susceptible according to EUCAST criteria)

	E.coli (n:10)		**K.pneumoniae (n:74)**	
	MIC≤2 (n:6)	MIC>2 (n:4)	MIC≤2 (n:20)	MIC>2 (n:54)
OXA + MBL	0	0	0	11 (%20)
OXA	3 (%50)	4 (%100)	18 (%90)	41 (%76)
MBL	0	0	0	1 (%2)
AmpC + Porin Loss	0	0	0	1 (%2)
Susceptible	3 (%50)	0	2 (%10)	0

Conclusion: Our results showed that, rectal microbiota investigation by using rectal swabs may be a useful method to determine a kind of dangerous bacteria, CRE's, even if they have enzymes with weak-hydrolytic activity.

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LIRAGLUTIDE CHANGES GUT MICROBIOTA AND REDUCES HEPATIC STEATOSIS AND FAT MASS IN TWO MODELS OF OBESITY MICE

MOREIRA, Gabriela (1); AZEVEDO, Flavia (2); SANTOS, Andrey (3); GUADAGNINI, Dioze (3); GAMA, Patricia (1); LIBERTI, Aparecido Edson (1); SAAD, Mario (3); DONATO, José (1); CARVALHO, Carla (1)
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Herein, we analyzed the liraglutide effect in the gut microbiota of ob/ob mice and high fat diet fed mice. Despite the improved glucose tolerance and insulin sensitivity in both mice models by liraglutide, there were great reductions in the density of lipid droplets in hepatocytes to less than 20% in both animal models accompanied by reduced adiposity in the high fat diet obese mice. Liraglutide treatment also induced inflammatory infiltrates attenuation in the liver of both rodent experimental models, and promoted epithelial goblet cells increased in diet induced obese animals. There were also changes in the intestinal flora within the liraglutide treatment in both animal models: reduced detection of Actinobacteria phylum. Accompanied by increased detection of Akermansia muciniphila in the diet induced obese mice, and reduced Proteobacteria phylum in the Ob mice. These data show that liraglutide is able to reverse the non-alcoholic steatohepatitis (NASH) and modify the intestinal flora of obese animals, beyond the known effects such as reduction of body weight, blood glucose control and insulin resistance. Furthermore, this drug can have a proliferative effect on the goblet cells of the intestinal mucosa.

SELECTION OF LACTIC ACID BACTERIA FOR THE PREVENTION OF DENTAL CARIES

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Presented by CASTRO, De Lourdes Erica

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The objective of this study was to isolate oral lactic acid bacteria, selecting those with a higher anti-cariogenic potential. 72 LAB were obtained from the dental surface of healthy children, 9 strains of which were preselected and subjected to further analysis. Antimicrobial activity was quantified against 8 native Streptococcus mutans isolates and the strain ATCC S. mutans 25175. Strains CB43, CB44, and CB45 inhibited the growth of each tested pathogen. Using the E-test technique, all 9 strains were susceptible to 13 of the 21 tested antimicrobial agents. A cariogenic model was made using human dental pieces and artificial saliva. Estimated specific growth rate for strains adhered to dental pieces was 0.63h-1±0.09(R2=0.89) for strain CB44, and 0.70h-1±0.18(R2=0.78) for strain CB45. Innocuousness of strains CB44 and CB45 was proved by treating Balb/c mice with 1x109 CFU of each strain. Animals treated with CB44 and CB45 showed no signs of disease. There were no significant differences in the hematocrit, total and differential leukocyte counts, neither IL-6 concentration in the spleen, lymph nodes, and intestines of treated mice when compared to the control group. Strains CB44 and CB45, identified as Lactobacillus fermentum, show potential to be part of a safe oral probiotic product.

ABUNDANCE AND VARIANCE IN TAXONOMY (‘AVEIT)

CHAKRABARTI, Anirikh; SIDDHARTH, Jay; CHOU, Chieh-Jason; LAUBER, Christian Lynn; PARKINSON, Scott James
Presented by CHAKRABARTI, Anirikh

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NGS has revolutionized explorations and analysis in the fields of microbiology, microbial ecology, contributing to our deeper understanding of health, and disease. The quality and quantity of processed sequencing data in the form of microbial taxa to represent diversity estimates of microbial communities, which is trending towards increasing number of samples and increase in sequencing depths, are in need of further attention and scrutiny. We present ‘AVeIT (Abundance and Variance In Taxonomy), an unbiased method to use all the available metrics (i.e. relative abundances in a sample, variance in a sample and abundance and variance in the whole study) in unison to identify members of microbial communities which could be potentially erroneous. In comparison to currently available strategies, at the level of taxa’s, in ‘AVeIT we systematically and reproducibly use the inherent abundance and variance of taxa in a given dataset, instead of using a priori thresholds, to determine the inclusion or rejection of taxa for further downstream analysis. While on one hand, our framework provides inferences that cannot be achieved using preexisting approaches; it does not affect the conclusions from traditional downstream analysis like PCoA.

VIRAL DETECTION IN AML GENOMES

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Presented by CHO, Eun Mi

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Background Human cancers, about ten to fifteen percent, are caused by viruses. Next generation sequencing technologies (NGS) enabled genome-wide virus detection studies in human cancer. A TCGA metagenome study drew a landscape of viral expressions in 19 cancer types by using whole transcriptome data. But, the result did not show viral etiology in acute myeloid leukemia (AML).

Method We performed paired-end whole genome sequencing for each 5 acute myeloid leukemia bone marrow and normal population blood samples on Illumina HiSex X Ten platform. We detected virus infection and integration sites in those ten samples by using iterative reference sequence customization method.

Results Enterobacteria phage subtype, Semliki forest, and Autograph californica nucleopolyhedro were commonly detected from AML and normal samples. Lcatococcus prophage subtype (requency 2/5), Coliphage subtype (frequency = 2/5), Hepatitis C (HCV) for Hubei (frequency = 3/5) and Moloney murine leukemia (MLV) (frequency = 1/5) viruses were detected only from AML patients.

Conclusion HCV is known as hepatocellular carcinoma and some lymphoma virus and MLV as leukemia virus in murine. But, our study suggests they may have viral etiology in human leukemia too. Further studies with more samples are warranted to validate the results.

CULTURE AND MOLECULAR ANALYSIS OF MICROBIAL COMPONENTS OF THE HUMAN OUTER ERA IN THE HEALTY, INFLAMED AND HEARING AID USERS

CONTE, Pia Maria (1); ALEANDRI, Marta (1); AMBROSI, Cecilia (1); MARAZZATO, Massimiliano (1); LEPANTO, Stefania Maria (1); LONGHI, Catia (1); BUSSU, Francesco (2); ANZIVINO, Roberta (2); GALLUS, Roberto (2); PALAMARA, Teresa Anna (1)
Presented by CONTE, Pia Maria

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The ear canal is an important ecological niches that is colonized by microorganisms that are still to characterize. The use of hearing aids is a predisposing factor to develop acute otitis externa and microorganisms responsible are poorly identified. The aim of this study was to determine microbial communities in the human ear canal and to identify specific bacterial communities that may be associated with otitis, in individuals wearing hearing aids or not. A total of 115 samples, 56 ear swabs and 59 hearing aids, fit open and closed, were collected. Microorganisms were identified by conventional phenotypic systems and by 16S rDNA gene sequence analysis. All samples with a high bacterial load had a polymicrobial profile, suggesting a possible synergy among different bacterial species. Coagulase negative staphylococci were the most frequent microbial isolates. Pathogens, such as Staphylococcus aureus, Streptococcus agalactiae, and opportunist, such as Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Corynebacterium pseudodiftericum, Corynebacterium afermentans, Bacillus spp., and Enterococcus faecalis were also recovered. This study describes the microorganisms retrieved from canal ear and hearing aid (HA). With the exception of coagulase negative staphylococci, each HA analyzed reflected a unique, varied and mostly polymicrobial bacterial composition, deeply different from non HA users.

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VIRULENCE, ANTIMICROBIAL AND HEAVY METAL RESISTANCE CHARACTERIZATION BY A NEW MICROARRAY AND WHOLE GENOME SEQUENCING IN SALMONELLA ENTERICA: IS THERE SIGNIFICANCE FOR THE COMMENSAL MICROBIOME?

