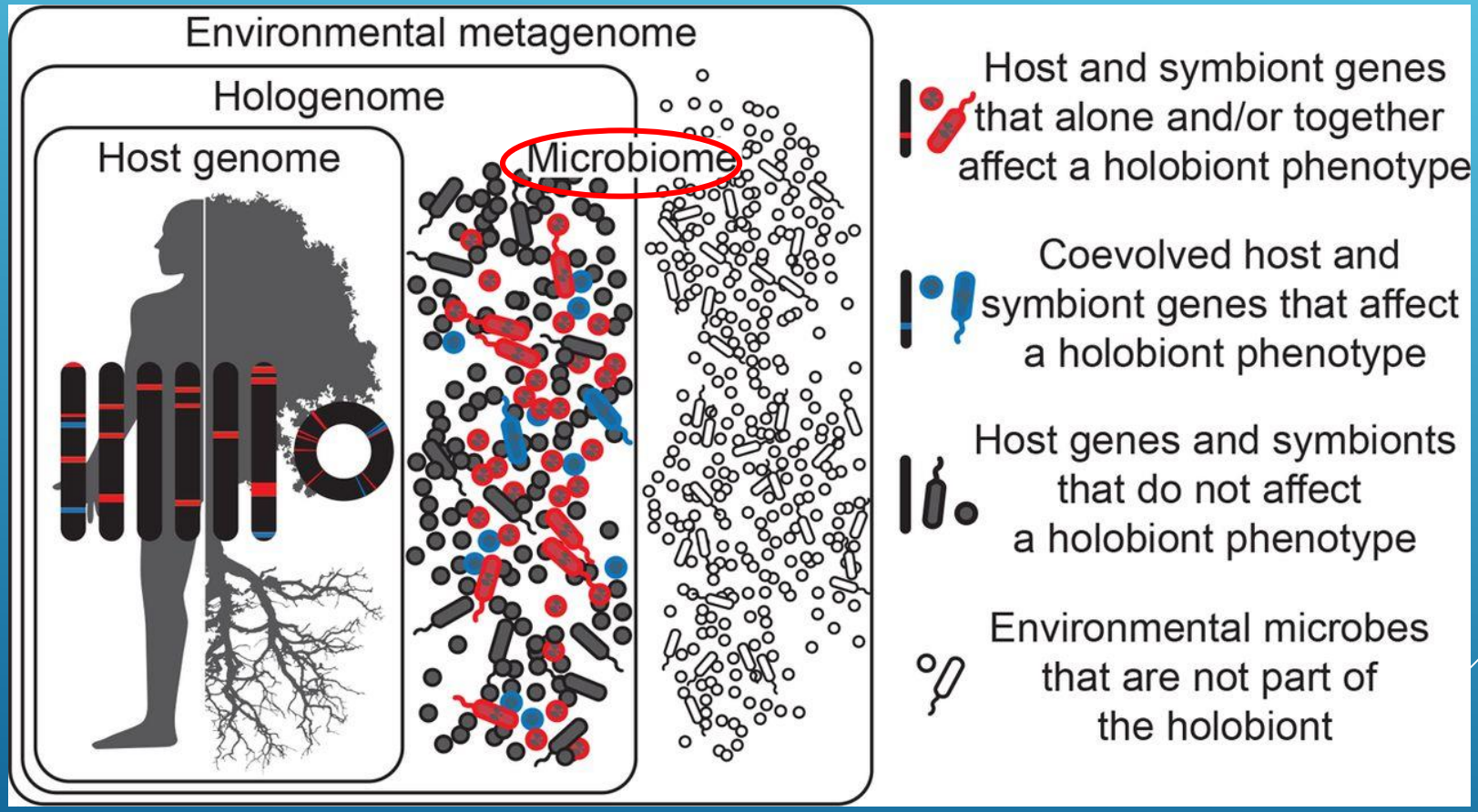
Il Microbiota intestinale: applicazioni cliniche

Dott.ssa Federica Del Chierico

Unità del microbioma umano

Ospedale Pediatrico Bambino Gesù

Holobiont concept

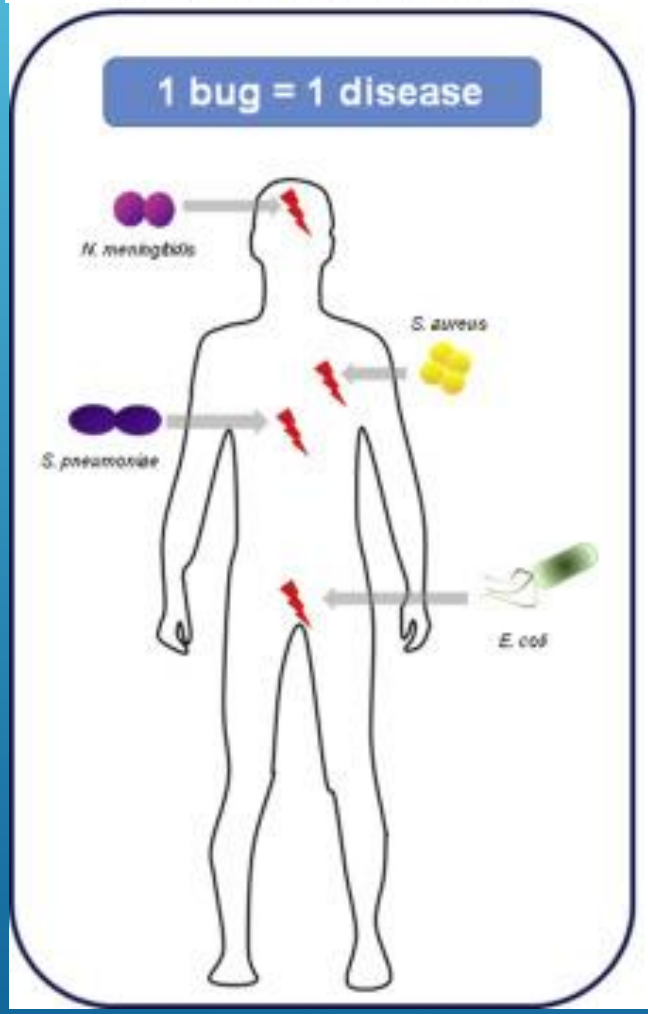


ASM Journals mSystems Vol. 1, No. 2 Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes

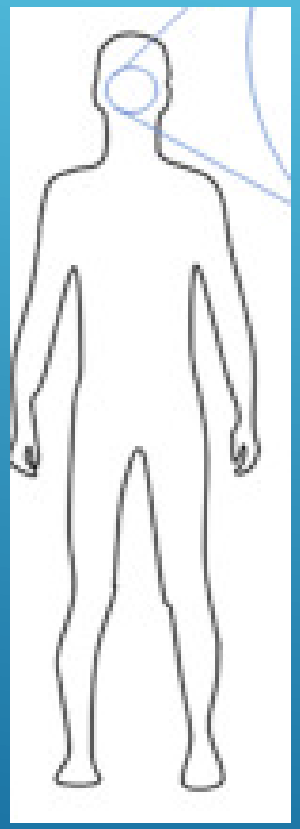
Evolution of the Koch postulates over time

Evolution over time

KOCH'S POSTULATE



CURRENT VISION

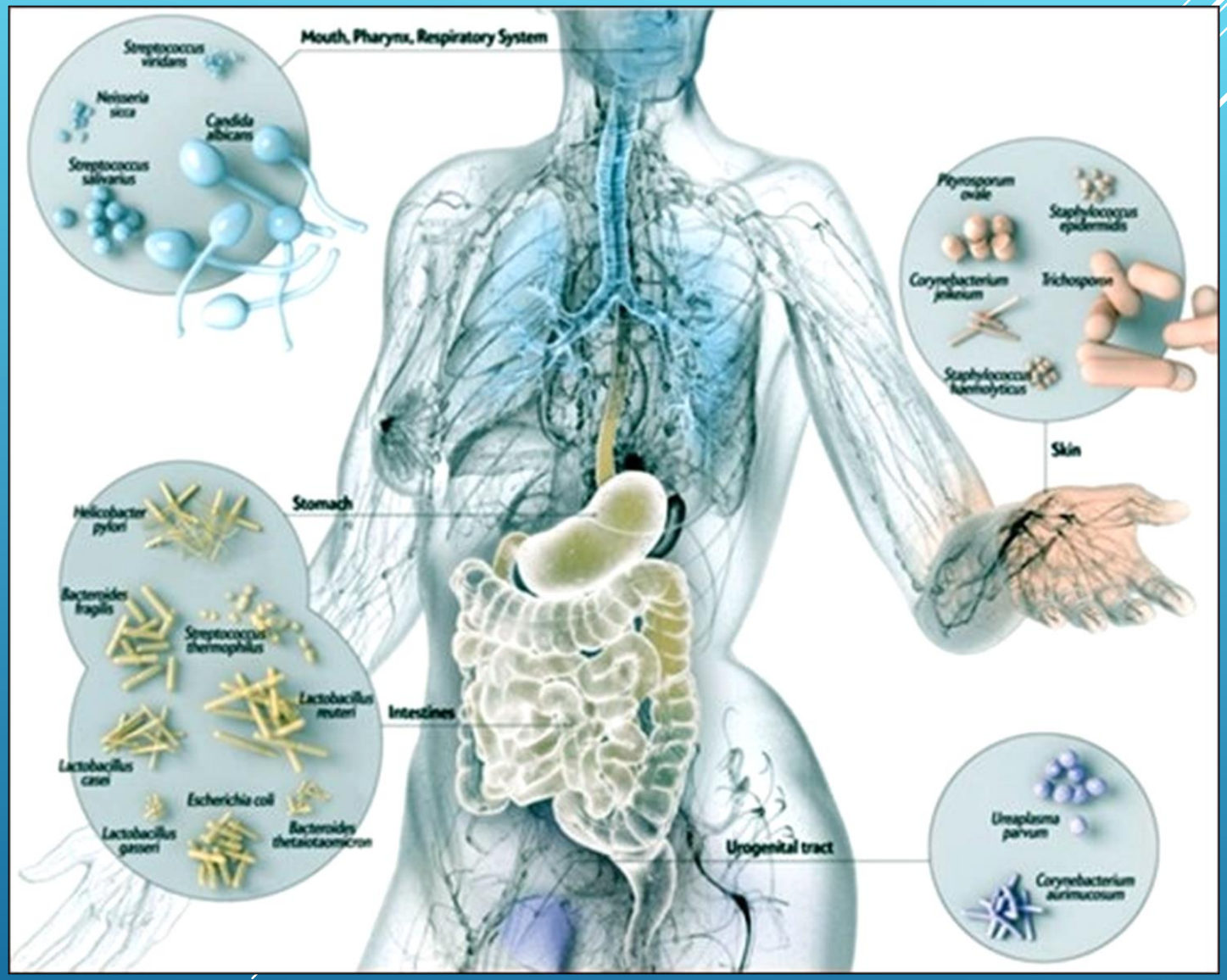


- Competition between pathogens
- Dysbiosis
- Inflammation
- Intestinal permeability
- Reservoir of Multi drug resistant organisms

Adapted fro: Lagier J., Dubourg G., Amrane S., Raoult D. Koch postulate: why should we grow bacteria? Archives of medical research 2017 48: 774-779

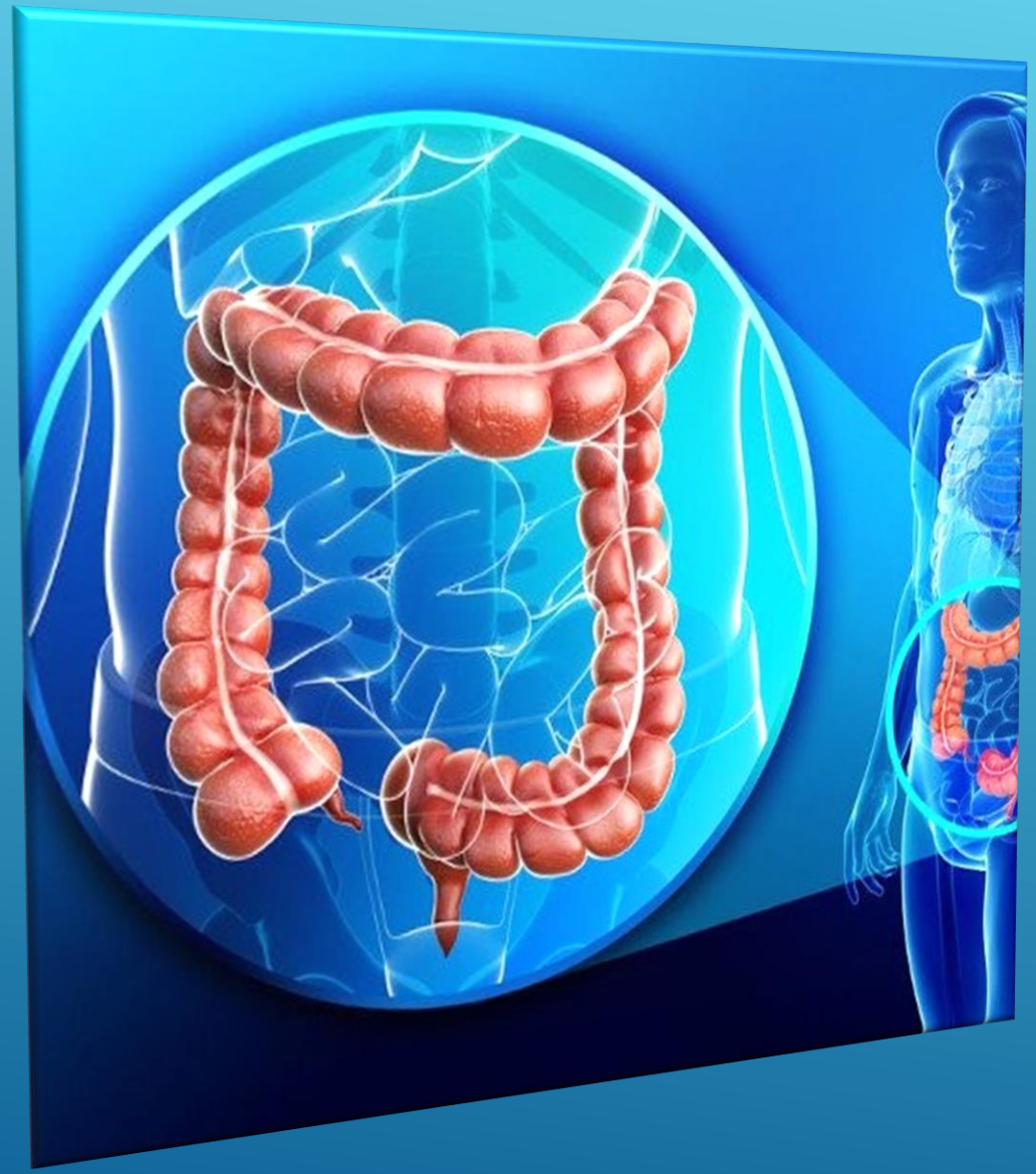
Il microbiota umano

I nostro corpo è popolato da un grandissimo numero di batteri, virus e funghi che sono variamente distribuiti nell'organismo.