FIGUEIREDO, Rui (1); CARD, Roderick M (2); NUNES, Carla (1); BAGNALL, Mary C (2); ABUOUN, Manal (2); NUNEZ, Javier (3); MENDONçA, Nuno (1); ANJUM, Muna F (2); DA SILVA, Gabriela Jorge (1)
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Gut inflammation can fuel horizontal gene transfer between pathogens and other gut bacteria (1). This study characterized the molecular mechanisms of virulence, antimicrobial and heavy metal resistance (AMR/HMR) of 106 Salmonella enterica strains of food origin. A high throughput virulence genes microarray was designed, and whole genome sequencing was used in strains with atypical microarray results (2). Salmonella Pathogenicity Islands and adherence genes were highly conserved, in contrast with prophages and virulence plasmid genes. The cdtB toxin gene and other Salmonella Typhi-related genes were detected in S. Typhimurium (Sal199). The cdtB gene was upstream of transposase IS911, and co-expressed with other Typhi-related genes. Infection with Sal199 induced cell cycle arrest, cytoplasmic distension, and nuclear enlargement in HeLa cells, and increased mortality in Galleria mellonella. AMR and HMR genes were associated with mobile genetic elements (MGE) such as transposons and integrons, located in IncHI2 and IncF plasmids that were easily transferred by conjugation to Eschericha coli J53. The findings indicate that S. Typhi specific virulence traits can disseminate to other serovars. The presence of virulence, AMR and HMR genes in MGE highlights their potential for dissemination, and reveals the likely contribution of gut pathogens to the evolution of commensal microbiome.

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BENZO[A]PYRENE IMPACTS HUMAN GUT MICROBIOTA FUNCTION

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Polycyclic Aromatic Hydrocarbons (PAH) are carcinogenic pollutants arising from oil and derivatives along with incomplete organic matter combustion. Population exposure occurs mainly with contaminated food products, leading pollutants to end up in the digestive tract. Benzo[a]pyrene (B[a]P), being the most studied PAH due to its high toxicity, has been chosen as marker of PAH occurrence and toxicity. While its metabolism by host cells is well known (Shimada, 2006), the impact on the gut microbiota, which play a key role in human health is still unexplored. By means of 16S barcoding, metatranscriptomics and metabolomics, we studied, in an in vitro model assay, the impact of B[a]P on two distinct gut microbiota on a structural, functional, and metabolic side. B[a]P didn't shift significantly the microbial structure but some trends appeared as the increase of the genus Sutterella, Sporanaerobacter and Oscillospira and the reduction of Rikenellaceae and Blautia. Some functional pathways were heighten such as protein export, secretion systems, cell wall compound biogenesis, DNA repair systems and hydrocarbon degradation pathways. Furthermore, the microbial volatile metabolome was altered. Those primary findings showed that food pollutants, such as B[a]P, may modify our gut microbiota and could contribute to disturb the gut homeostasis.

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HIGH SUCROSE MEDIATED ALTERATIONS IN GUT MICROBIOTA DISSOCIATE HEPATIC STEATOSIS FROM LIVER INFLAMMATION IN NAFL

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Introduction: Non-alcoholic fatty liver (NAFL) is the most common liver disease in affluent societies. Western-type diets and changes in gut microbiota have been link to obesity, the major risk factor for NAFL. Therefore, we examined the effects of a sugar-rich diet (HS) on gut microbiota in mice.

Methods: Adult C57BL6 mice were fed a normal chow (NC) or a HS diet for 16 weeks. Gut microbiota were analysed by 16S ribosomal RNA gene sequencing of DNA extracted from cecal contents.

Results: Hepatic steatosis was detected in HS diet group by H&E staining. However, analysis of mRNA expression of IFN-γ, Trail and IL-10 indicated an anti-inflammatory response within the liver. NC and HS diet fed mice showed clear differences in microbiota composition (p<0.001, F=21.2, one-way PERMANOVA). A reduction in mean abundance of bacteria belonging to the family S24-7 (20.7%), and an increase in mean abundance of Lachnospiraceae (10.9%), and Allobaculum (7.7%) contributed to the majority of dissimilarity between diet groups.

Conclusion: A HS diet induces hepatic steatosis without inducing inflammation. We detected alterations in gut microbiota in mice fed the HS diet. Higher rates of short chain fatty acids production by Firmicutes could be the possible mechanism for our findings.

IMPACT OF BARIATRIC SURGERY ON THE GUT MICROBIOTA OF CHILEAN PATIENTS

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Obesity is a serious health problem worldwide, including Chile. Bariatric surgery (BS) is a highly effective treatment for severe obesity, characterized by a marked and sustained weight loss. In addition to reduced caloric intake, certain studies have identified the intestinal microbiota as a contributing factor in weight loss in BS. An increase in Bacteroidetes and Proteobacteria has been observed in patients after BS. Here we compared the effects of three obesity treatments on the metabolic profiles and gut microbiota in Chilean patients. Patients undergoing gastric bypass (GB,N=5), sleeve gastrectomy (SG,N=5) and medical treatment (MT,N=6) were included in this study. Anthropometric parameters and plasma markers were determined at 0 and 6 months, and major phyla of the gut microbiota were quantified by qPCR. GB and SG were more effective in weight loss compared to MT, also improving glucose and lipid metabolism. Bacteroidetes, but not Enterobacteriaceae were increased in patients who underwent GB. The Firmicutes/Bacteroides ratio was increased in SG patients, but decreased in SG. Finally a negative correlation was found between Firmicutes levels and body weight. This study indicates that BS is more effective in weight loss, but GB and SG change the gut microbiota differently.

PIGS FED A WESTERN DIET DEVELOP FEATURES OF METABOLIC SYNDROME AND A MICROBIOME ANALOGOUS TO HUMAN OBESITY

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The Total Western Diet (TWD) for pigs was formulated using the principle of nutrient density (mass of nutrient/kcal/day), using the 50th percentile daily intake levels for macro and micronutrients from the National Health and Nutrition Examination Survey and translating that information to a pig diet. To determine effects of the TWD on metabolism and the microbiome, ten female pigs were assigned to either the TWD (n=5) or a control (CON), low-fat commercial sow diet (n=5). Pigs were fed for 12 weeks and allowed ad lib access to diets. Pigs fed the TWD gained significantly more weight, 92.8 ± 9.5 vs. 54.2 ± 11.2 kg ($p < 0.001$) and had an increased fasted blood glucose, 123.2 ± 13.7 vs 91.4 ± 7.9 mg/dl ($p < 0.01$) compared to control pigs. Pigs fed the TWD shifted from a Bacteroidetes to Firmicute dominated microbiome after 12 weeks on treatment ($p < 0.01$).

The microbiome of pigs fed the CON diet remained Bacteroidetes dominant. These data suggest that the American diet pattern when fed to pigs is obesegenic, diabetogenic and causes shifts in the microbiome associated with obesity in humans.

METHODS FOR INVESTIGATING MITOCHONDRIAL-MICROBIAL CROSS-TALK IN THE AGEING COLONIC EPITHELIUM

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Introduction: Evidence from animal models has shown that microbial-derived shortchain fatty acids (SCFAs), in particular butyrate, stimulate mitochondrialoxidative metabolism, resulting in enhanced HIF1-alpha stabilisation andincreased epithelial barrier function (1). A reduction in butyrate-producingbacterial species is associated with a number of inflammatory bowel conditions,further implicating butyrate in metabolism, mitochondrial function and bowelhealth (2,3). We have previously shown that the ageing colon contains a highproportion of epithelial crypts which have lost oxidative phosphorylation (OXPHOS)capacity due to the accumulation of pathogenic mutations in the mitochondrialDNA (4). RNA seq analysis of these crypts has revealed alterations in theexpression of genes involved in epithelial barrier function (unpublished). Furthermoremouse models of mitochondrial dysfunction (MD), with a premature agingphenotype have a large number of intestinal crypts with OXPHOS dysfunction (5) andan impaired ability to absorb fat. Aims: To investigate whetherage-related changes in intestinal mitochondrial capacity can influence thecomposition of the gut microbiome in PolgA mut/mut mice. In addition we willinvestigate whether exercise alters mitochondrial function. Methods: 16s rRNA, SCFA and inflammatory biomarkers from faecal samples andplasma will be measured, respectively.

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ANALYZING THE IMPACT OF BIOACTIVE COMPOUNDS FROM MUSCADINE GRAPE SKIN EXTRACT ON GENE EXPRESSION OF HELICOBACTER PYLORI

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Food consumption can affect the biodiversity of microbiota inside human gut. The human pathogen Helicobacter pylori infects > 50% of world population as a major causative agent of gastritis, peptic ulcers, and gastric carcinoma. In this study, the effects of sub-inhibitory concentrations of muscadine grape skin extract (MGSE) and constitutive polyphenols quercetin and resveratrol on H. pylori transcripts were determined by microarray analyses. Following 15 min exposure, 63, 39, and 29 genes were found to be differentially expressed in MGSE-, quercetin- and resveratrol-treated cells, respectively, with only one overlapping gene detected between resveratrol and quercetin treatments. Of the genes showing altered transcript amounts in response to each treatment, 22% encoded proteins involved in transcription/translation, 15% encoded proteins involved in energy production/metabolism, ~30% encoded hypothetical proteins, and ~34% genes related to other cellular processes, amino acid biosynthesis, etc. In addition, cagA and ureA were repressed in MGSE-treated cells whereas cagA, ureE and I, were repressed following quercetin and resveratrol treatment, respectively. Our results further support the potential use of bioactive plants for H. pylori prevention, and this microarray method may be useful for analyzing primary response mechanisms in H. pylori and/or microbiota upon exposure to bioactive food products.

FAECALIBACULUM RODENTIUM GEN. NOV., SP. NOV., ISOLATED FROM THE FECES OF A LABORATORY MOUSE

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Presented by KIM, Byoung-Chan

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The effect of the intestinal microbiota on animal health including human can be caused by interactions between microbes and the host. In addition, aging of the host seems to be related with the composition of the intestinal microbiome. The purpose of our study was to isolate novel gut microbes from laboratory mice with varying ages. Especially we were focusing on isolating some dominant and representative strains from middle and old mice. Langile et al. (2014) reported that Erysipelotrichaceae was one of the dominant families for representing middle aged mice, which was exactly corresponding to our unpublished data for microbial diversity of 18-month-old mice feces. A novel strict anaerobic bacterial strain ALO17T was isolated from 18-month old mice feces using the DSM 104 medium. Based on the 16S rRNA gene sequence phylogenetic analysis, the novel strain ALO17T obviously belong to the family Erysipelotrichaceae but was distant from all the members of this family. On the basis of polyphasic evidence from this study, the isolate should belong to a novel genus within the family Erysipelothricaceae. We propose the name Faecalibaculum rodentium gen. nov., sp. nov. to accommodate strain ALO17T (= KCTC 15484T = JCM 30274T) as the type strain.

CAN YOUR GUT MICROBIOTA EXPLAIN YOUR PERSONALITY?

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There is a growing awareness that microbiota influence gut-brain communication in health and disease. Recent studies indicate that gut microbiota may play a pivotal role in brain chemistry and consequently behavior. We investigated that whether gut microbiota differ depending on personality traits. We sought to identify gut microbiota with significant effects on the five NEO personality traits by regression analyses of individuals scoring in the extremes of these traits. Each subject provides a stool sample and bacterial DNA is extracted. The V3 and V4 hypervariable region of bacterial 16S rRNA gene are sequenced using Illumina MiSeq platform. The resulting paired reads are assembled using PANDAseq, demultiplexed, and analyzed using QIIME software. For quality filtering, the default parameters of QIIME are maintained. Chimeric sequences are identified and removed using usearch61. To idenfify OTUs from the non-chimeric sequences we use an open reference-based picking approach using usearch61. In the current study, highly agreeable man significantly increased the relative proportion of butyrate-producing bacteria such as Bacteroidales S24-7. Our findings might explain how the microbiota affect brain-gut communication.

SUBSPECIES-LEVEL DYSBIOSIS OF FAECALIBACTERIUM PRAUSNITZII IN THE HUMAN GUT MICROBIOME UNDERLIES ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a serious global epidemic associated with a modern life style. Although aberrant interactions between gut microbes and the intestinal immune system have been implicated in this skin disease, the nature of microbiome dysfunction underlying the disease remains unclear. Here, we show that enrichment of a subspecies of the major gut species Faecalibacterium prausnitzii is strongly associated with AD. The AD microbiome was enriched in genes encoding the utilization of nutrients that are released from damaged gut epithelium, reflecting a bloom of auxotrophic bacteria. AD fecal samples showed decreased levels of butyrate and propionate, which have anti-inflammatory effects. This is likely a consequence of an intra-species compositional change in F. prausnitzii that reduces the number of high butyrate and propionate producers. Together, the data suggest that feedback interactions between dysbiosis in F. prausnitzii and dysregulation of gut epithelial inflammation underlie the chronic progression of AD by resulting in impairment of the gut epithelial barrier, which ultimately leads to aberrant T-helper 2-type immune responses to allergens in the skin.

LYMPHOTOXIN MEDIATED T CELL-DEPENDENT IGA INDUCTION IN VIVO AND ITS RELEVANCE FOR MICROBIOTA CONTROL

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Production of immunoglobulin A (IgA) at the mucosal surfaces contributes to host defense against intestinal pathogens and governs commensal microbiota composition (1, 2). IgA is induced by two distinct T cell-dependent and T cell-independent pathways. We have recently developed novel mouse model based on cell-type specific Lymphotoxin beta (LTb) ablation. We found that ablation of LTb in type III innate lymphoid cells (RORgt+ ILC3) resulted in selective loss of T cell-independent intestinal IgA production (3), whereas T-cell dependent IgA can still be induced. Further analysis of mcirobiota coating by IgA using flow cytometry revealed that lack of LTb in ILC3 resulted in a distinct profile of anti-microbial IgA antibodies produced in the gut, indicating that T-cell dependent intestinal IgA recognize particular microbes in the intestine. Finally, ablation of LTb in ILC3 lead to the development of anti-pancreatic IgG autoantibodies and their induction depends on microbiota. Altogether, our data indicate that T-cell dependent anti-microbial IgA repertoire is distinct from WT repertoire and perturbances in IgA mediated control of microbiota may result in autoimmunity development.

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PROFILING OF SERUM MCP-1, IFNγ AND GUT MICROBIOTA IN TYPE 2 DIABETES MELLITUS PATIENTS FROM A TERTIARY CARE CENTRE IN INDIA

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Objective: To determine and correlate pro-inflammatory cytokines MCP-1 and IFNγ with gut microbiota in patients with type 2 diabetes mellitus and euglycemics.

Methodology: The study included 30 subjects. Human MCP-1 and IFN γ ELISA was performed for 23 serum samples. Fecal DNA was extracted and PCR done using fusion primers. Metagenomic analysis was performed using ion torrent sequencing.

Table1: Correlation of MCP-1, IFNγ and gut microbiota with HbA1c

		Mean HbA1c (%)	Mean MCP1 (pg/ml)	MCP1 Range (pg/ml)	Mean IFNγ (pg/ml)	IFNγ range (pg/ml)	Predominant Phyla	Predominant Genus
Euglycemic	HbA1c < 6.5% (n=11)	5.6	137.3	80.4-276.4	17.395	13.93-20.63	Firmicutes (63.63%)	*Fecalibacterium Eubacterium*
Type2 DM	**Group 1** HbA1c 6.5% -8% (n=7)	7.04	204.5	114.1-294.9	24.214	13.61-35.74	Proteobacteria (71.42%)	*Escherichia, Fecalibacterium*
	Group 2 HbA1c >8% (n=5)	9.7	178.2	98-206.8	19.033	15.83-20.47	Proteobacteria (60%)	*Escherichia, Prevotella*

Conclusion:

In mild to moderate (group 1) MCP-1, IFNγ levels are elevated and phyla recovered is Proteobacteria. The profile of gut microbiota in severe T2DM is predominant with gram negative bacteria than gram positive bacteria, with mildly elevated MCP-1 and IFNγ levels.

RELATION BETWEEN MEAT CONSUMPTION AND MOOD: POTENTIAL ROLE OF BACTERIAL INDOLE ?

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The NutriNet-Santé Study is a French web-based prospective cohort study on relationships between nutrition and health outcome. Firstly, on subjects of this cohort, we studied the relationship between mood, assessed by 2 repeated Center for Epidemiological Studies Depression Scales (CES-D) questionnaires, and dietary intakes. We selected 1554 participants based on the following inclusion criteria: female, aged 45-60 years, with urinary samples available in the cohort biobank. Statistical analysis showed a positive association between the risk of poor mood (score <17) and meat consumption but no association between dietary fibre consumption and mood. Secondly, since in a previous experiment in rats we had shown that indole production from tryptophane by intestinal microbiota induced anxiety and depression-like behaviours, we undertook to analyse indoxylsulfate, the major detoxification metabolite of indole, in the urine of the selected participants. First results obtained on a subset of 140 samples indicate that the urinary concentrations of indoxylsulfate increase with meat consumption and decrease with dietary fibre consumption. They also tend to be higher in women with a poor mood. If these results were confirmed they would indicate that the relationship between meat consumption and mood involve the microbiota, and more particularly a bacterial metabolite, the indole.