Il microbiota intestinale

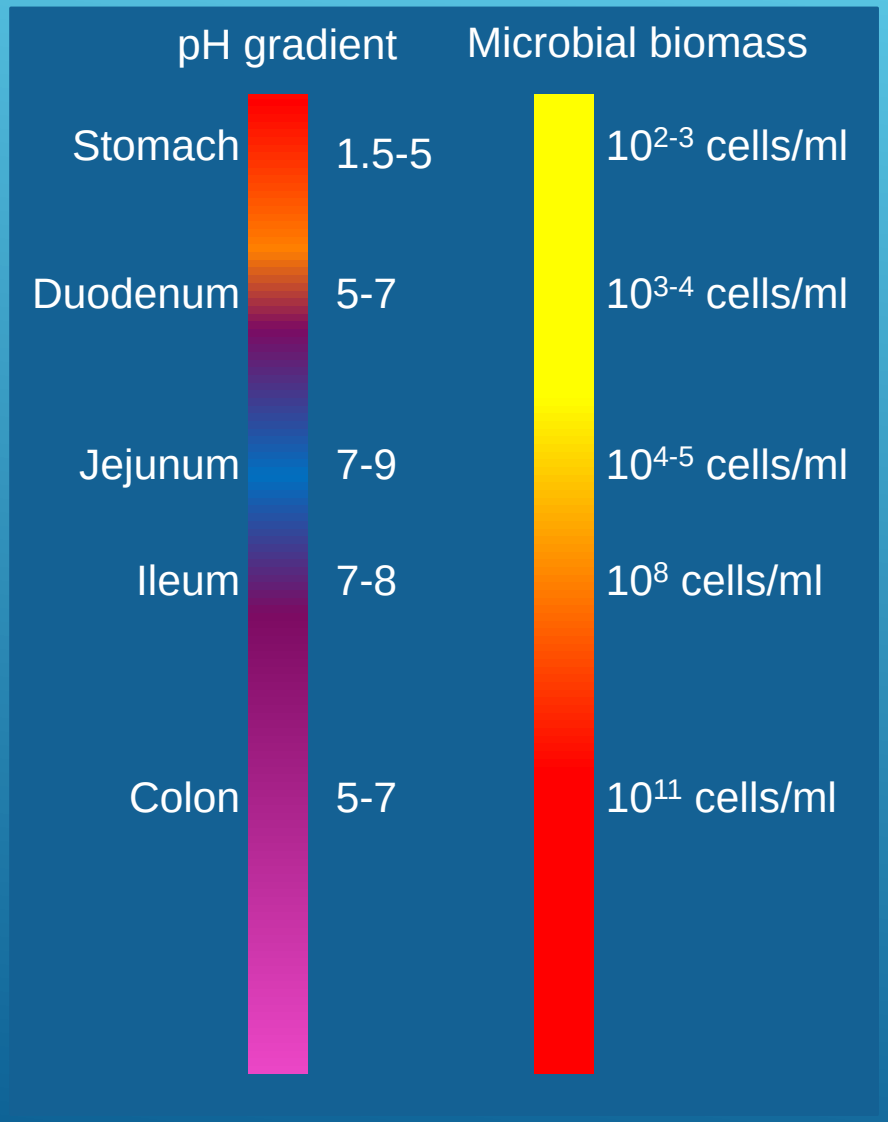
Oltre il 70% del totale di questi batteri si trova nell'apparato digerente, dove costituiscono il **microbiota intestinale umano**, quello che una volta si chiamava, in maniera non del tutto corretta, flora batterica intestinale.

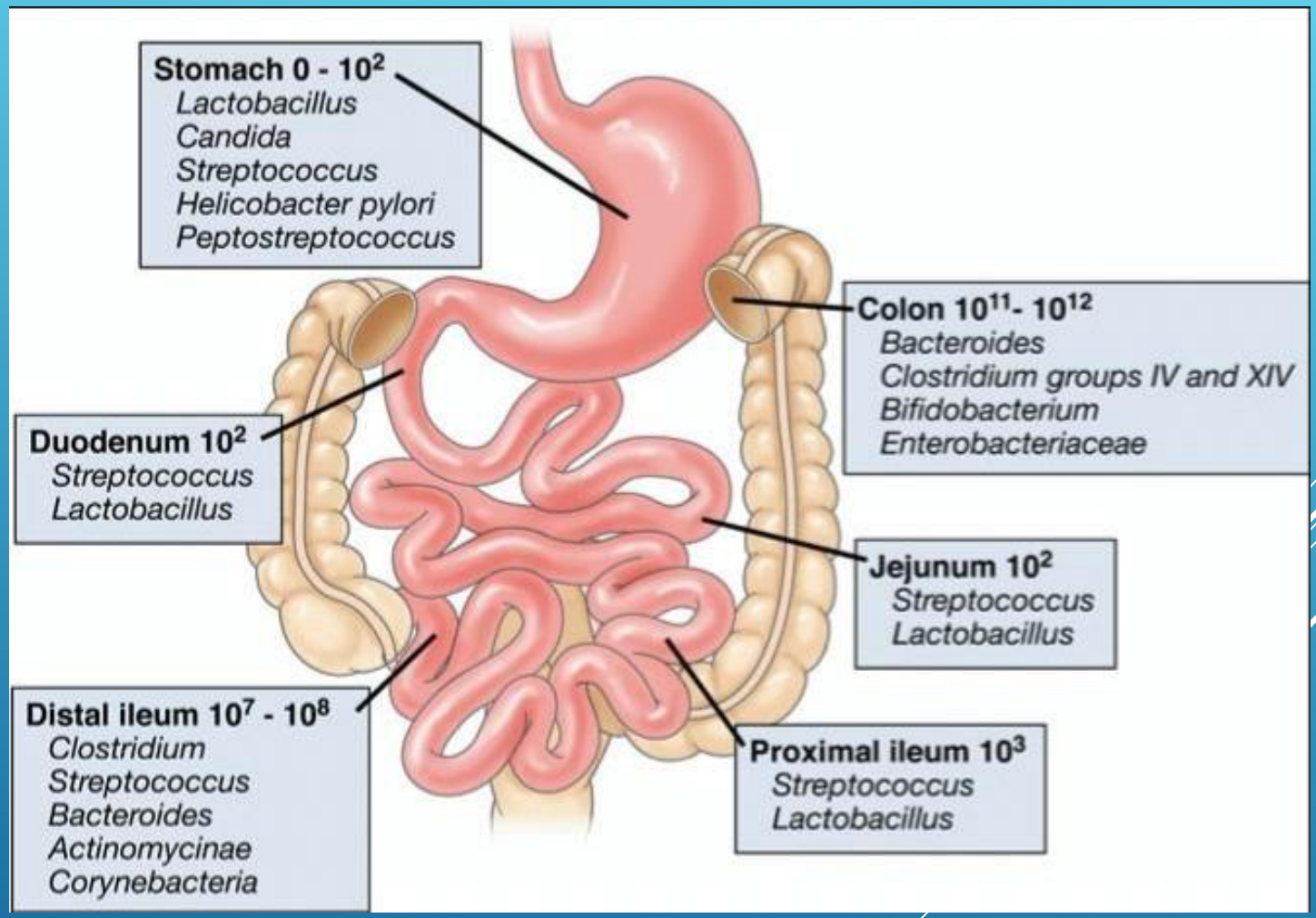


Tutte le malattie hanno origine nell'intestino …

IPPOCRATE 460 A.C. – 377 A.C.

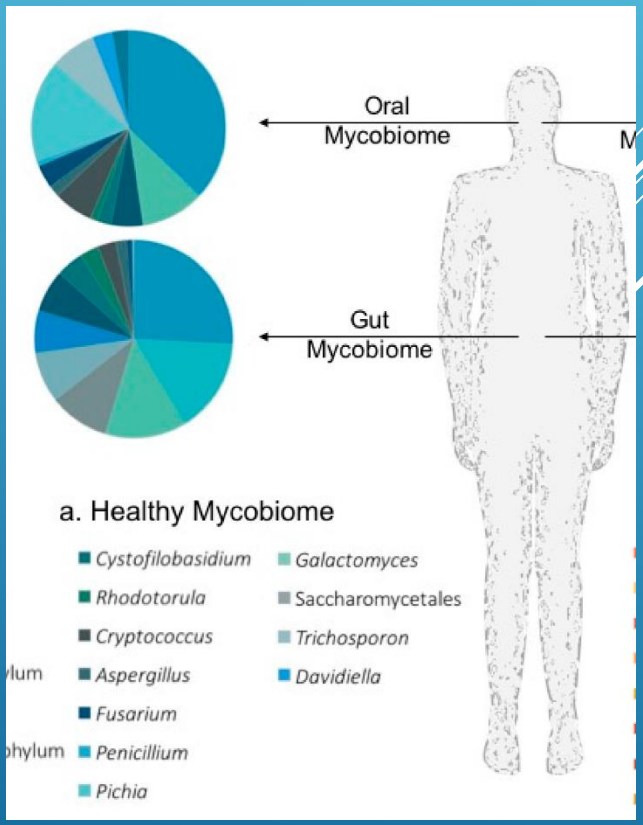
Il microbiota intestinale





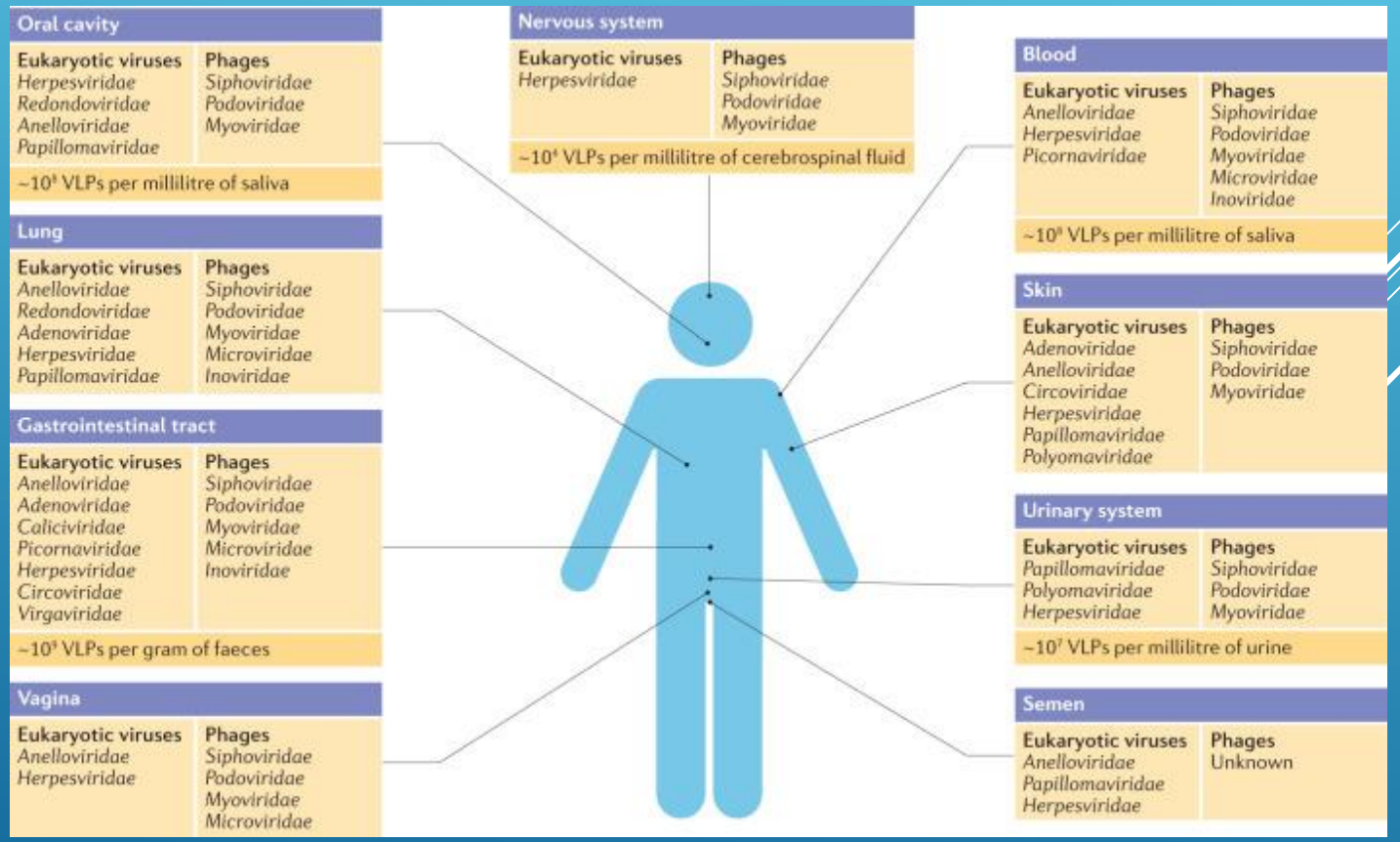
NON SOLO BATTERI….