ANTI-INFLAMMATORY EFFECT OF HALOFUGINONE FOR DSS-INDUCED COLITIS IN MICE THROUGH INHIBITION OF HYPOXIA-INDUCIBLE 1ALPHA AND FATTY ACID OXIDATION

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Dextran sulfate sodium (DSS)-induced colitis in mice has been well recognized as the inflammatory bowel disease animal model of ulcerative colitis (UC). Here, we report that halofuginone treatment exerts anti-inflammatory effect in DSS-induced colitis in mice. Alleviation of colonic damage was observed upon HF treatment. Intriguingly, hypoxia-inducible 1alpha (HIF-1α) and tumor necrosis factor alpha (TNFα) was suppressed by HF in colon tissues. Moreover, we also performed mass spectrometry-based metabolomics for liver, spleen and colon tissues; and found that DSS elevated levels of acylcarnitines while HF and positive control decreased the acylcarnitines in colon tissues, indicating that fatty acid oxidation (FAO) was inhibited upon HF treatment as the tissue-specific metabolic characterization. In this regard, we further hypothesized that HF exerts anti-inflammatory effect in DSS-induced colitis in mice through inhibition of HIF-1α and FAO. To test this hypothesis, lipopolysaccharides and cobalt (II) chloride were used to induce HIF-1α expression in HCT116 colon cancer cells by mimicking hypoxic condition with inflammatory response. As a result, HF treatment, as expected, reduced HIF-1α, carnitine palmitoyltransferase 1C (CPT1C) as well as TNFα expressions in cells, to support our notion that HF exerts anti-inflammation through inhibition of HIF-1α and FAO in colon of DSS-induced colitis.

ADHERENT/INVASIVE ESCHERICHIA COLI LF82 STRAIN CAN INVADE AND PERSIST IN HUMAN PROSTATE CELLS

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Adherent/invasive Escherichia coli (AIEC) pathovar strains are associated to dysbiosis of gut microbiota in Crohn's disease (CD) patients in which they show increased abundance. In particular, AIEC LF82 strain shares many genetic and phenotypic characteristic of extraintestinal pathogenic E. coli (ExPEC) such as uropathogenic E. coli (UPEC), representing an important etiological agent of bacterial prostatitis. Hence in addition to their suspected role in CD, AIEC strains could be involved in extraintestinal disease, including urinary and urologic infections. The reference AIEC LF82 and UPEC EC73 strains were compared for their adhesion, invasion and persistence ability in RWPE-1 cells. Phylogenetic groups, virulence-gene carriage, intracellular localization, and cellular response such as signal transduction pathways, cytokines expression and production were also evaluated. Results show that LF82 is able to adhere, invade and survive in RWPE-1 cells. Furthermore LF82 strain shares B2 phylogroup, strong biofilm production and virulence genes with UPEC EC73 strain. Infections with both E. coli strains induce IL-8 and IL-6 expression and production, although AIEC LF82 strain provokes stronger induction than EC73 strain and possess different ability in modulating NF-κB and MAPK phosphorylation in RWPE-1 cells.

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CHARACTERIZATION OF LIFESTYLE AND FAECAL MICROBIOTA FROM COLORECTAL CANCER PATIENTS

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Colorectal cancer (CRC) is the third most commonly diagnosed cancer and one of the leading cause of cancer-related deaths worlwide. It is accepted that diet and lifestyle have a major impact on risk of developing this disease and, recently, imbalance of gut microbial has been reported in CRC. This work aims to describe lifestyle from CRC patients attending in Barretos Cancer Hospital and characterize faecal microbiote. The faecal DNA was extracted by PowerSoil DNA kit and submitted to PCR using primers for V4 and V5 regions of 16S rRNA amplification, library construction and sequencing by Ion Torrent. Our preliminary results include 9 patients (3 female, 6 male) and 6 controls (2 female, 4 male), all of them married and, mean age 52 ± 2.0 years for patients, and 61 ± 2 years for controls. Regarding to lifestyle for cases x controls: 100% x 66.7% intake alcohol, 44.4% x 83.3% are non-smoker and, 33.3% x 100% do physical activity. Concerning vegetables, fresh fruit and meat consumption, both groups intake most of days. After amplification, 2/2 samples analyzed were positive. In summary, more studies are needed to improve the prevention of CRC and, possibly, will include intervention for modulating intestinal microbiote.

ANTHOCYANINS AS MICROBIOTA MODULATORS IN OBESITY

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Anthocyanins may be subjected to the gut microbiota metabolism and they or their metabolites may modulate bacterial growth. The aim of this study was to analyze whether obesity modify the acute effect of blackberry anthocyanins on the gut microbiota composition. A total of 8 normoweight (23.2 ± 1.9 kg/m2) and 10 overweight/obese (30.3 ± 3.8 kg/m2) participants were recruited to consume 250 ml of blackberry juice with or without 12 % ethanol (to mimic the concentration presented in red wine). Fecal samples were collected before and at least 6h after juice consumption. DNA was extracted from stool for gut microbiota analysis by real-time PCR. Firmicutes, Bacteroidetes, Firmicutes to Bacteroidetes ratio, Bacteroides spp., Lactobacillus spp. and Bifidobacterium spp. were not significantly different between the two groups of participants at baseline. Blackberry juice reduced Lactobacillus spp. in overweight/obese individuals (from 1.86 ± 0.82 to 1.64 ± 0.49 log10 16S rRNA gene copies/20 ng DNA) especially when ethanol was present (from 2.17 ± 1.08 to 1.71 ± 0.68 log10 16S rRNA gene copies/20 ng DNA). We conclude that body weight and the presence of ethanol interfere in the gut microbiota modulation by anthocyanis.

FRUCTOSE CARAMEL SUBCLINICAL ORGAN DAMAGE ASSOCIATED TO HOST-MICROBIOTA CO-METABOLISM

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Fructose caramel is globally used in Western culture food processing and soft drinks. Although the health risks of massive use of fructose caramel have been suggested, comsumption by healthy people is regarded as safe by the food industry. Among the populations at risk, it is difficult to asses the impact on young adults. In this work, we show that fructose caramel induced fatty liver and metabolic disorders in young rats, The metabolomic profile of these rats is closer to diabetic rats than to control rats. In addition, the multivariate analysis of this profile exhibit signfiicant changes in metabolites important for host-microbiota co-metabolism. Both the organ damage and the metabolic alterations can be detected by NMR metabolomics of normal clinical blood samples. Overall, we detected important detrimental metabolic shifts in young rats on a fructose caramel supplemented diet that can be associated to changes in host-microbiota co-metabolism.

FECAL MICROBIOTA TRANSPLANT DONOR SELECTION BASED ON FECAL MICROBIOTA COMPOSITION: STUDY PROTOCOL

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Introduction: Lack of clinical disease does not correlate with having a 'normal' microbiota. In addition, even if the donor is clinically healthy and the microbiota composition is assessed to be 'normal'; it is imperative to select the donor based on the expected beneficial alterations that are exerted on the patient microbiota.

Objective: is to identify donor candidates based on gut microbiota structure.

Methods: We will screen 50,000 participants of the Golestan Cohort Study (GCS) (1) for age, cigarette smoking, opium consumption, alcohol consumption, family history of cancer, body mass index, and history of cardiovascular disease, stroke, diabetes mellitus, and hypertension. Donor candidates will be contacted through the Health Network of the Ministry of Health and the GCS network. Anthropomorphic, life style, and medical status will be updated. Serologic and fecal bacteriologic tests will be conducted as previously described (Table1). Gut microbiota composition will be examined. Based on the sequencing results, individuals will be categorised according to the bacterial diversity as well as composition into distinct groups.

Conclusion: we will identify a group of universal fecal donors based on their gut microbiota composition; which can direct the choice of donor selection for the disease(s) under investigation in FMT trials.

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VIRGIN OLIVE OIL AND RED WINE POLYPHENOLS MODULATE FECAL MICROBIOTA AND REDUCE RISK METABOLIC MARKERS IN HIGH INSULIN RESISTANT PATIENTS

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Objectives: This study evaluated the prebiotic effect of a moderate intake of virgin olive oil and red wine polyphenols on modulating the gut microbiota composition and the improvement of the metabolic risk factors in subjects with high (HIR) or low (LIR) insulin resistance.

Methodology: Ten HIR patients and ten LIR subjects were included in a randomized and controlled intervention study. After a washout period both study groups consumed a virgin olive oil rich diet and the same diet plus red wine over a 30-day period for each.

Results: The dominant bacterial composition differ significantly between study groups after the two diet intake periods and within each group with respect to their basal level. At Phylum level we observed an improvement in the profile of Bacteroidetes, Actinobacteria and Firmicutes in HIR patients after both diet treatments. In HIR patients, polyphenols from olive oil and red wine significantly increased the number of fecal bifidobacteria an intestinal barrier protectors.

Conclusion: The changes in gut microbiota in these HIR patients could be responsible for the improvement in the metabolic markers. Modulation of the gut microbiota by virgin olive oil and red wine polyphenols could be an effective strategy for managing metabolic diseases.

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ANTIMICROBIAL ACTIVITY OF A BOVINE MYELOID ANTIMICROBIAL PEPTIDE (BMAP-28) AGAINST ENTEROCOCCI

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Antimicrobial peptide (AMP) plays a key role in mammalian innate immunity. A newly developed AMP such as BMAP-28 is expected to be an effective compound against antibiotics-resistant bacteria. This study aims to assess the bactericidal effect of BMAP-28 against enterococci, a commonly causative pathogen of nosocomial infection around the world. Eneterococcus faecalis and E. faecium (with or without multidrug-resistant gene, mprF) were used. No difference was recognized in the component of membrane phospholipid between two species. Four analogs of BMAP-28 (GGLRSLGRKILRAWKKYGPIIVPIIRI) were designed according to hydrophobic and hydrophilic properties. E. faecium are susceptible (MIC: 5-20 mg/ml) to BMAP-28 and its analogues. Membrane damage (97.7%) was also observed on that species. In contrast, E. faecalis was resistant. In the presence of valinomycin, BMAP-28 (10-20 mg/ml) inhibited the growth of E. faecalis. Our results suggested that BMAP-28 is effective against enterococci, although varying among different species. Polarity and hydrophobicity of analogues did not alter susceptibility of enterococci. Relevance of BMAP-28 and enterococci could be explained by somewhat beyond the factors. Further study on membrane potential allows us to figure out the essence of the interaction of BMAP-28 to bacterial cell.

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INTESTINAL MICROBIOTA TRANSPLANTATION: A NEW OPTION IN THE TREATMENT OF IRRITABLE BOWEL SYNDROME?

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Objective: Irritable bowel syndrome (IBS) is a clinical entity that effects up to 20% of the population (1). The etiology and pathophysiology is multifactorial why specific treatment options are limited. However, numerous reports in the literature support that the gut microbiota is of great importance for the development of IBS (1,2).

Methods: 20 patients fulfilling the Rome III criteria of IBS, all of them assumed to have a gastrointestinal dysbiosis, most likely initiated in conjunction with antibiotics or traveller´s diarrhoea. An Anaerobic Cultured Human Intestinal Microbiota (ACHIM) was administered on two occasions through a gastroscope in the descending duodenum. One week before treatment and four weeks after the last administration of the microbiota the patients scored their symptoms (from 0 to 500) using a standard translated version of IBS-SSS (3). Data are expressed as mean ± standard deviation. Statistical differences were calculated using Wilcoxon signed rank test and Student's t test.

Results: In our cohort 16/20 patients reduced their symptom score with >50 points. Mean value for the group before and after treatment were 322±64 and 214±134, respectively (p=0.0003).

Conclusion: Our results indicate an underlying dysbiosis in cases of IBS patients. Larger clinical studies with longer follow-up time are warranted.

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CHARACTERIZATION OF LIFESTYLE AND INTESTINAL MICROBIOTA FROM BRAZILIAN TYPE 1 DIABETES PATIENTS

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Aberrant gut microbiome has been reported in pre-diabetic children who possess autoantibodies against pancreatic beta-cells, suggesting that the dysbiosis may contribute to autoimmune attack in type 1 diabetes (T1D). The aim of this work is to describe the lifestyle and characterize the intestinal microbiota from Brazilian T1D patients. This study was approved by the local ethics committee. Faeces bacterial DNA was extracted by using PowerSoil DNA kit and V4 and V5 regions from16S rRNA were amplified by PCR. Amplicons will be used for DNA library construction and sequencing by using Ion Torrent platform. Our preliminary results include 14 T1D patients, 9 females and 5 males, aged 21.4 ± 8.0 years. Regarding to lifestyle, 62.5% of patients uses alcohol, none of them is smoker and 58.8% practice physical activity. Most patients ingest vegetables daily (76.5%), fresh fruit (35.3%), meat (41.2%), carbohydrates (41.2%) and milk (58.8%). After amplification using V4 and V5 primers, all of the samples were positive for 300 bp bands. More patients will be recruited and the next steps include sequencing and bioinformatics analyses. In summary, more studies are needed, and the prevention of T1D in the future, possibly involves intestinal microbiota modulation.

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EVALUATION OF HUMAN FAECAL MICROBIOTA CONTENT BY 16S RRNA ANALYSIS USING DIFFERENT COLLECTION, STORAGE AND DNA EXTRACTION METHODS – OMNIGENE.GUT CASE STUDY

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Methodologies for collection and storage of human faecal samples as well as DNA extraction govern the quality of DNA obtained and present crutial steps in obtaining representative models of bacterial community structure after 16S rRNA gene sequencing. Collection of faeces in the clinical settings necessitates the need for uniform, reliable, robust and patient compliant procedures. In this study we compared two different approaches for faeces collection and storage and three commercially available bacterial faecal DNA extration kits. Faeces from 6 healthy donors were collected fresh and in OMNIgene.GUT system, then stored for 14 days at -20oC and at room temperature, respectively. DNA yield and quality varied between DNA extration kits in the order MP Biomedicals>QIAGEN>MoBio with OMNIgene.GUT preserving integrity of the obtained DNA. Donor-specific bacterial diversity was maintained irrespective of the collection, storage or DNA extraction method used. However, kit-based trends were observed displaying different levels of abundance among the most prevalent families, notably Bacteroidaceae and Lachnospiraceae. Although OMNIgene.GUT faeces collection system slightly decreased the richness of detected bacteria, the change was not statistically significant. Overall, OMNIgene.GUT was proven as convenient and reliable human faecal collection, transport and storage system for human faecal microbiota analyses.

GENE EXPRESSION PROFILING OF ZEBRAFISH IN RESPONSE TO NUTRITIONAL SUPPLEMENTATION WITH MASTIC ESSENTIAL OIL

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The present study aimed at evaluating the effects of mastic essential oil supplementation on gene expression profile of zebrafish (Danio rerio). Mastic essential oil is composed of several components, mainly volatile terpenes, with distinct established biological roles. This unique composition accounts for the multiple uses of mastic oil and its constituents in food industry and healthcare. Previous work has shown that mastic oil fights helicobacter pylori bacteria, cure peptic ulcers (1, 2, 3) and exhibits anti-bacterial properties against distinct bacterial strains, including S. aureus and B. subtilis (4). Mastic oil was extracted from the mastic resin by direct gum distillation and its compostiton was analysed by GC/MS. To study the gene expression profile of zebrafish fed with mastic oil supplemented diet, mRNA was isolated at 42dpf and subjected to DNA microarray analysis. Gene ontology characterisation indicated that most of the differentially expressed genes were related to the immune defense against pathogens, immunometabolic processes and mucin physiological functions. The microarray data were validated by qRT-PCR of selected target genes. Our results open up the possibility that mastic essential oil supplementation affects the composition and/or production of mucus, which in turn, lead to changes in the establishment and fluctuation of the microbiota.

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PROBIOTIC BACTERIUM STIMULATE THE IMMUNE RESPONSE OF CAENORHABDITIS ELEGANS THROUGH NUCLEAR HORMONE RECEPTOR

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Even though the immune response of Caenorhabditis elegans to pathogen infections is well established, very little is discovered about the impacts of health promoting-probiotic bacteria on C. elegans host responses. We determined on the survivals of C. elegans by plate method and transmission electron microscopy (TEM). In addition, we employed DNA microarray, quantitative real time-polymerase chain reaction (qRT-PCR), and transgenic worms assay for exploring health-promoting pathways via probiotic bacteria in C. elegans. We showed that conditioning with L. acidophilus strain A4 strongly colonized in the C. elegans nematode intestine (plate count and TEM image), significantly enhanced the resistance of nematodes exposed to Staphylococcus aureus as well as prolonged the lifespan of C. elegans. Importantly, whole transcriptome analysis and transgenic worm assays showed that health-promoting effects of L. acidophilus strain A4 were mediated by the presence of nuclear hormone receptor (NHR) family. Taken together, we describe a new C. elegans based system for the screening of novel probiotic activity and our findings demonstrate that pre-conditioning with probiotic bacterium L. acidophilus strain A4 may positively stimulate the longevity and resistance to foodborne pathogen infections of C. elegans host.