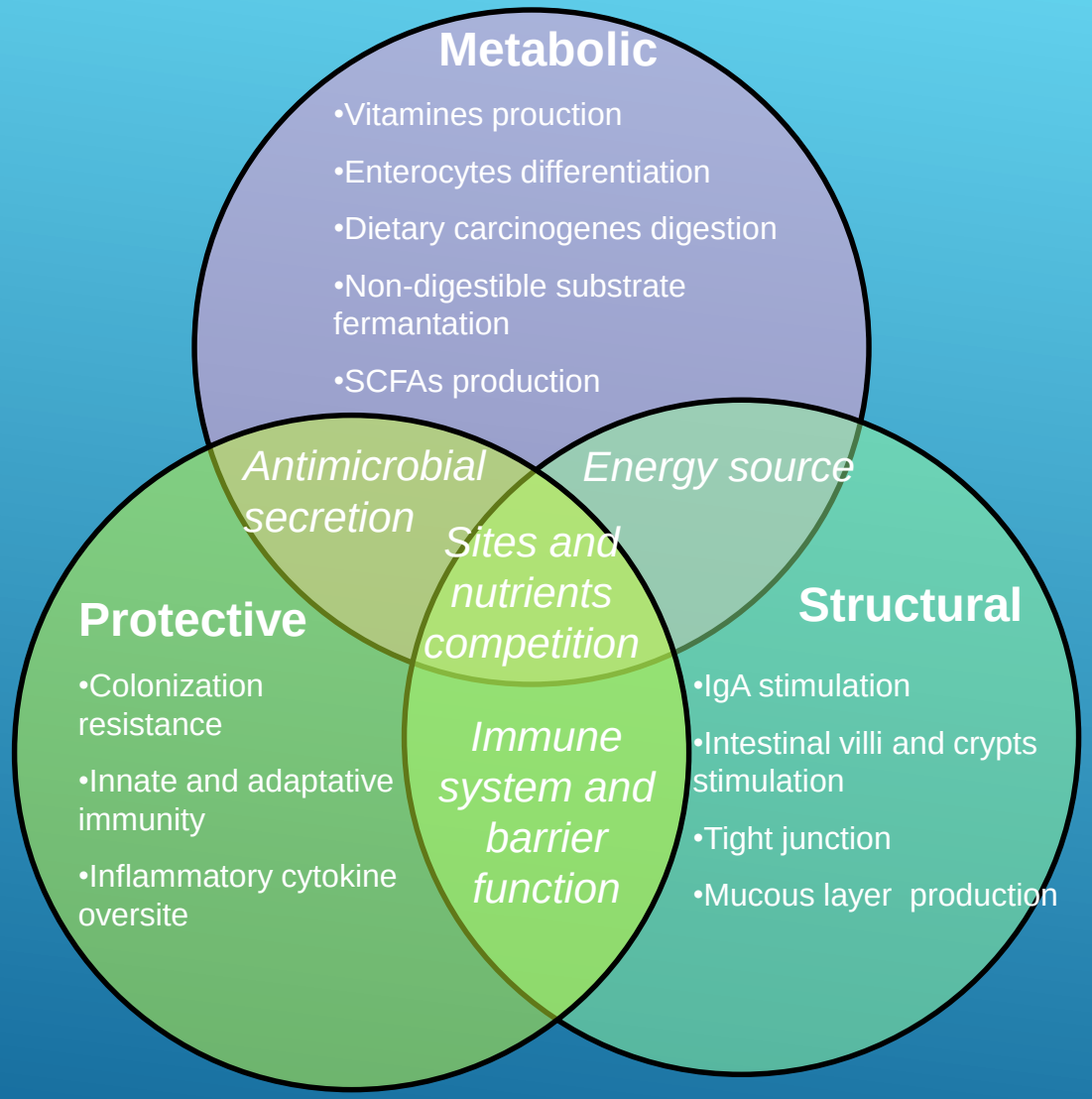
IL MICOBIOMA



IL VIROMA

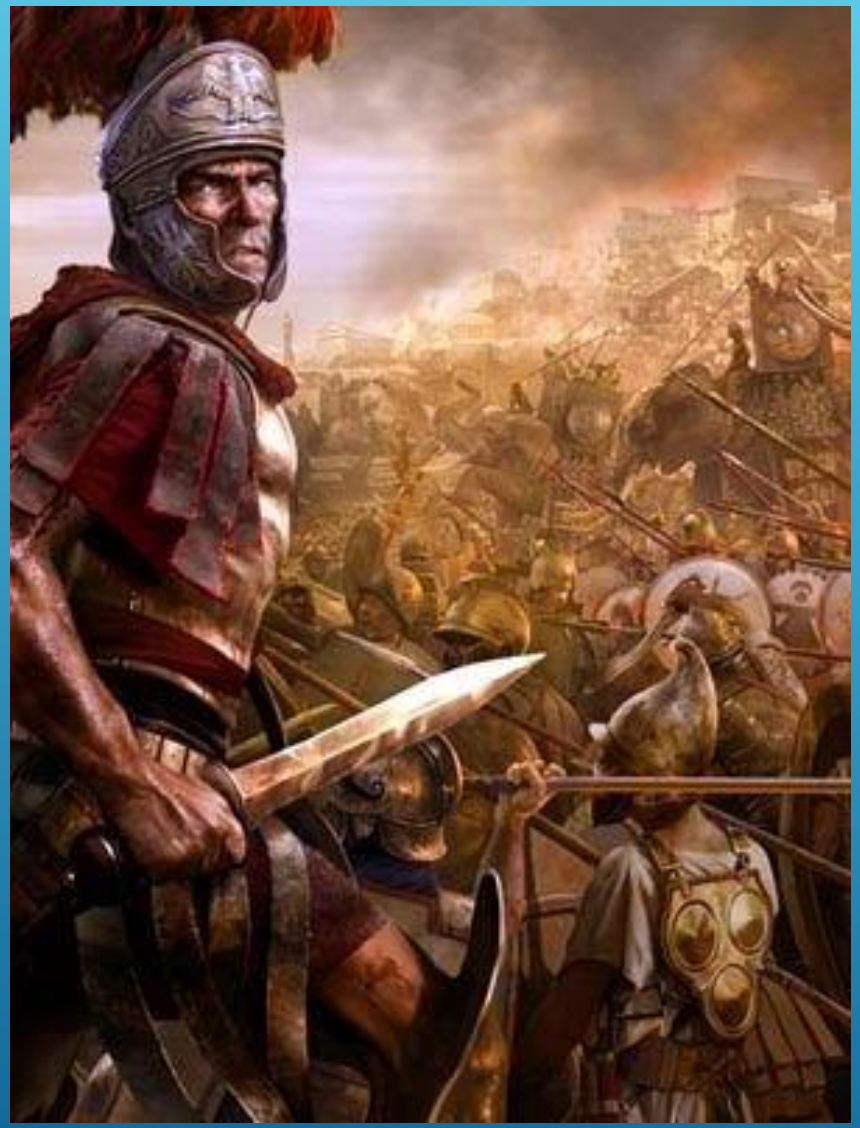
Nonostante la loro ubiquità, la conoscenza della **diversità genomica virale nel microbioma umano** è limitata e la maggior parte delle sequenze virali non sono rappresentate nei database esistenti del genoma.





COSA FA IL MICROBIOTA

- La relazione che esiste tra ciascuno di noi e il proprio microbiota è una relazione di **simbiosi**, una situazione che è vantaggiosa per entrambi i partecipanti. Il nostro ruolo è quello di fornire un ambiente caldo, protetto e ricco di cibo ai batteri che, in cambio, contribuiscono alla nostra salute.



Ruolo protettivo

I batteri intestinali sono una efficace **barriera** che inibisce ingresso e sviluppo di microrganismi provenienti dall'esterno e di specie opportuniste la cui crescita è normalmente ridotta.

Il microbiota forma una vera e propria barriera aderendo alla mucosa intestinale, impedendo quindi adesione e ingresso di specie patogene.

Varie specie batteriche producono sostanze in grado di inibire la crescita dei competitori, le **batteriocine**.

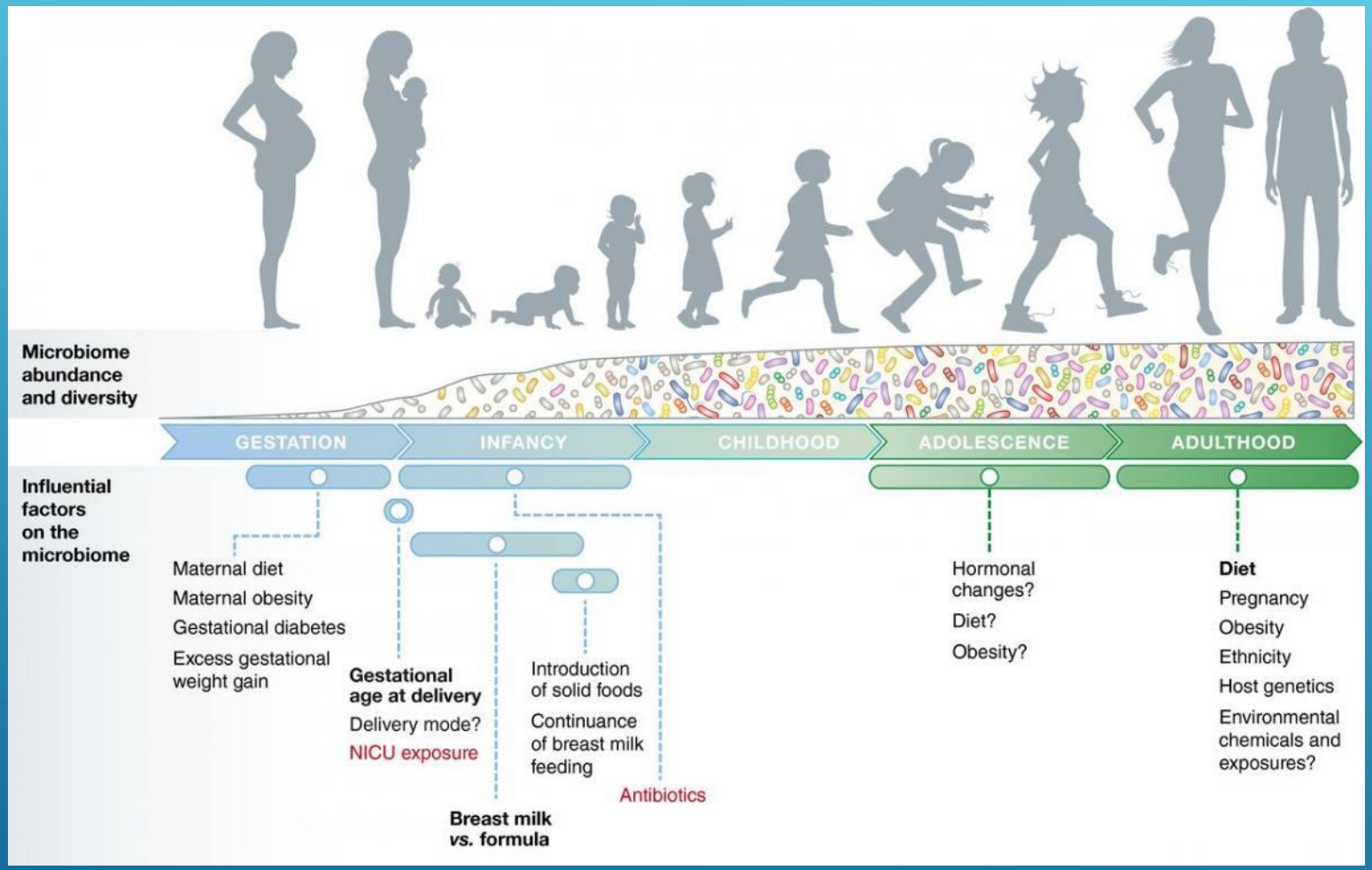
RUOLO METABOLICO

- Le diverse centinaia di specie presenti presentano un corredo enzimatico estremamente vario che è profondamente diverso dal nostro.
- Il nostro colon, con la sua ricca presenza di batteri, è in pratica un bioreattore dove i residui non digeriti o non digeribili del cibo consumato diventano il substrato, il pane quotidiano, del microbiota.

CONTRIBUISCE ALLO SVILUPPO DEL SISTEMA IMMUNITARIO

- Il microbiota lavora con gli elementi del sistema immunitario per raggiungere due obiettivi:
- - da una parte previene una risposta immunitaria eccessiva che potrebbe danneggiare i batteri commensali presenti,
- - dall’altra garantisce un’azione di controllo che eviti crescita eccessiva o trasferimento in altri siti di batteri.

DA DOVE VIENE IL MICROBIOTA?



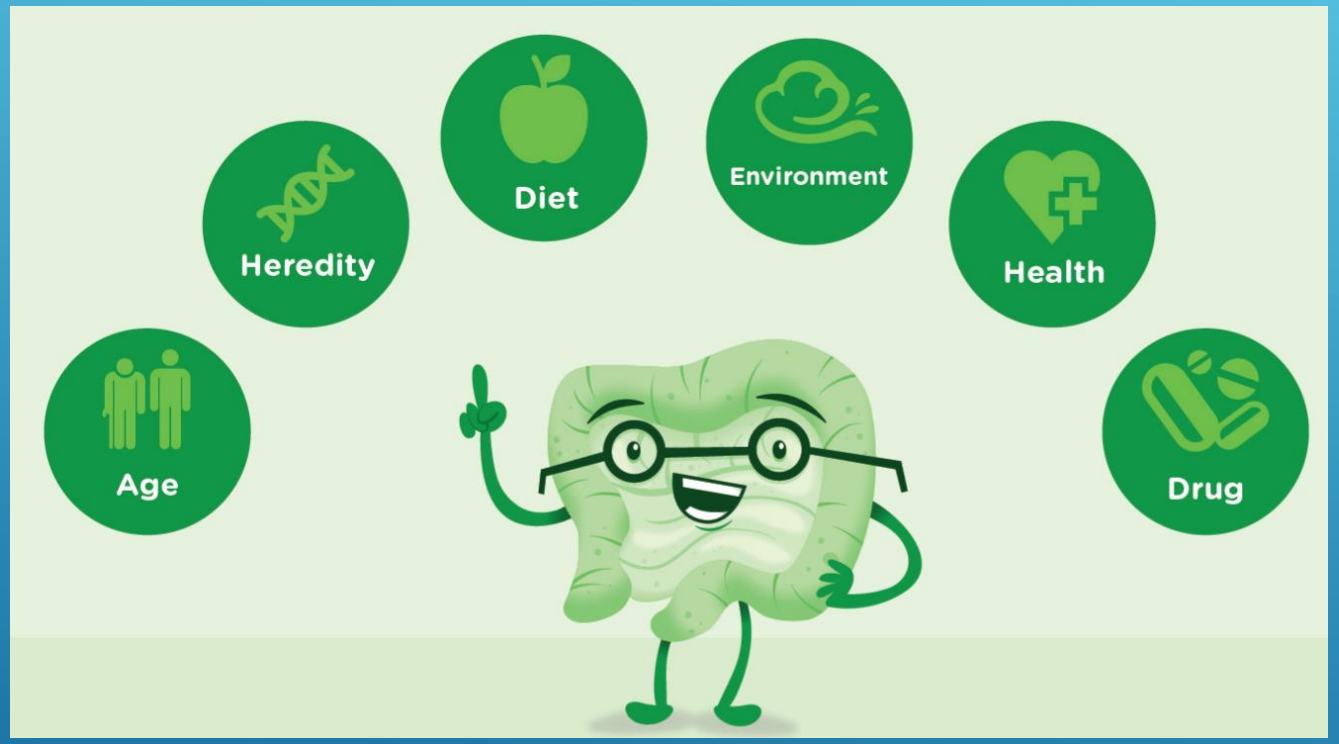
La colonizzazione vera e propria inizia con il passaggio nel canale del parto. Nei primi tre anni di vita, la popolazione batterica cambia notevolmente e dopo i 3 anni tende a diventare simile a quella che sarà tipica dell'età adulta.

Sapiamo che ci sono differenze tra il microbiota dei bambini nati con parto normale e quelli nati da parto Cesareo.

L'allattamento al seno o utilizzando latte artificiale, il tempo e la modalità di svezzamento ed i cibi utilizzati, ed eventuali terapie antibiotiche sono fattori importanti che influenzano la composizione del microbiota.