SPECIFIC GUT MICROBIOTA ASSOCIATED WITH INSULIN RESISTANCE IN APPENDIX SAMPLES FROM MORBIDLY OBESE PATIENTS.

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Objectives: Identify the gut microbiota associated with insulin resistance in morbidly obese patients classified in 2 groups, high (IR-MO) and low insulin-resistant (NIR-MO), and to determine the possible association between these gut microbiota and variables associated with insulin resistance and the expression of genes related to inflammation and macrophage infiltration in adipose tissue.

Methodology: Microbiome composition of appendix samples was determined by 16S rRNA pyrosequencing and bioinformatics analysis by QIIME.

Results: Chao and Shannon indices of each study group suggested similar bacterial richness and diversity between both study groups. 16S rRNA pyrosequencing showed that IR-MO group had a significant increase in the abundance of Firmicutes, Fusobacteria, Pseudomonaceae, Prevotellaceae, Fusobacteriaceae, Pseudomonas, Prevotella, Veillonella and Fusobacterium compared to NIR-MO group. In IR-MO group we found a positive and significant correlation between the abundance of Prevotella, Succinovibrio, Firmicutes and Veillonella and the visceral adipose tissue expression level of IL6, TNF alpha, ILB1 and CD11b respectively, and significant negative correlations between the abundance of Butyricimonas and Bifidobacterium, and plasma glucose and insulin levels, respectively.

Conclusion: Appendix dysbiosis occurs in IR-MO patients, with loss of butyrate-producing bacteria, essential to maintenance of gut integrity, together with an increase in mucin-degrading bacteria and opportunistic pathogens.

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THE ROLE OF GUT MICROBIOTA PRODUCED SHORT CHAIN FATTY ACID IN ADIPOSITY AND INFLAMMATION MEDIATED INSULIN RESISTANCE

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Objectives: To study the effect of alteration the gut microflora by administration of spectrum specific antibiotics delivery in HSD (High sucrose diet) induced obesity and type 2 diabetes rat model. Methodology: Rats were fed HSD with or without antibiotics for 60 days. The physiological and biochemical parameters, fecal microbiota composition, Short chain fatty acids (SCFAs), Liver biochemistry, histopathology and gene expression of various Pathogen recognition receptors (PRRs) and G-protein coupled receptors (GPCRs) and various adipokines were investigated. Results: Simultaneous administration of HSD and antibiotics has shown significant improvements in glucose tolerance and obesity associated parameters like hypercholesterolemia and hypertryglyceremia as compared to HSD. The qPCR study of fecal samples showed increased in gram positive bacteria and reduction in gram negative bacteria, which shows that restoration of commensal microflora in direction to improvement in obesity and insulin sensitivity. Treatment had reduce the effect of Lipopolysaccharide content, which further reduced immune receptors, GPCRs expression along with reduced the expression of proinflammatory cytokines. PRRs and inflammatory mediators also decrease in antibiotic treated group.Conclusion: Spectrum specific antibiotic modulate gut microflora diversity and their metabolites. Alteration of metabolites of microflora improves the insulin sensitivity through adipose tissue markers and decreases the inflammatory markers.

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EFFECTS OF SYNBIOTIC SUPPLEMENTATION ON GUT PERMEABILITY AND LOW-GRADE SYSTEMIC INFLAMMATION OF COMMUNITY- DWERLING ELDERLY AT FRAILTY RISK

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Background: Recent knowledge of gut microbiota has lead to set up strategies to preserve this complex microbial asset. Symbiotic intake is one of these strategies. Aims: to analyze the effects of symbiotic (SYN) supplementation on the gut permeability and the inflammatory status of elderly at risk of frailty. Methods: double blind, randomized clinical trial (U1111-1155-0607), including community-dwelling elderly (50 participants 65-90 years, both genders, from São Paulo, Brazil), pre-frail, randomized in two groups: SYN [FOS (6g) + L.Paracasei + L. Rhamnosus + B. Lactis]; and placebo (PLA), for 6 months. The elderly were investigated for - gut permeability [serum lipopolysaccharides (LPS), intestine fatty acids binding protein (iFABP) and diamine oxidase (DAO)]; and –systemic inflammation (serum IL-10, IL-10 and TNF-α). The data were analyzed by means comparison, and by relative risk (RR). Main Results: 37 participants finished the study. SYN presented lower reduction, lower RR in IL-10 (regulatory cytokine); higher reduction, lower RR in IL-6 (inflammatory cytokine) than PLA. Both groups had increased the TNF-α. Gut permeability did not show any difference between the groups. Conclusion: SYN seemed to be efficient in modulating regulatory cytokines, and these effects did not show any association to gut permeability.

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BACTERIAL FLAGELLIN INDUCES ADIPOSITY AND METABOLIC IMPAIRMENT

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Toll-like receptor 5 (TLR5) has been traditionally linked to innate immunity due to its ability to recognize pathogen-associated flagellin molecules (1). Our recent findings suggest that adipose tissue TLR5 is associated with metabolic impairment in humans (2). In cultured adipocytes, flagellin seems to induce insulin resistance (IR) and inflammation (2). In this study, we examined in vivo the effects of prolonged exposure to flagellin. For 12 weeks, C57BL/6N mice on high-fat diet were injected intraperitoneally flagellin (n=6, FLG) or PBS (n=6, Control). FLG group had higher subcutaneous fat mass characterized by increased inflammation and decreased hormone-sensitive lipase activity, which together may explain the adipose tissue expansion. While no differences between the groups were found in visceral fat mass, in FLG group there were signs of IR accompanied with increased inflammation. FLG group had higher serum levels of lipolysis end product, glycerol. Finally, flagellin regulated vascular adhesion protein 1 (VAP-1) expression in subcutaneous adipocytes; in response to flagellin, VAP-1 expression was decreased. In agreement with the role of VAP-1 in regulating glucose uptake, its expression correlated negatively with serum glucose levels. In conclusion, flagellin induces adiposity, inflammation and metabolic impairment including IR, increased lipolysis and defective glucose uptake.

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PBA AND PIOGLITAZONE, DIFFERENT CLASSES OF ANTIDIABETIC ADJUVANTS DRUGS, ALTERED THE COMPOSITION OF GUT MICROBIOTA IN HIGH FAT-INDUCED MICE.

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Recent evidences indicate that the composition of the gut microbiota contributes to the development of obesity and type 2 diabetes (T2D) by affecting the physiology and metabolism of the host. 4-phenyl butyric acid (PBA) and Pioglitazone are different classes of antidiabetic adjuvants drugs. The present work's main objective was to determine whether the antidiabetic effects of PBA and Pioglitazone are related to alterations of the gut microbiota composition. Male C57bl/6JUnib mice were submitted to a high-fat diet (HFD) or standard chow (CTL group) for 16 weeks. After this period, some HFD animals received PBA (500 mg/kg)(PBA group), Pioglitazone (20 mg/kg/dia)(PIO group) by gavage for 30 days while the rest did not receive any drug (HFD group). Then we extracted the colon feces to metagenomic analyzes. PBA and Pioglitazone treatment significantly altered the composition of gut microbiota by increasing Akkermansia muciniphila and decreasing Lactobacillus johnsonii in HFD. While PIO group had increased Allobaculum stercoricanis, PBA group had increased Rikenella microfusus. Our results show that modulation of the gut microbiota induced by PBA and Pioglotazone may contribute to antidiabetic effects, but the mechanisms of action of these therapeutic drugs in T2D though the gut microbiota still needs to be elucidated.

CHANGES OF ANTIMICROBIAL PEPTIDE ALPHA-DEFENSIN AND MICROBIOTA DURING GROWTH IN YOUNG MICE

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Aims: Alpha-defensin, an antimicrobial peptide secreted from Paneth cells in small intestinal crypts, is known to be involved in regulating the microbiota, and decreased expression of alpha-defensin owing to Paneth cell dysfunction has been reported in disorders associated with dysbiosis such as obesity and IBD(1). In this study, we aimed to reveal the relationship between microbiota and alpha-defensin levels during growth of mice.

Methology: Pregnant BALB/C mice were purchased and postnatal mice were kept with mothers until 3 weeks and then housed in an individual cage until 8 weeks of age. Fecal and ileal samples were collected from postnatal mice at 1, 2, 3, 4, 6 and 8 weeks of age for analysis of gut microbiota and the level of alpha-defensin by western blotting and sandwich ELISA.