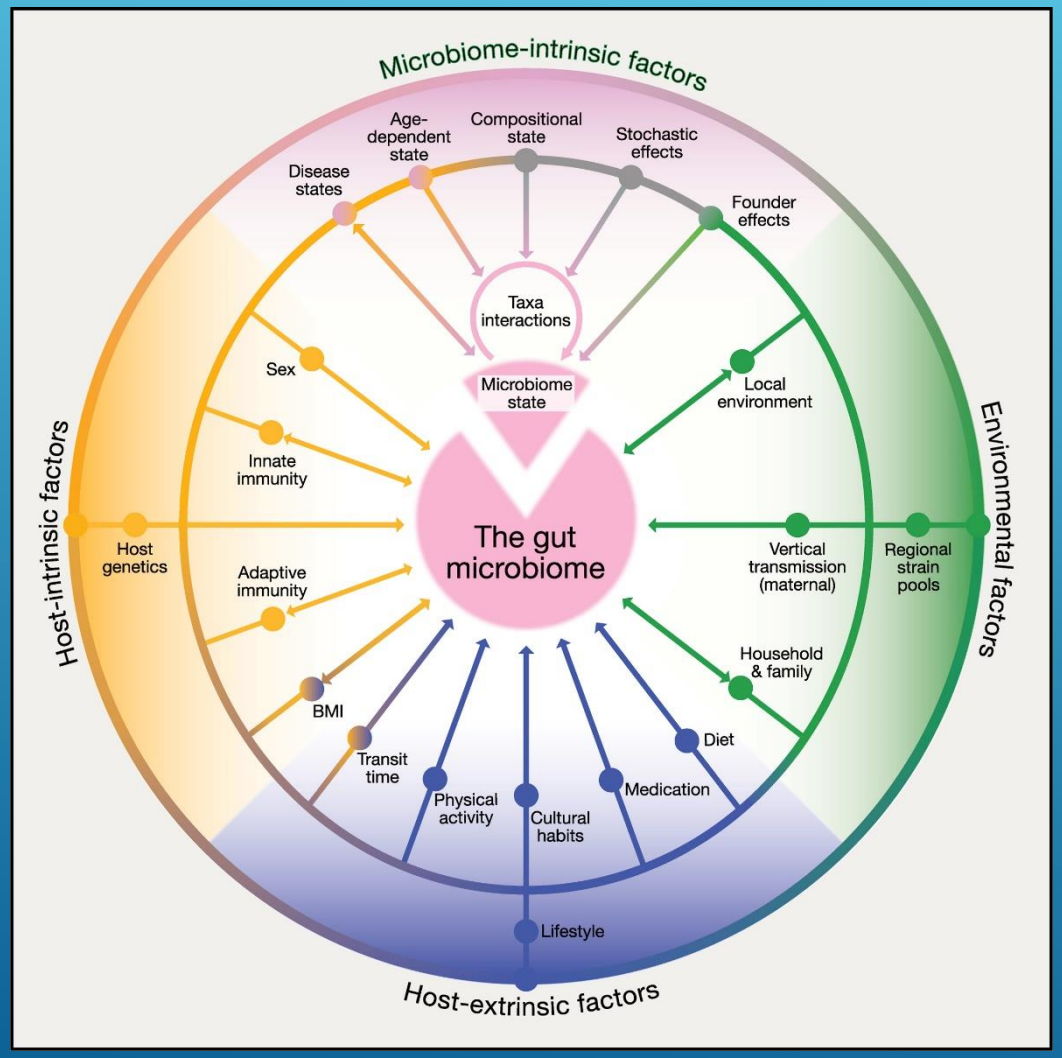
COME MANTENERE IL MICROBIOTA IN SALUTE

- La **dieta** rimane il fattore predominante nel determinare l'equilibrio del microbiota.
- **Un'alimentazione ricca di verdura, frutta e cereali integrali garantisce un elevato apporto di quelle fibre che sono garantiscono massima ricchezza e diversità del microbiota**.
- Uno stile di vita sano contribuisce a mantenere un microbiota sano.



Fattori che influenzano la composizione del microbiota intestinale

Microbiota Composition Is Associated to Several Known Co-variates

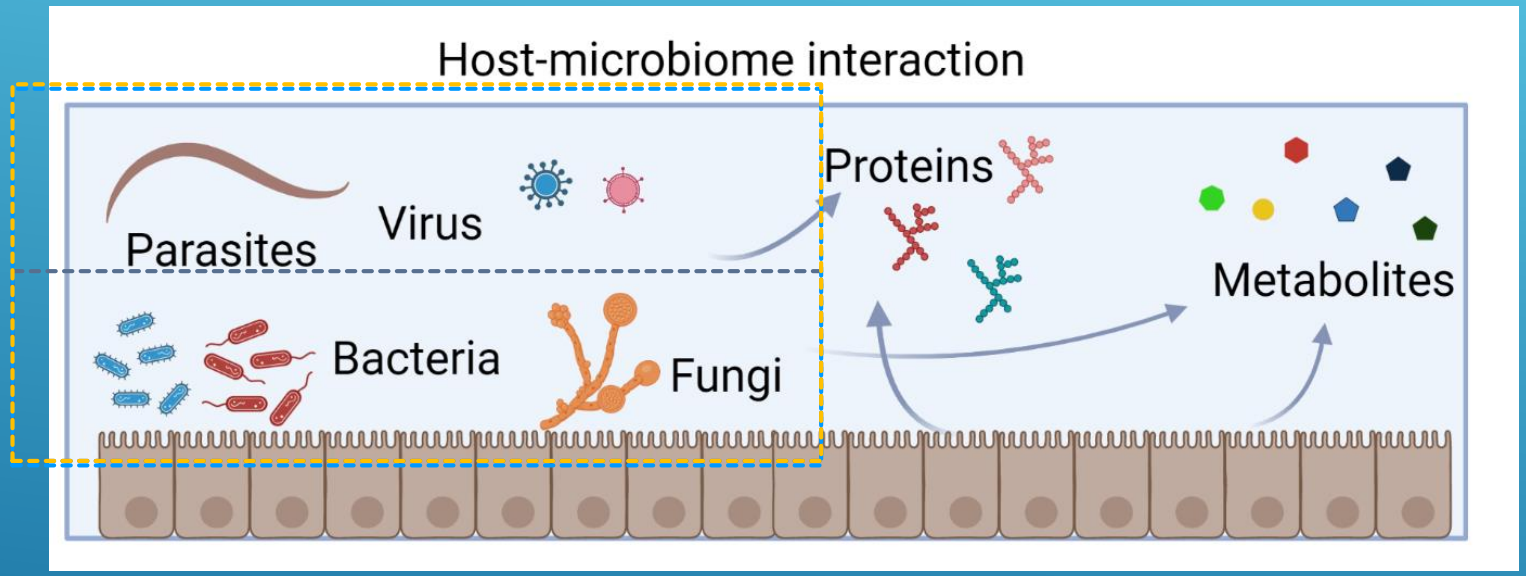


Schmidt TSB, Raes J, Bork P. The Human Gut Microbiome: From Association to Modulation. Cell. 2018 Mar 8;172(6):1198-1215. doi: 10.1016/j.cell.2018.02.044. PMID: 29522742.

Meta-omics approaches for the host-microbiome association studies

Targeted-sequencing

Taxa composition



Metagenomics

Taxa composition and functional capability

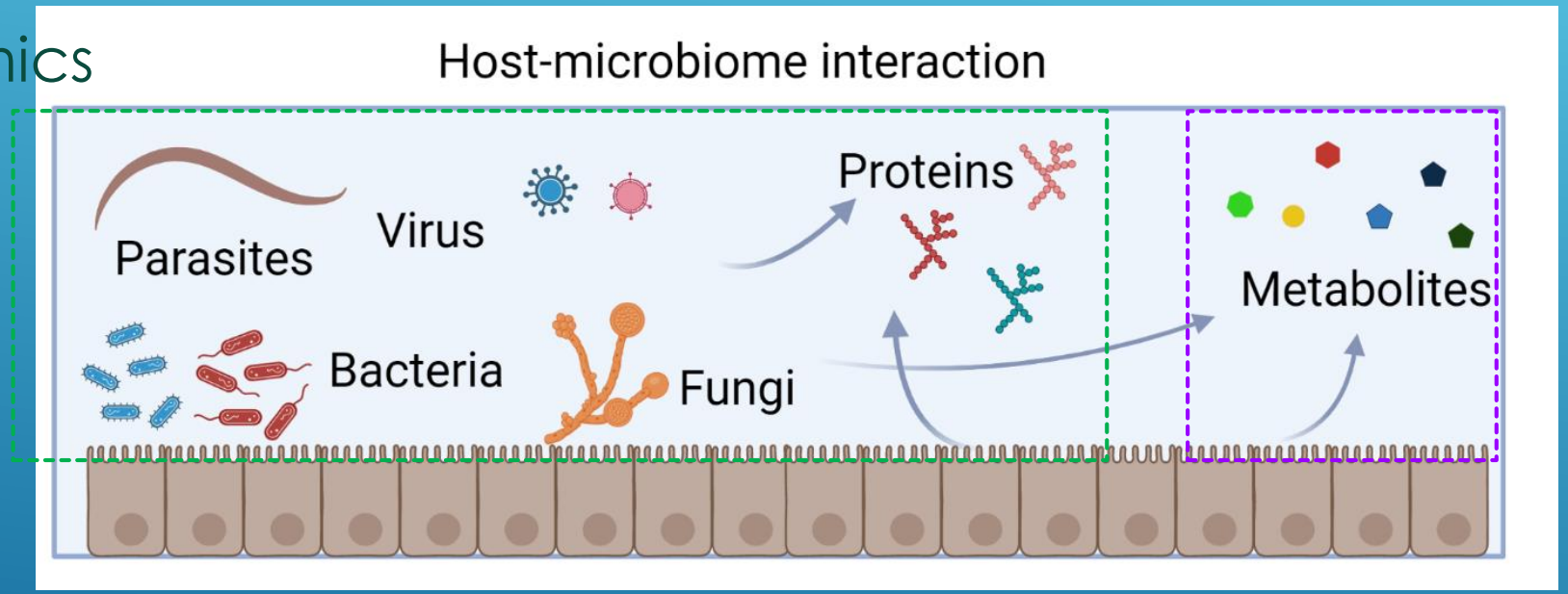
Metatranscriptomics

Taxa composition and functional information

Meta-omics approaches for the host-microbiome association studies

Metaproteomics

Taxa composition and function



Metabolomics

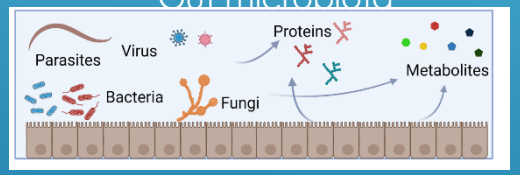
Function

Integration of -omics data

Clinical data

Diet and lifestyle data

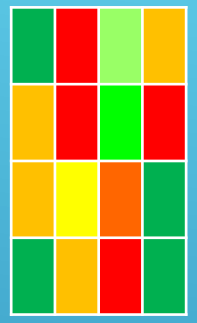
Gut microbiota



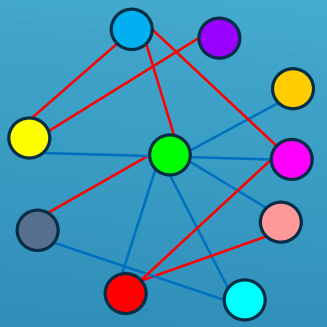
-Omics data

Cytokines profiles

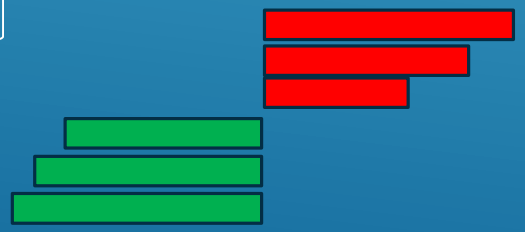
-Omics integration



Correlation heatmaps



Functional networks



Biomarker discovery

Machine learning
Predictive modelling

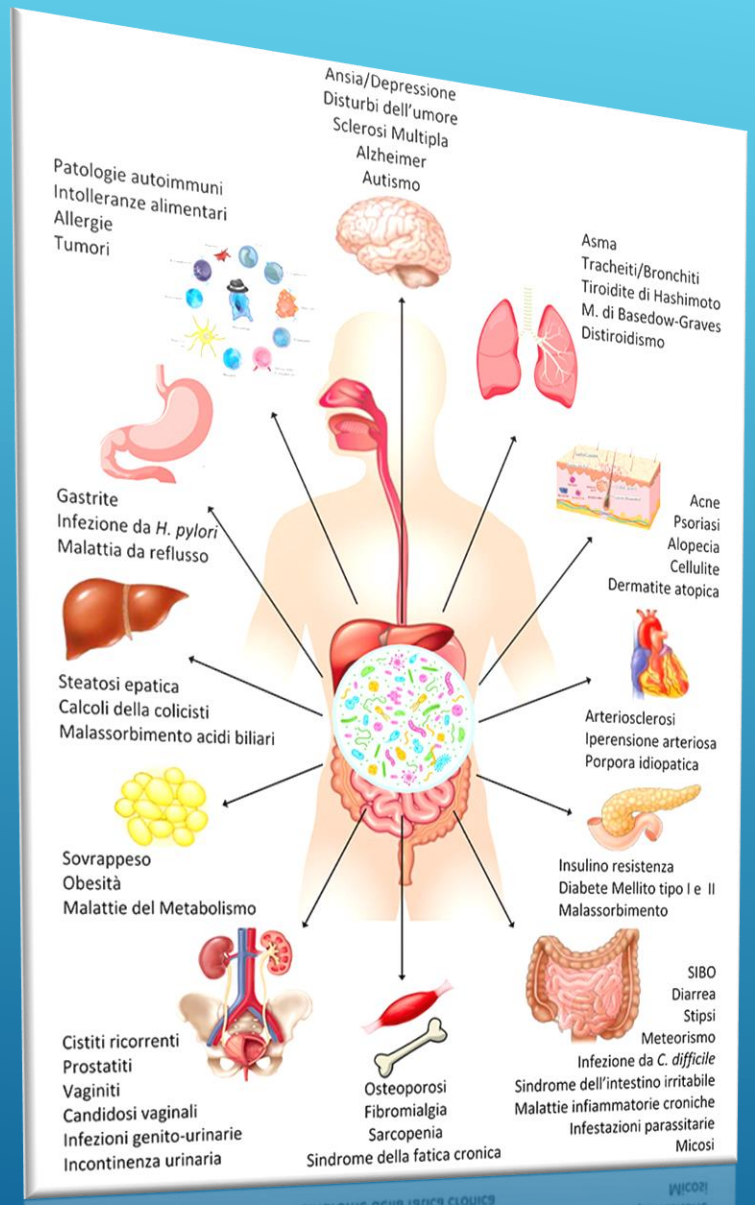
Results validation

Clinical application

Data di pubblicazione:
10 dicembre 2018
ultimo aggiornamento:
13 settembre 2021

https://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=2800

MICROBIOTA E MALATTIE



- Visti i diversi ed importanti ruoli che il microbiota ricopre, è stato evidenziato un possibile ruolo del microbiota intestinale in un nutrito e variegato gruppo di malattie.

LA DISBIOSI

Fattori genetici che possono alterare la barriera intestinale

Crescita smisurata di batteri patogeni

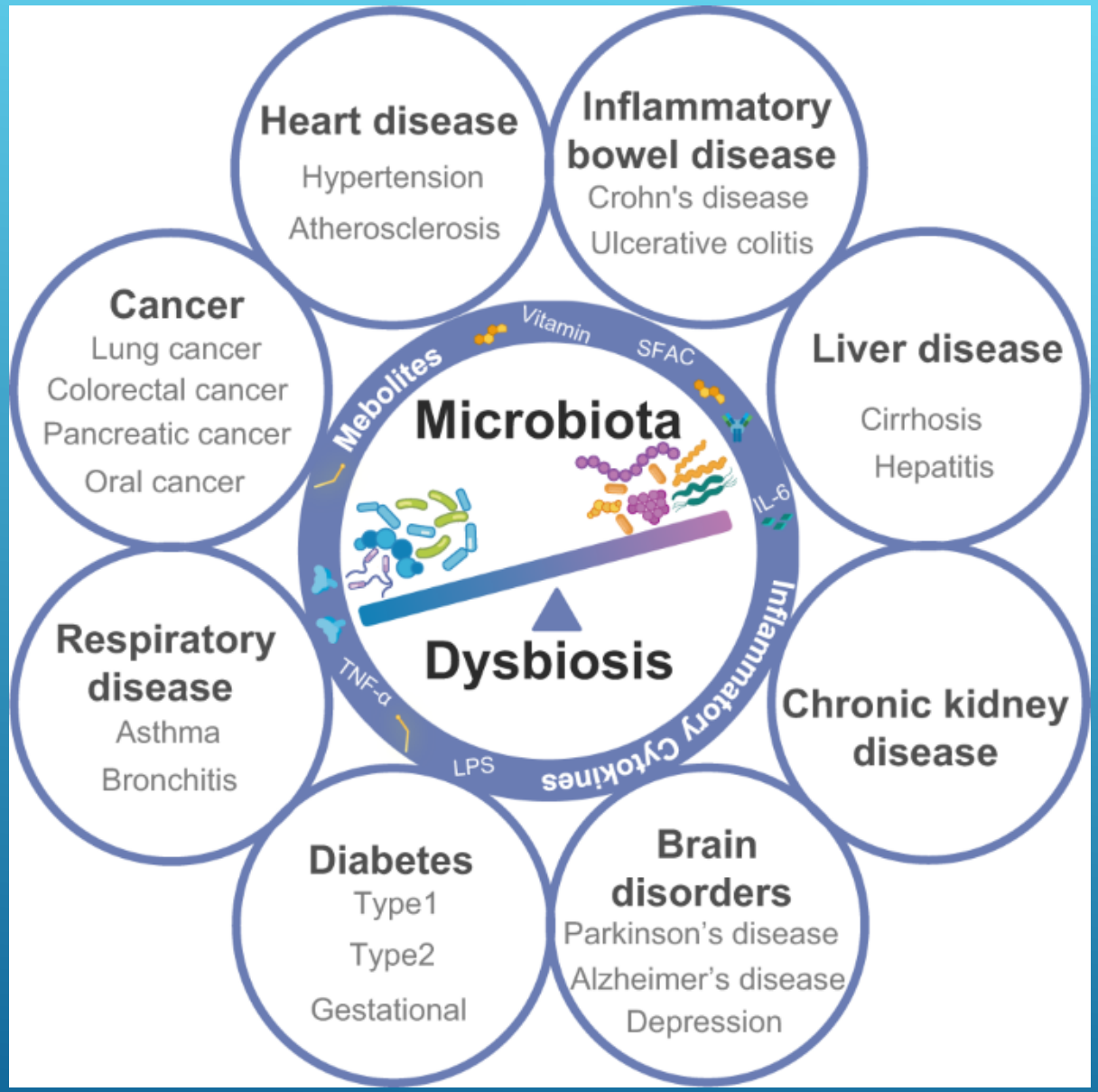
Traslocazione di batteri o prodotti batterici

Attivazione immunitaria produzione di citochine pro-infiammatorie

Infiammazione cronica che porta alla distruzione dei tessuti

La sindrome dell'intestino permeabile

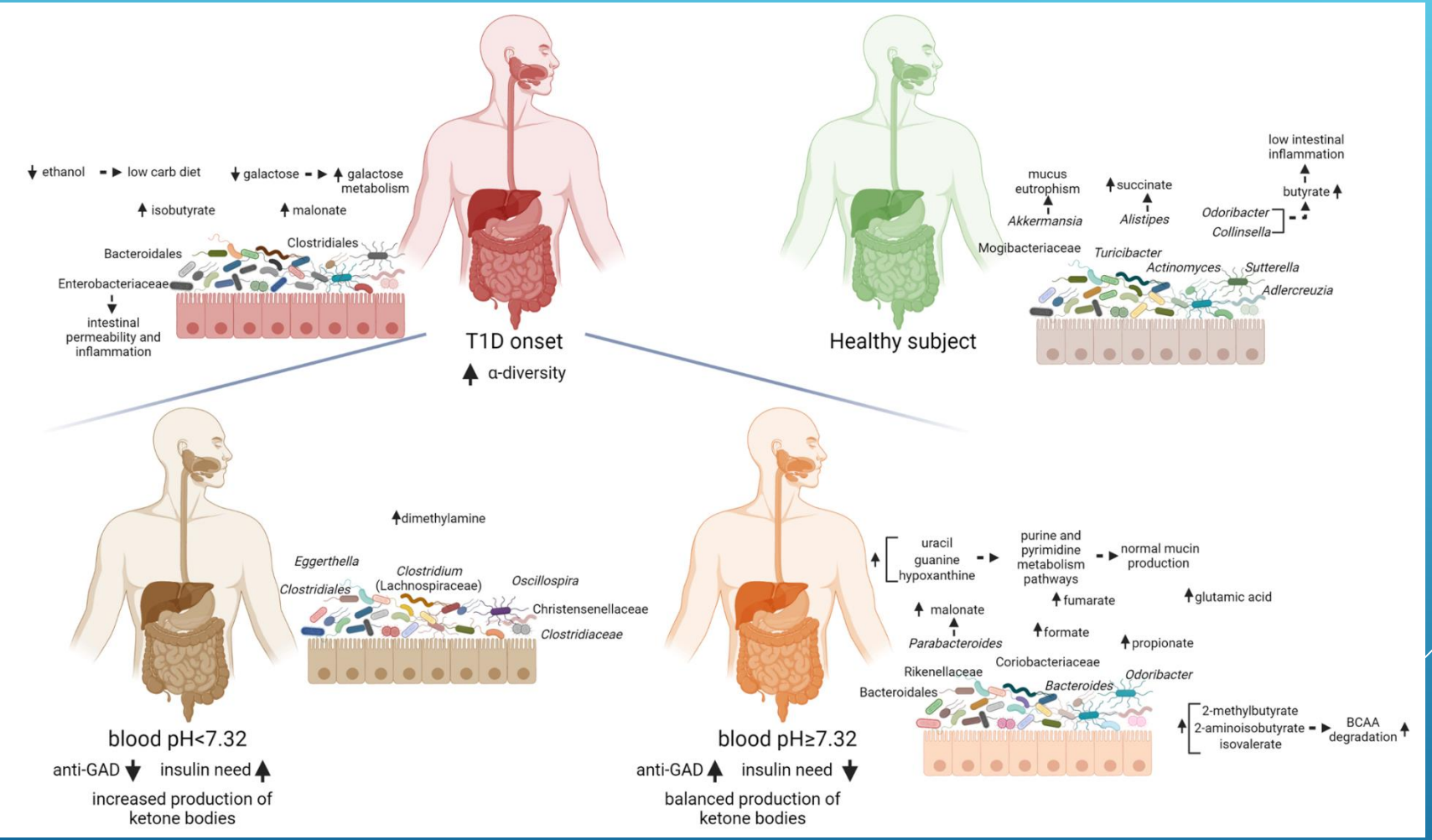
DISEASE-RELATED PROFILING



Hou, K., Wu, ZX., Chen, XY. et al. Microbiota in health and diseases. Sig Transduct Target Ther 7, 135 (2022). https://doi.org/10.1038/s41392-022-00974-4

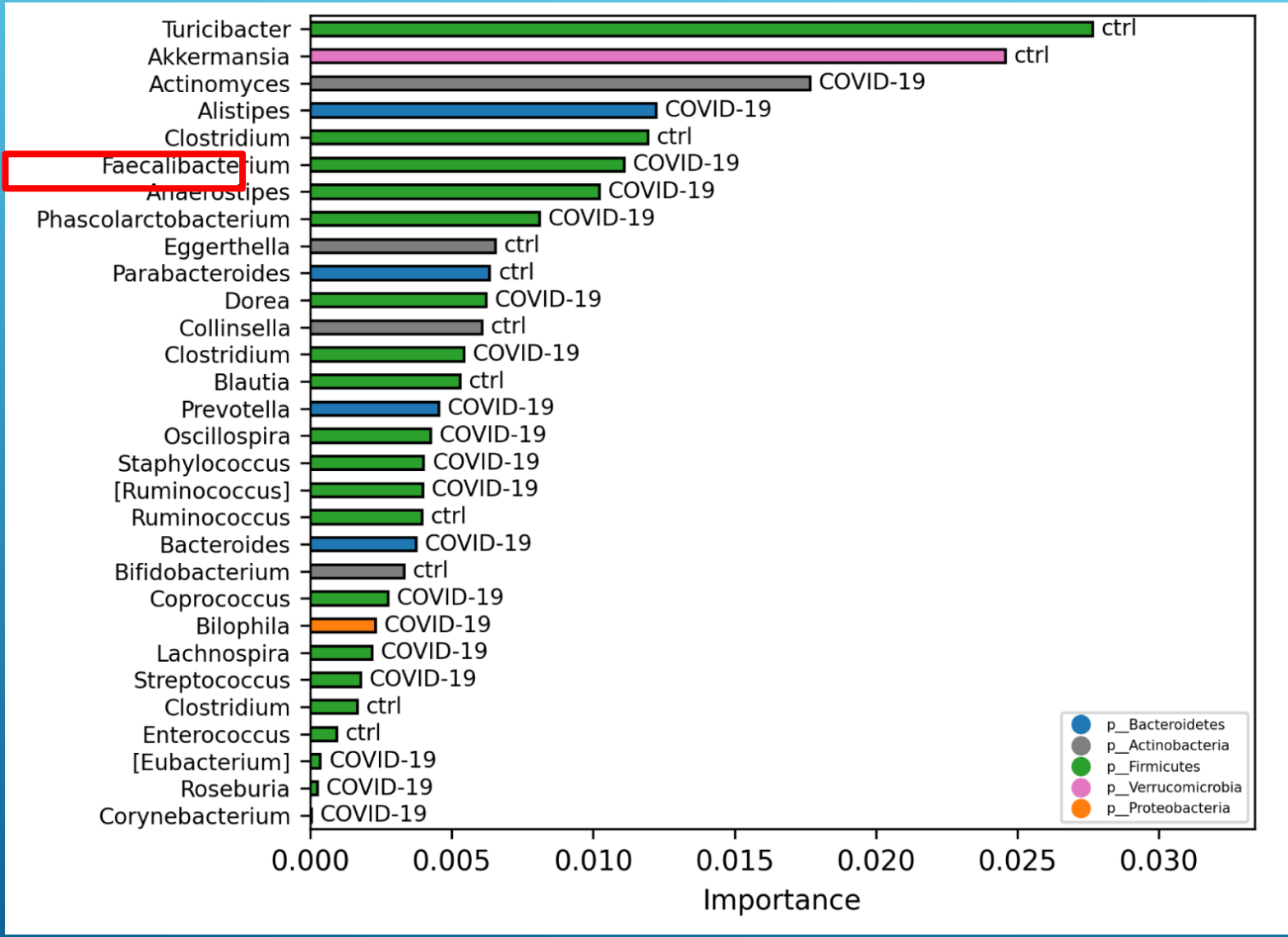
Integration of meta-omics approaches in host-microbiome association study

Type 1 diabetes



Del Chierico, F.; Conta, G.; Matteoli, M.C.; Fierabracci, A.; Reddel, S.; Macari, G.; Gardini, S.; Guarrasi, V.; Levi Mortera, S.; Marzano, V.; et al. Gut Microbiota Functional Traits, Blood pH, and Anti-GAD Antibodies Concur in the Clinical Characterization of T1D at Onset. *Int. J. Mol. Sci.* **2022**, *23*, 10256. https://doi.org/10.3390/ijms231810256

GUT MICROBIOTA IN SARS-COV-2 INFECTION

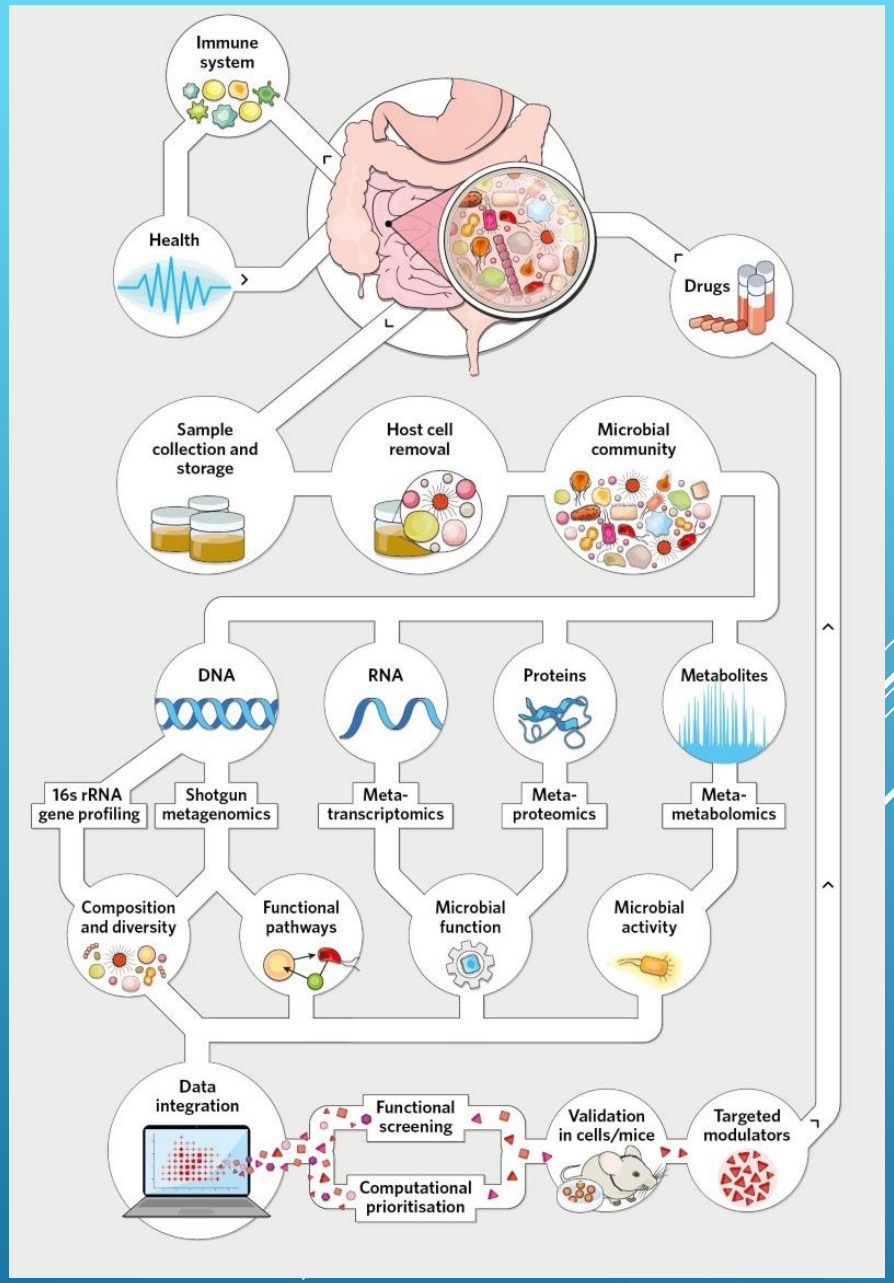


- ↓Faecalibacterium in adult COVID-19 GM
- Anti-inflammatory and fermentative properties of Faecalibacterium
- Reinold et al 2021: ↓Faecalibacterium as discriminant for severe disease