Results and conclusion: The composition of gut microbiota changed drastically at 3 weeks. The levels of alpha-defensin increased significantly at 3 weeks and were negatively correlated with the abundance of Enterobacteriaceae and Lactobacillaceae, which were found to be sensitive to alpha-defensin in bactericidal assay. Furthermore, the diversity of microbiota increased at 3 weeks. These results suggested that alpha-defensin functions as a regulator of microbiota when microbial diversity increased after weaning.

Salzman NH and Bevins CL., Dysbiosis--a consequence of Paneth cell dysfunction., Semin Immunol. 30;25(5):334-41 2013

SHORT CHAIN FATTY ACIDS INDUCE CUTANEOUS REGULATORY T CELLS IN MICE

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Presented by SCHWARZ, Agatha

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A well characterized subset of Treg is the CD4+CD25+ population expressing the transcription factor Foxp3. Commensal microbes induce Treg in the colon via short chain fatty acids (SCFA). The effects of SCFA are dependent on the G protein-coupled receptors 41 and 43. Thus, we postulated that SCFA produced by commensal skin bacteria may also activate resident skin Treg that are diminished in certain inflammatory dermatoses. To address this issue, sodium butyrate was injected subcutaneously into the ear of hapten-sensitized mice. After challenge 24 later, ear swelling was measured. Mice, which were injected with sodium butyrate before challenge, responded with a significantly reduced CHS reaction compared to the positive controls. Transcription of foxp3 and il-10 was determined by quantitative real time PCR. Their expression was upregulated upon treatment with sodium butyrate, suggesting that skin Treg were activated. Furthermore, immunofluorescence analysis of ear tissue revealed the presence of enhanced numbers of Foxp3-positive cells in sodium butyrate injected ears. Finally, we examined the expression of GPR43 on skin T cells. FACS analysis revealed that murine Treg as well CD4+CD25- express this receptor. Incubation of CD4+CD25- cells with sodium butyrate significantly upregulated expression of GPR43.

LOW COMPLEXITY OF THE DUODENAL MICROBIOTA IN CHILDREN WITH NEWLY DIAGNOSED ULCERATIVE COLITIS

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Background: Inflammatorybowel disease (IBD) is characterized by inappropriate immuneresponses to gut bacteria. The smallintestine is central in induction and control of mucosal immune responses andmicrobiota composition could modulate these functions. Despite this, theupper small intestinal microbiota in IBD has received sparse attention.

Methods: Duodenal fluids were collected from children withsuspected IBD during diagnostic work-up. The samples were analyzedby quantitative culture and pyrosequencing (average 32,000 reads/sample, 500-ntin length).

Results: Culture revealed nodifference in duodenal bacterialpopulation levels between the clinical groups. However, pyrosequencing revealedthat the duodenal microbiota ofchildren with ulcerative colitis contained fewer Operational Taxonomic Units(OTUs) per individual than those of controls (P=0.005). This reduced diversity was seen across three majorphyla, i.e., Firmicutes, Actinobacteria and Bacteroidetes. Several bacterialgenera were isolated less frequently from children with ulcerative colitis thanfrom controls, including Collinsella (P=0.001), Lactobacillus (P=0.007) and Bacillus (P=0.007) aswell as a non-identified member of the order Sphingobacteriales (P=0.007). The microbiota in Crohn's disease was lessaberrant, but Collinsella and Campylobacter were reduced compared tocontrol subjects (P=0.001).

Conclusions: The duodenal microbiota inchildren with ulcerative colitis shows reduced overall diversity despitethe fact that the disorder is located in the colon. Whether this contributes to the disease processremains to be determined.

STABILITY OF REPEATED FECAL DNA PREPARATION USING THE GA-MAP™ DYSBIOSIS TEST

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In recent years, repeated reports elevate the importance of standardized sample preparation when working with fecal samples. DNA extraction is one of the main causes for low reproducibility of microbiota results between laboratories and/or methods. A standardized method for extracting fecal DNA has been developed for the GA-map™ Dysbiosis Test1, which comprises 16S rRNA amplification and a set of 54 selected probes targeting gut bacteria and bacteria groups important in human health. First, fecal DNA was extracted 10 times from three donors and processed according to GA-map™ Dysbiosis Test protocol. All 10 fecal aliquots per donor showed identical Dysbiosis Indices (DI) with standard deviation (SD) ≤0.15. Next, fecal samples from eight donors were analyzed at two laboratories (Norway and Germany). DNA was extracted in duplicate and analyzed in triplicate (n=48). DI values and bacteria profiles were compared between laboratories, and of 42 overlapping QC approved samples, 35 DI values fall within a 2SD limit with a pass rate of 83%. Likewise, 35/42 microbiota profiles (83%) were equivalent between laboratories.

The results show good reproducibility, repeatability and precision, both within run and between sites, for the fecal sample DNA preparation method used for the GA-map™ Dysbiosis Test.

Casén, C. et al. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment. Pharmacol. Ther. 1–13 (2015). doi:10.1111/apt.13236

THE LUNG MICROBIOTA ASSESSED THROUGH SPUTUM SAMPLES IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Background: Sputum induction is a non-invasive method for obtaining information on secretion in the lower airways(1). That spontaneous sputum is a valid surrogate when studying inflammation and microbiota is not a given. The aim of this study was to compare microbiota in induced and spontaneous sputum sampled on the same consultation either in stable state or during exacerbations of COPD.

Methods: 30 COPD-patients were included, contributing 72 sputum samples. They had GOLD stage II-IV COPD, participating in the large longitudinal cohort studies BCCS/BCES during 2006/2007(2, 3). Induced and spontaneous sputum were later processed for extraction of bacterial DNA and 16S rRNA sequencing using Illumina MiSeq. QIIME was used for bioinformatics and statistical analyses.

Results: Taxonomic differences of clinical interest were found only for Streptococcus (genus) and only in exacerbarated patients (Induced>Spontaneous, p=0.03). Alpha- and beta-diversity measurements were not significantly different (p>0.05) between sputum types, neither during stable state nor exacerbations.

Conclusion: We did not find differences in microbiota between induced and spontaneous sputum, implying that spontaneously sampled sputum could be a valid surrogate. Sampling by sterile procedures from the lower airways is needed for comparison with the traditionally used expectorate.

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MUCOSA-ASSOCIATED MICROBIOTA STRUCTURE AND METHYLATION STATUS OF GENES INVOLVED IN THE INFLAMMATION RESPONSE IN CHRON'S DISEASE

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Presented by TOTINO Valentina

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Introduction. Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract, where there is a deregulated immune response to the intestinal microbiota. In CD microbiota is suspected to play an important role. Objectives. The project is aimed to evaluate the impact of microbiota on expression of genes involved in the inflammation response in CD pathology.

Methodology. Starting from total DNA extracted from CD ileal biopsies and healthy subjects, we analyzed: i) the mucosa-associated microbiota through Next-Generation-Sequencing; ii) the methylation level of the genes involved in the host inflammatory response through the plate/array "The Human Inflammatory Response & Autoimmunity EpiTect Methyl II Signature PCR Array" to estimate the methylation status of 22 promoter genes involved in inflammation.

Results. Preliminary results shown a mucosal dysbiosis in CD patients, with higher relative abundance of *Pseudomonas* genus in CD and *Veillonella* in controls. A significant decrease in methylation levels of *IL13RA1* and *InhA* promoters was observed.

Conclusion. A minor methylation level of the two promoter genes in CD, related to an increased expression, could represent a new connection between bacteria and innate immune system; a new therapeutic targets and/or diagnostic biomarkers to be used.

EATING HABITS AND INTESTINAL MICROBIOTA COMPOSITION OF HEALTHY FRENCH PEOPLE: PRELIMINARY STUDY

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By the past, studies have shown that diet modulate intestinal microbiota. Our objective was to compare intestinal microbiota composition of healthy subjects, according to food groups' consumption. Our study includes 134 individuals from the Milieu Intérieur Healthy Donor Cohort, mean age 40.4y; 57.5% women. Eating habits were assessed using a food frequency questionnaire. Composition of the faecal microbiota was established by 16S rRNA gene sequencing. OTU abundance was log10 transformed and OTUs were grouped by phylum and genus. We compared taxa abundance according to eating habits of subjects (ANOVA tests – age and gender adjusted). Compared to individuals that consume more frequently the food group concerned, those who eat less often fish had more Eubacterium spp., Ruminococcus spp., Streptococcus spp. and Actinobacteria. Those who eat less often cheese had less Coprococcus spp., Faecalibacterium spp., Prevotella spp., and Bacteroidetes, and more of Actinobacteria and Verrucomicrobia. Those who eat less often deep-fried foods had less Coprobacillus spp., Turicibacter spp. and Tenericutes. This preliminary study highlights associations between eating habits and microbiota composition. Investigation of associations between gut microbiota composition and diet patterns rather than specific food compounds could be useful to identify microbiota/nutrition signatures related to health.