Disease-associated microbial markers

microbiome-based medicines studies

Experimental validation of results



https://www.nature.com/articles/d42473-020-00214-9

Experimental validation

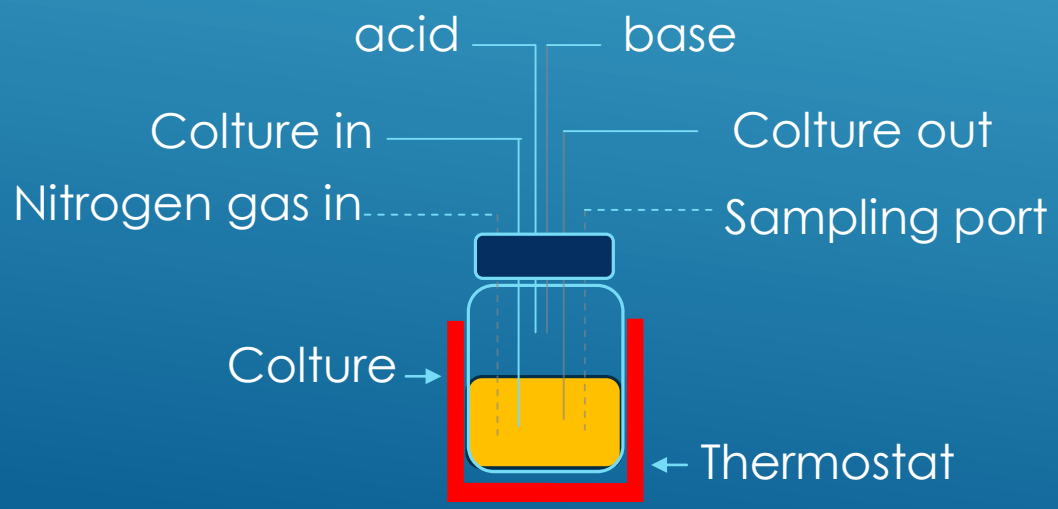
In vitro model: in vitro culture model of the human gut microbiota

Batch cultures

Continuous Single-Stage Culture models

Twin-vessel single-stage chemostats system

MiniBioReactor arrays



PROS

No confounding factors

test of different substrates on the gut microorganisms' physiology and biodiversity

CONS

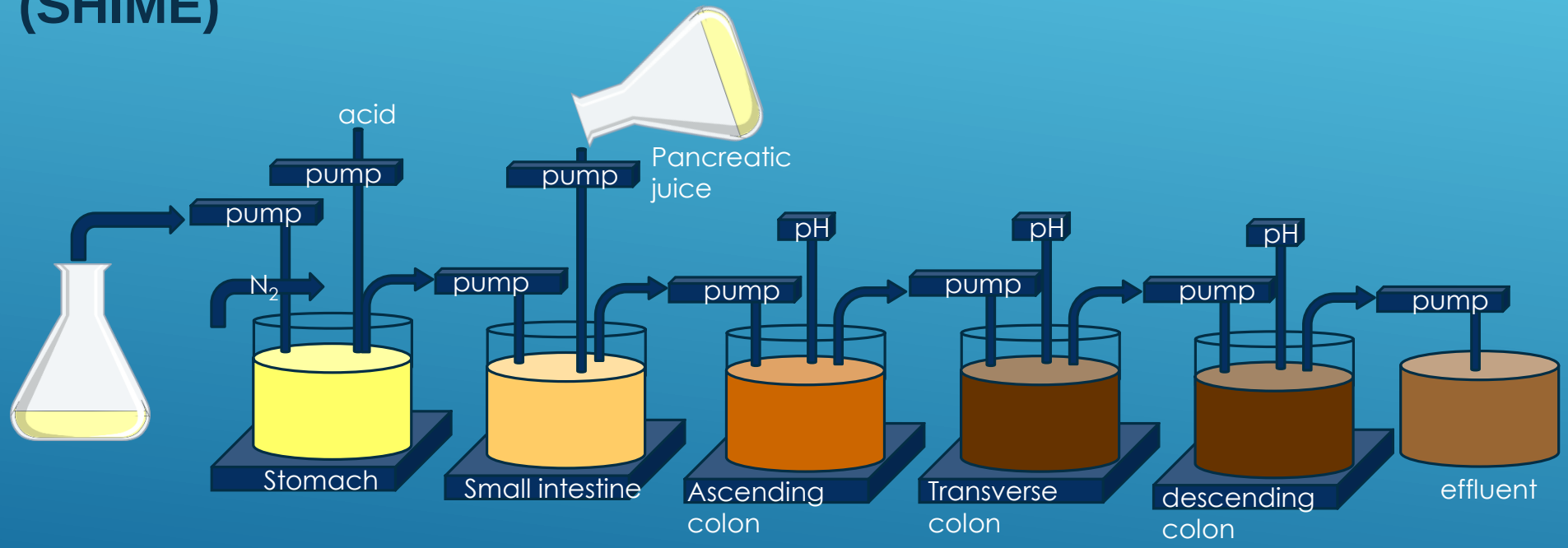
single colon zone simulation

lacking information on the microbial dynamics

behaviour along the GI tract

Experimental validation

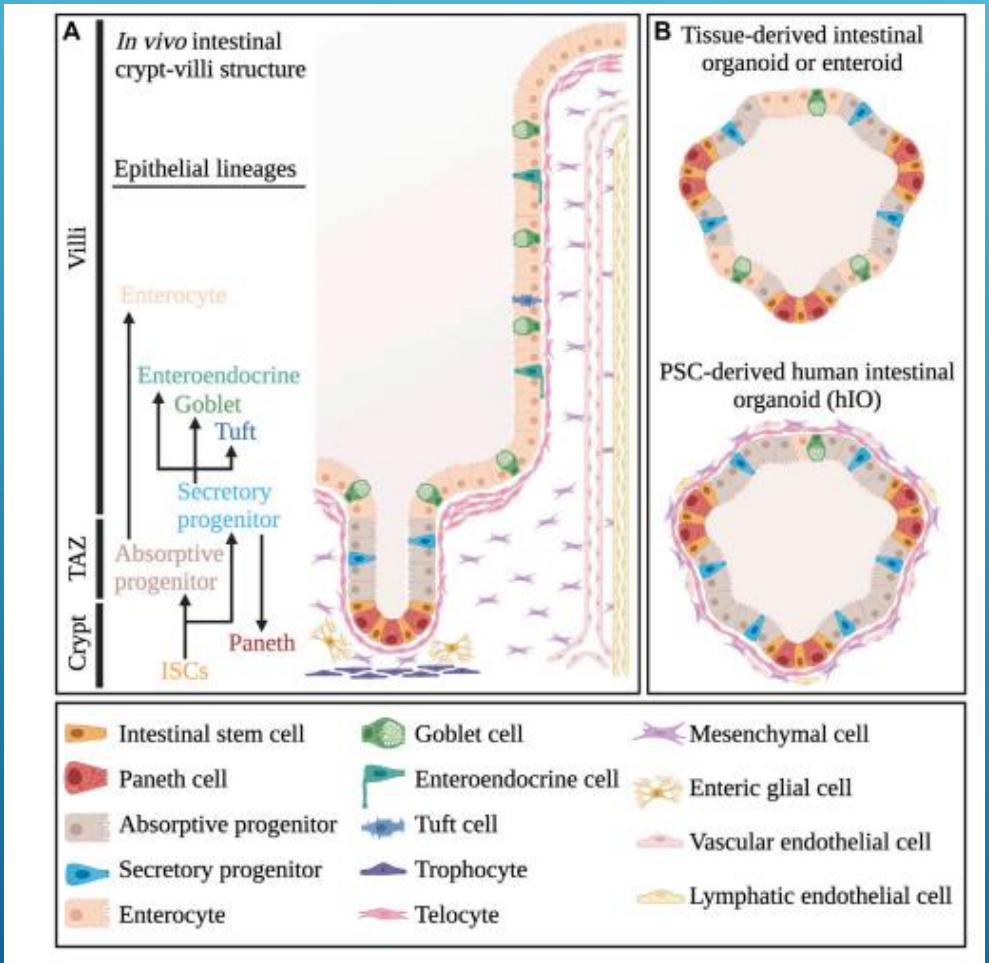
In vitro model: Simulator of the Human Intestinal Microbial Ecosystem (SHIME)



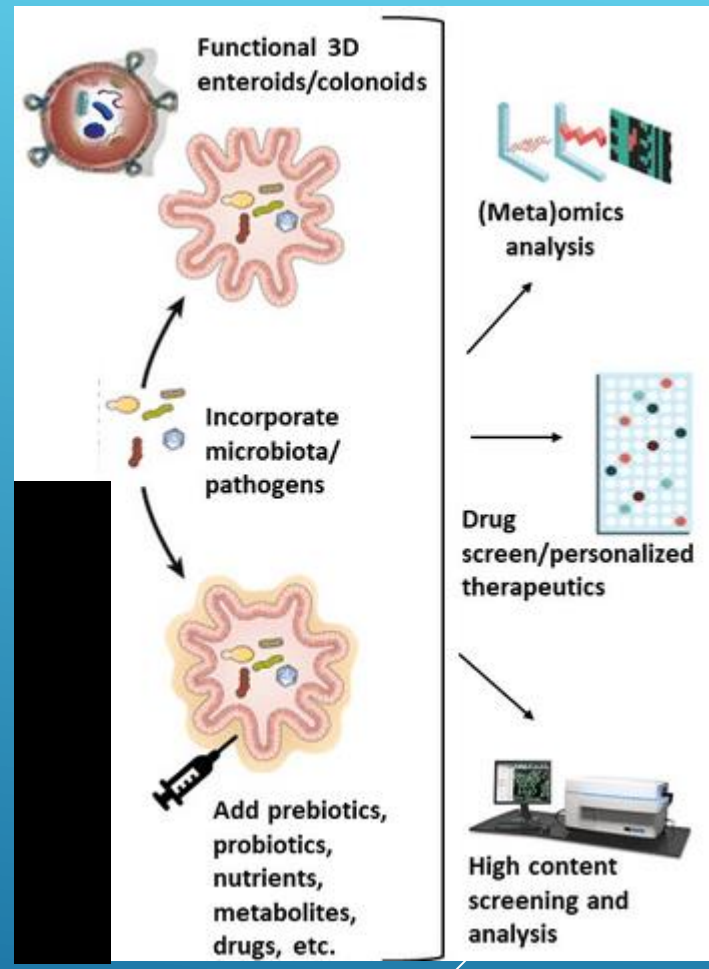
To study effects of probiotic strains and SCFA production

Experimental validation

In vitro models: organoids



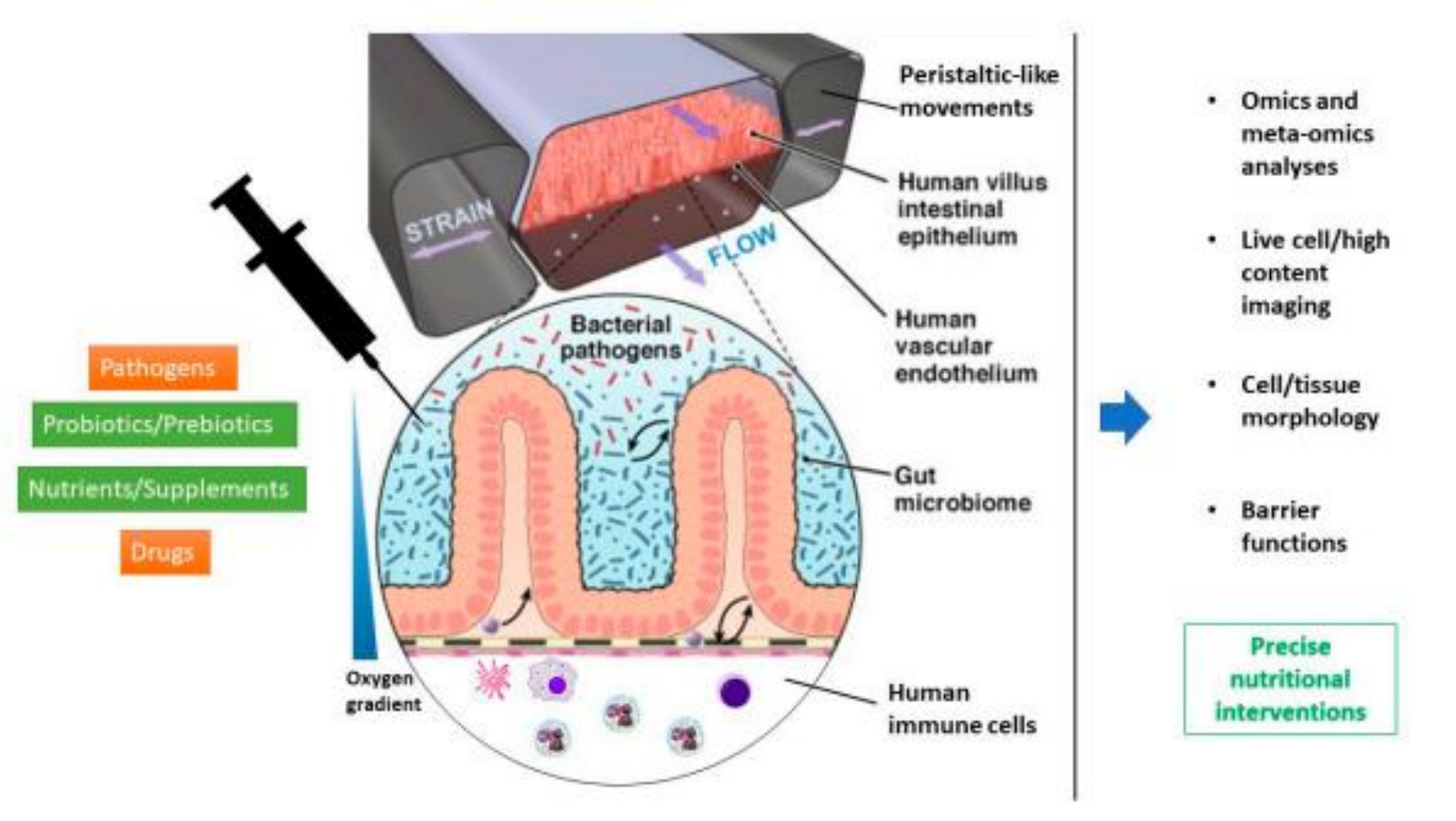
Front. Cell Dev. Biol. 2022
https://doi.org/10.3389/fcell.2022.854740



Links between Nutrition, Infectious Diseases, and Microbiota: Emerging Technologies and Opportunities for Human-Focused Research. Nutrients. 2020. doi: 10.3390/nu12061827.

Experimental validation

In vitro models: gut on a chip



Links between Nutrition, Infectious Diseases, and Microbiota: Emerging Technologies and Opportunities for Human-Focused Research. Nutrients. 2020 doi: 10.3390/nu12061827.

Experimental validation

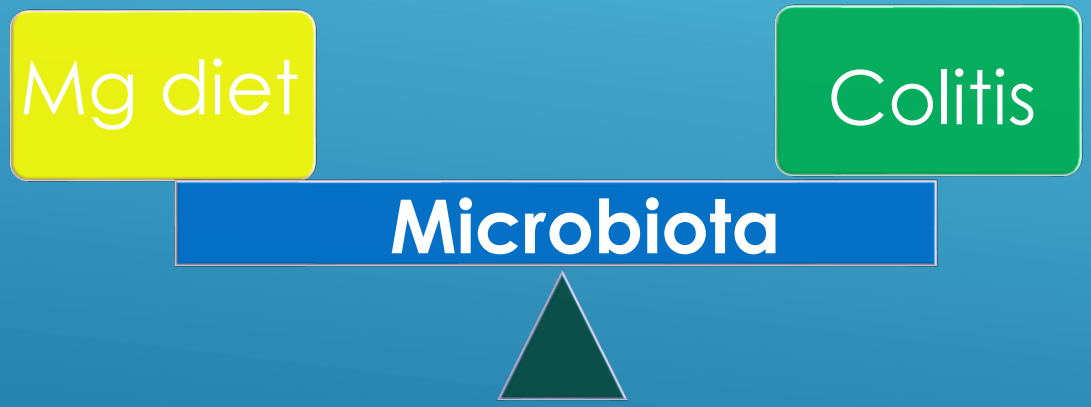
In vivo models: animal models

THE EFFECT OF DIETARY MAGNESIUM ON GUT MICROBIOTA

- Magnesium (Mg) is the second most abundant intracellular cation after potassium and plays an essential role in numerous fundamental cellular reactions
- Approximately 40% to 50% of dietary Mg is absorbed by duodenum, jejunum and ileum
- Mg deficiency is associated with several metabolic disorders, including type 2 diabetes metabolic syndrome dyslipidemia and hypertension.
- systemic inflammation has been observed in subjects with hypomagnesemia
- The mechanism underlying the immunomodulation occurring during Mg deficiency remains poorly described.

AIM

to assess the role of dietary Mg content in gut microbiota modulation



a mouse model of DSS induced colitis

METHODS

Animal model

27 mice

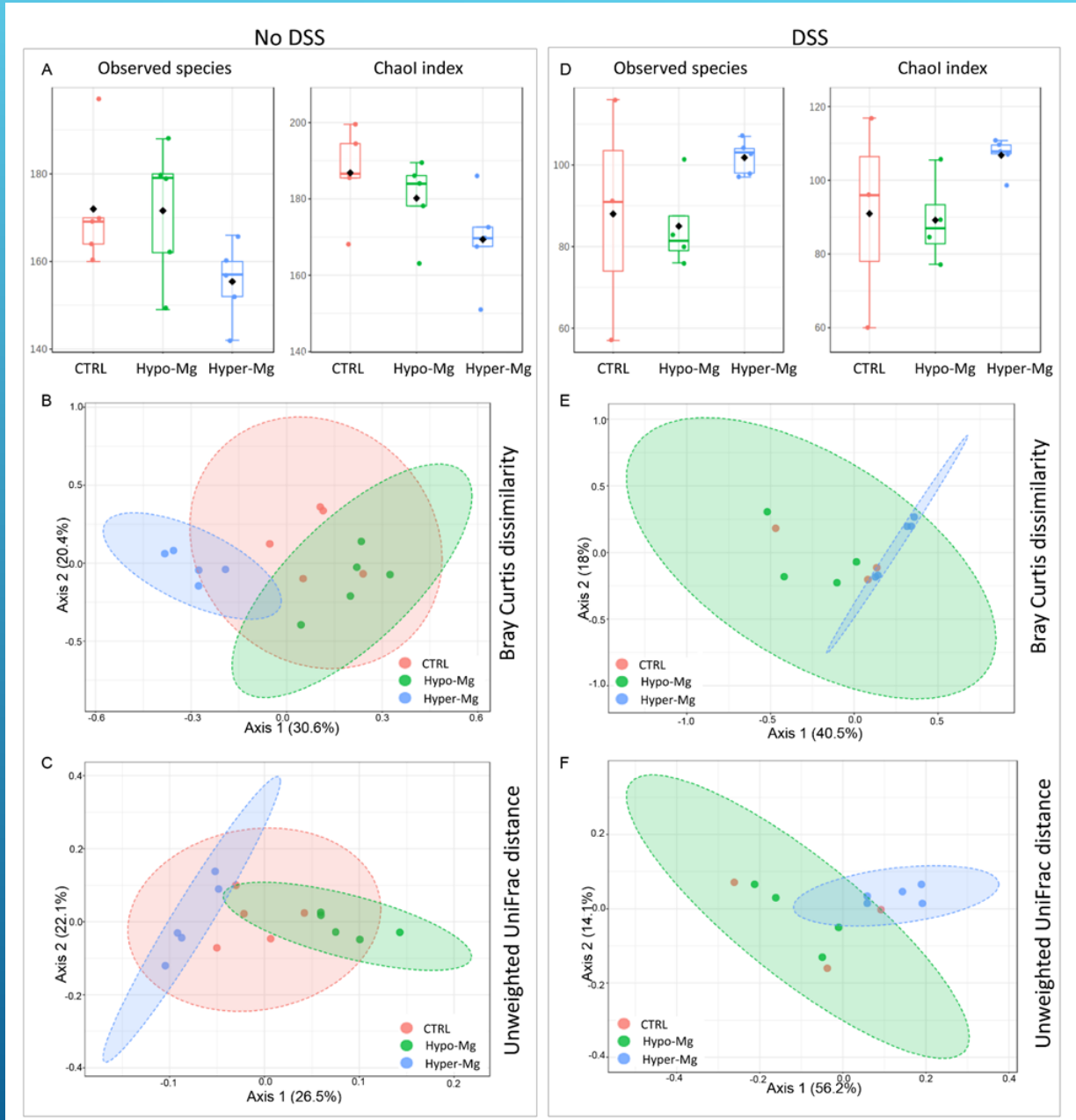
Female 7/8- week-old C57BL/6 mice

Hypo-Mg Diet 30 mg/Kg Mg	2.5% w/v DSS	Mice N=4
	No DSS	Mice N=5
Hyper-Mg Diet 4 g/Kg Mg	2.5% w/v DSS	Mice N=5
	No DSS	Mice N=5
CTRL Diet 1 g/Kg Mg	2.5% w/v DSS	Mice N=3
	No DSS	Mice N=5

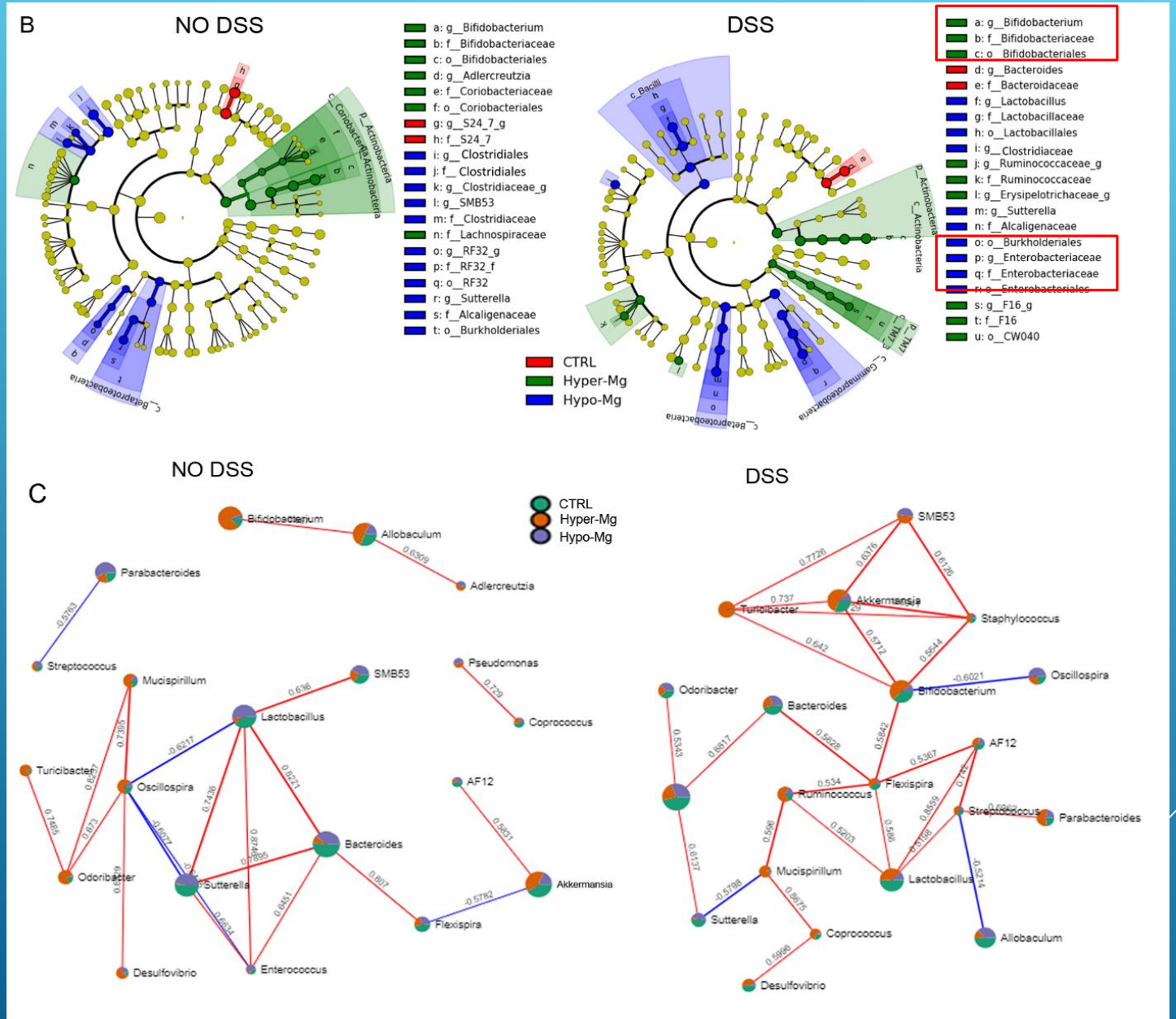
Diet

Composition of Semipurified Mg-Adjusted Diets	Hypo-Mg g/kg	CTRL g/kg	Hyper-Mg g/kg
Casein	200	200	200
Wheat starch	650	650	650
Fiber alphacel	50	50	50
Corn oil	50	50	50
Mineral mixe	35	35	35
Vitamin mixf	10	10	10
DL-methionine	3	3	3
Choline bitartrate	2	2	2
MgO	-	1.67	6.68

Dietary Mg enriches and shapes microbiota composition in colitic mice



Dietary Mg content modulates gut abundance of specific bacterial taxa and their interactions

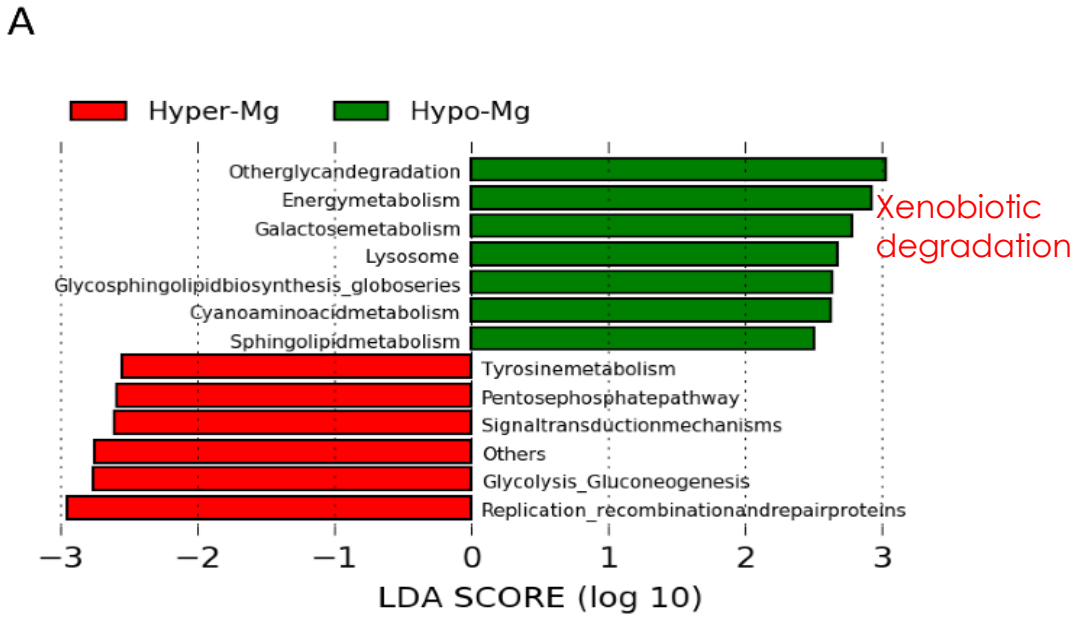


KEGG predictions

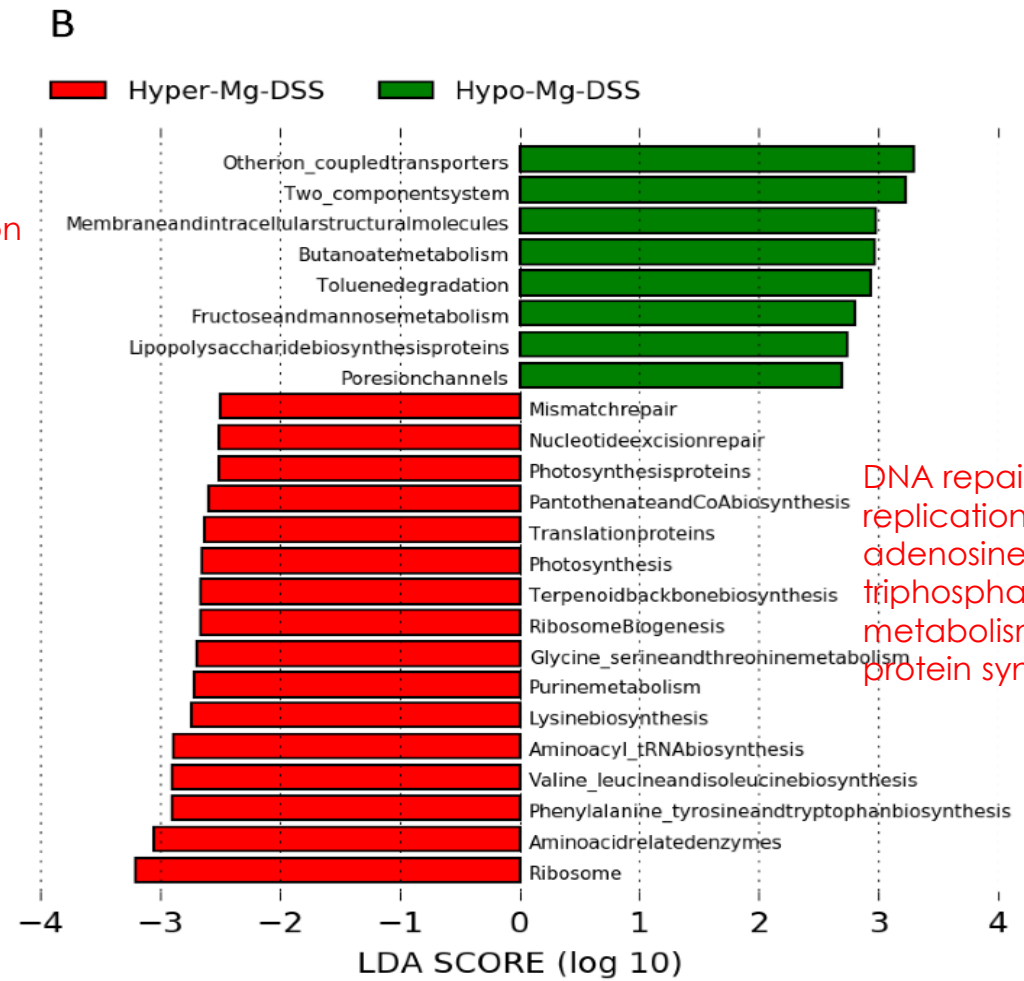
Dietary Mg content modulates specific bacterial functional pathways