LIMITED EFFECTS OF PICEATANNOL SUPPLEMENTATION ON GUT MICROBIOTA COMPOSITION IN OBESE ZUCKER RATS

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Background & objectives: Piceatannol, a natural polyphenolic stilbene present in grapes and red wine is an analog and metabolite of resveratrol. Piceatannol exhibits higher metabolic stability and bioavailability than resveratrol, suggesting a promising role in obesity and associated disorders. As gut microbiota is a potential contributor factor in obesity, it would be of interest to analyze the influence of piceatannol on gut microbiota composition in a model of obesity.

Methodology: A total of 30 male Zucker (Crl:ZUC-Leprfa) rats were randomly distributed in three experimental groups. Piceatannol was given orally (15 mg/kg and 45 mg/kg). After 6 weeks of treatment, cecal contents were removed. DNA was extracted and metagenomics analysis of microbiota was carried out.

Results: Bacterial composition from piceatannol treated rats was not significantly affected at the phylum level; only slight changes were observed in less abundant phyla and in several species belonging to Firmicutes and Bacteroidetes phyla.

Conclusion: Minor changes in gut microbiota composition were observed, similarly to what was observed when analyzing its in vitro antimicrobial activity. The physiological relevance of the bacterial species modified needs to be further determined, although they were associated with only modest mitigation of obesity complications in this model of genetic hyperphagia.

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OXIDATIVE STRESS PARAMETERS AND THE METABOLIC SYNDROME MANIFESTATIONS RELATIONSHIP

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Objectives. Oxidative stress (OS) parameters and metabolic syndrome (MS) induced dermal manifistations relationship. Methodology. Prospective clinical study, 94 respondents. Clinical evaluation: general examination (specifically for skin changes), anthropometric measurements, blood samples (glucose, cholesterol, triglycerides, lipoproteins, C-reactive protein, Vitamin D, OS biomarkers). Skin was evaluated through dermatoscopy; structural changes and skin immunity was evaluated by immunohistochemistry of punch-bioptates. Immunohistochemistry included CD1a, CD31, CD34, CD3, CD8. Results. OS related parameters were higher among MS patients. Correlation has been identified between OS biomarker and certain skin changes (elastosis, keratosis, dispigmintation, lentigo) in patients with MS. Light microscopy revealed degeneration of dermal tissue, deposition of elastic fibers; stratum spinosum thickness positively correlated with increased OS parameters (p-value<0.01) and MS characteristics. Conclusion. Antioxidative defense system is weakened and/or ineffective in patients with MS. Under such stress, keratinocyte synthesis is reduced, transepidermal water evaporation intensifies. These changes lead to skin barrier impairment, microbiota imbalance, fungal skin infections and papilloma development. Restoration of epidermal lipid layer of the skin is a particularly urgent task in patients with metabolic syndrome.

A NOVEL APPORACH FOR THE SIMULTANEOUS ISOLATION OF DNA AND RNA FROM STOOL SAMPLES FOR METAGENOMIC AND METATRANSCRIPTOMIC PURPOSES

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Presented by WIDMANN, Philipp

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Objectives: The gut microbiome impacts on human health and disease. Next-Generation-Sequencing enables insights into microbial compositions, functional potentials, and metabolic functions. We present a novel nucleic acid extraction method for the simultaneous isolation of microbial DNA and RNA from a single stool aliquot into separate eluate fractions for NGS-based investigations on the human gut microbiome.

Methodology: The presented method incorporates a novel additive that prevents nucleic acid yield losses that commonly arise during inhibitor removal. PCR inhibitors are removed by spin columns that selectively bind nucleic acids. Fecal microbial DNA and RNA were isolated with the presented method and sequenced on an Illumina MiSeq system.

Results: Nucleic acid yields were in all cases sufficient for NGS downstream applications and generated 16S rRNA-, whole-genome-, and transcriptome sequences were of high quality. The microbial compositions determined from these datasets reflect the current literature. Our findings indicate that the presented method is suitable for extracting the collective microbial genetic material from human stool.

Conclusion: The herein presented method enables the simultaneous isolation of microbial DNA and RNA from a single stool aliquot into separate eluate fractions. The method lays the foundation for highly precise metagenomic/metatranscriptomic investigations on the human gut microbiome.

MANNOSE-BINDING LECTIN INHIBITS THE MOTILITY OF SALMONELLA THROUGH THE ALTERATION OF MEMBRANE POTENTIAL

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Bacterial motility has been believed as an essential factor of pathogenicity. In this study, we showed that a complement protein, mannose-binding lectin (MBL), impairs the motility of Salmonella. MBL is well-known in triggering opsonization and enhancing phagocytosis, which is a key substance in the complement system. We quantitatively analyzed the motility of Salmonella cells treated by MBL and found that swimming speeds and flagellar rotation rates were decreased. We also observed that the membrane voltage was significantly reduced upon addition of MBL. Since the membrane voltage is a crucial component of proton motive force powering flagella, the depolarization on bacterial cell membrane due to association with MBL would result in the decrease of motility. Aberrant switching of rotational direction of flagella was also observed in the presence of MBL: highly frequent switching or continuous counter rotation. The alteration in controlling of rotational direction was not observed in a mutant strain lacking chemotaxis, suggesting that MBL association can affect not only the membrane potential but also the chemotaxis system in Salmonella. Our findings indicate the possibility that MBL can directly attack microbial pathogens, bringing a new insight into a role of the complement protein in immune systems.

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DAMAGING MUTATIONS OF FLG2 AND TLR4 WOULD POSSIBLY INFLUENCE ON SKIN BARRIER AND INTERACTIONS BETWEEN MICROBIOME AND HOST IMMUNE SYSTEM IN SEVERE ATOPIC DERMATITIS PATIENTS

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Atopic dermatitis (AD) is an inflammatory skin disorder caused by dysfunction of skin barrier and interactions between microbiome and host immune system. Mutation of genes with epidermal barrier function make the host more susceptible to bacterial and viral skin infections and may influence on skin microbiome. Therefore, mutation screening of epidermal and immune response related genes would help us understanding underlying the etiology of AD. In this study, we sequenced exomes of 35 severe AD cases and 26 controls. We selected 16 epidermal and immune response related genes previously reported with host-microbial interactions. Among them, 71 exonic variants were found. Damaging mutations were screened based on the following criteria: 1) coding variants only, 2) mutant allele count ≥ 1 in cases while 0 in controls, 3) excluding common variants in the general populations (1,000 genomes phase 3 and ESP project). After screening, 5 variants were remained and annotated by snpSIFT software using dbNSFP v2.7 to study the predicted impact on gene function. As a result, we discovered two damaging mutations in FLG2 and TLR4). In conclusion, the present study provides valuable genetic mutations for further understanding the etiology of AD.

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FACTS AND ARTIFACTS ABOUT METAGENOMIC ANALYSIS IN HUMAN ADIPOSE TISSUE

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Presented by ZULIAN, Alessandra

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Objectives: The existence of a microbiota in the stroma-vascular fraction of human adipose tissue has been recently suggested. The present study was aimed to characterize viable cultivable bacteria and non-viable bacteria in human adipose tissue.

Methodology: The presence of bacteria was assessed through standard microbiological cultural techniques and 16S rRNA gene sequencing (Illumina technology) on DNA and RNA from a) whole abdominal subcutaneous (SAT) and visceral (VAT) adipose tissue of 14 obese and 5 normal-weight subjects, b) mature adipocytes isolated from these tissues after collagenase digestion and/or mechanical separation. Transmission electron microscopy (TEM) was used to identify bacteria at intracellular level.

Results: Microbiological cultures were negative in all samples. Contaminating bacterial DNA was amplified and sequenced in enzymatically isolated adipocytes. No amplification of the 16S rRNA gene was obtained from DNA and RNA of whole SAT and VAT and mature adipocytes mechanically isolated. Ultra-structure analyses by TEM did not show presence of bacteria within adipocytes.

Conclusion: Our results do not confirm the existence of human adipose tissue microbiota. Further investigations are needed to clarify whether the discrepancy between our and previous results is due to methodological differences.

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