NO DSS

DSS



Xenobiotic degradation



DNA repair and replication, adenosine triphosphate (ATP) metabolism and protein synthesis

High Mg intake in intestinal function

Increase of short-chain fatty acid-producing bacteria and Increase of mucin-degrading bacteria

Increase of intestinal health and glucose homeostasis

By the increase of Verrucomicrobia

Maintaining gut barrier homeostasis

Reduction of inflammatory cytokines and chemokines production

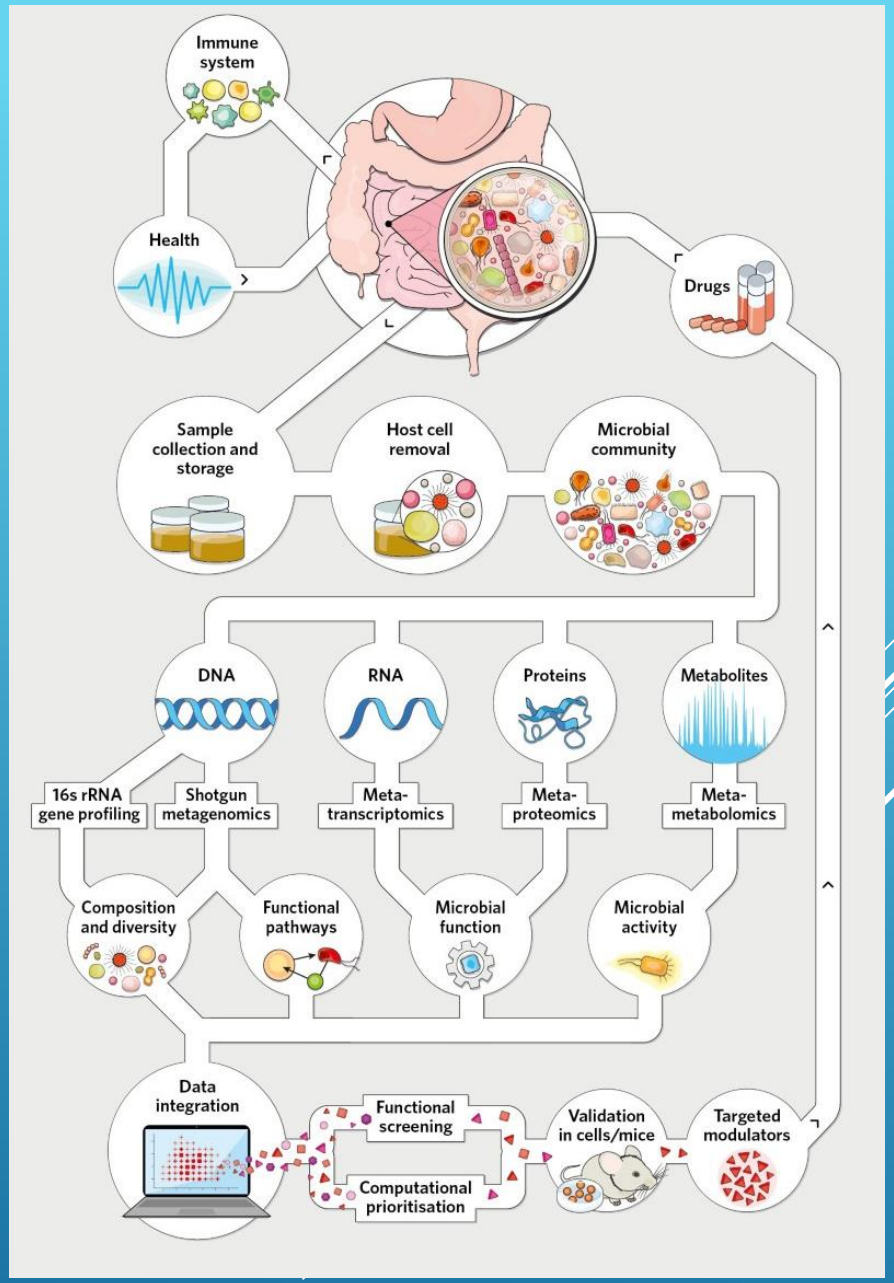
By the increase of Actinobacteria

Increase of gut barrier homeostasis and decrease of inflammatory cytokines and chemokines

By reduction of Enterobacteriaceae

microbiome-based medicines studies

Translation of the knowledge into clinical applications: diagnostics or therapies



https://www.nature.com/articles/d42473-020-00214-9

Potential clinical applications for metagenomics sequencing

Microbiomics

Community structure

microbiome

virome

parassitome

mycome

Disease related profiles

Dysbiotic status

Surveillance

Outbreak investigation

Multi drug resistance tracking

Strain identification

SNP typing

Pathogen genomics

Drug resistance gene mutation

Toxins/virulence factors gene description

Host response

Efficay of therapy

Disease status

Immune system/microorganism interaction

Microbiome-based therapies

Probiotics

Prebiotics

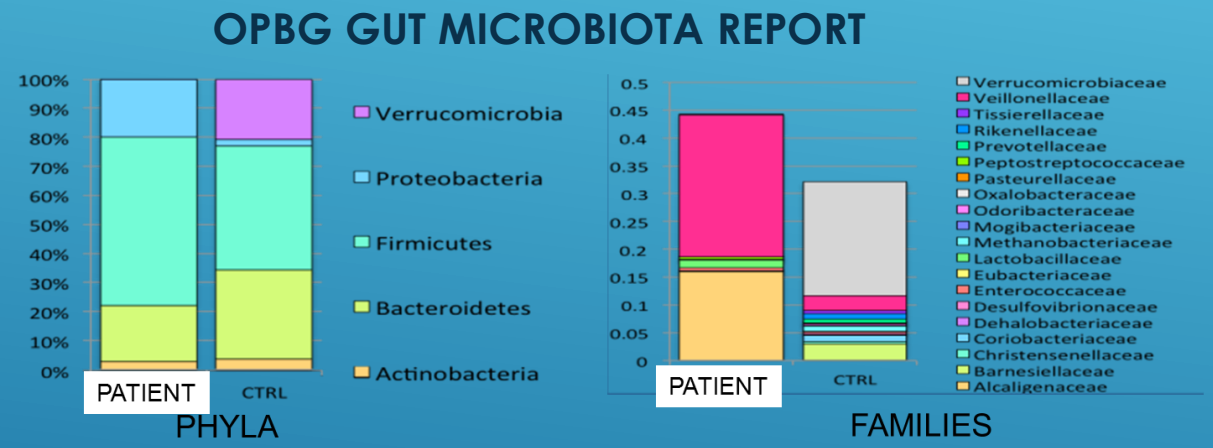
Postbiotic

Phage therapy

Fecal microbiota transplantation FMT

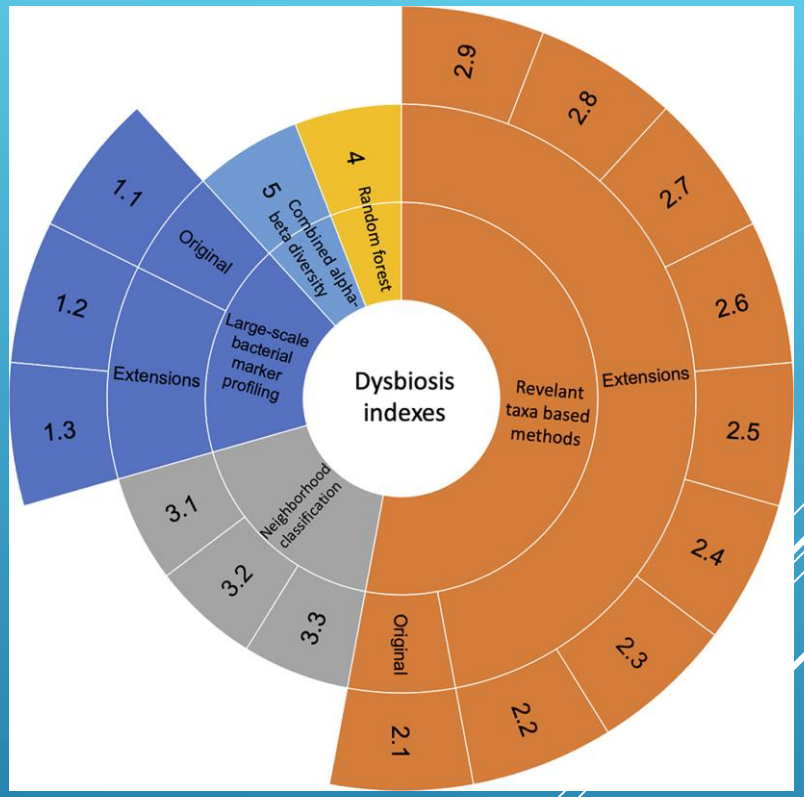
How to assess intestinal dysbiosis?

Patent: Metagenomic Method for the *in vitro* diagnosis of intestinal dysbiosis, PCT/IT2017/000119, June 2017



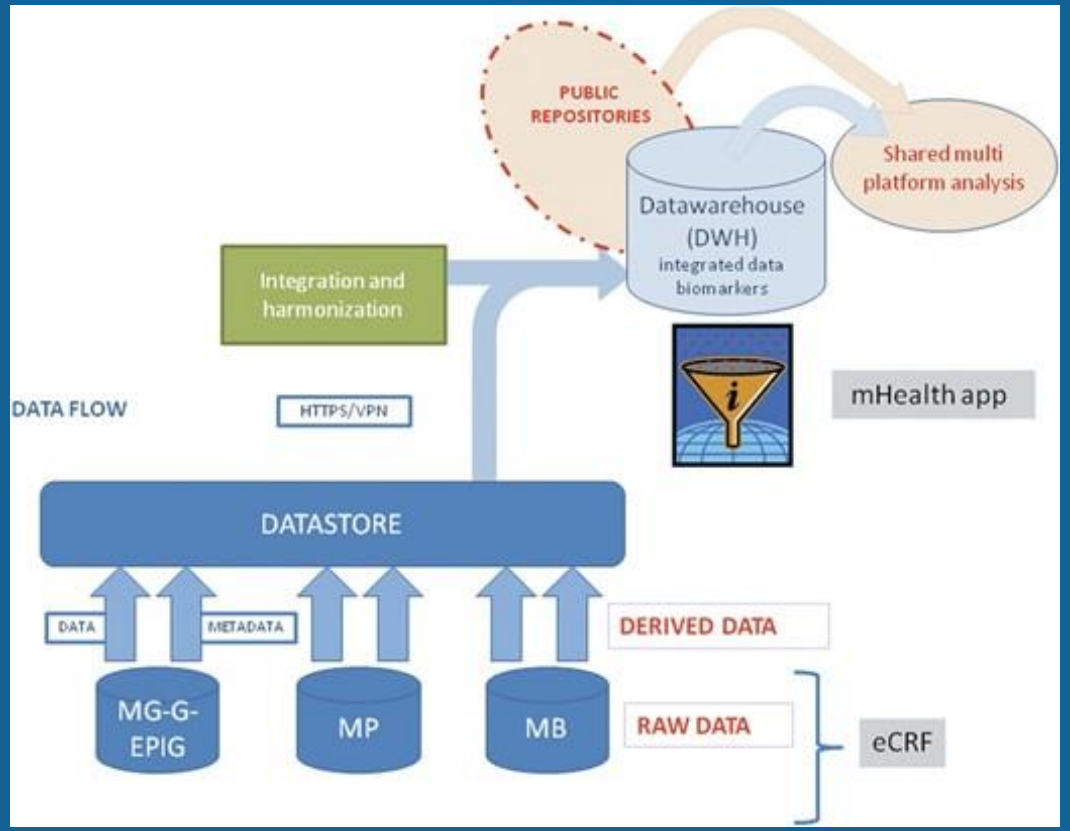
SPECIES/SUBSPECIES

Unità tassonomica	Unità tassonomica	PATIENT	CTRL	ANDAMENTO[2]
Actinobacteria	*Bifidobacterium*	0.03550	0.00621	+
Actinobacteria	*Bifidobacterium adolescentis*	0.02567	0.00027	+
Actinobacteria	*Bifidobacterium longum*	0.01599	0.00205	+
Actinobacteria	*Collinsella*	0.00092	0.00008	+
Euryarchaeota	*Methanobrevibacter*	0.00000	0.00912	-
Firmicutes	*Clostridium hiranonis*	0.00006	0.00000	+
Firmicutes	*Dorea formicigenerans*	0.00011	0.00000	+
Firmicutes	*Oscillospira*	0.00134	0.00753	-
Firmicutes	*Ruminococcus bromii*	0.00347	0.00005	+
Firmicutes	*Streptococcus*	0.00097	0.01670	-
Proteobacteria	*Acinetobacter*	0.00006	0.00001	+
Verrucomicrobia	*Akkermansia muciniphila*	0.00039	0.22569	-

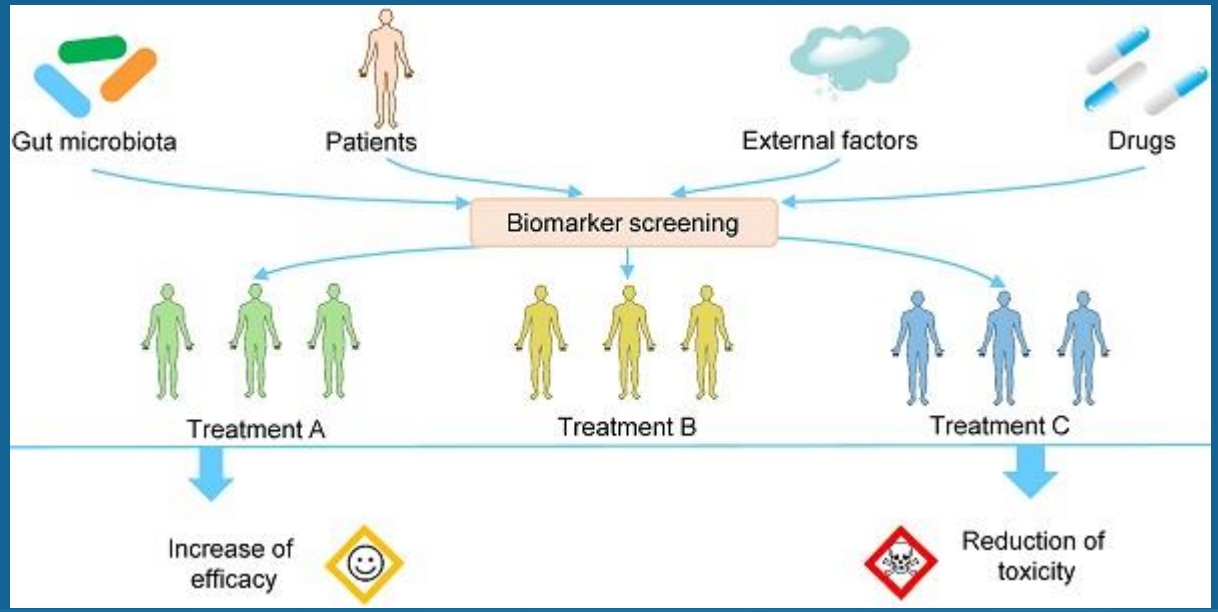


Wei S, Bahl MI, Baunwall SMD, Hvas CL, Licht TR. Determining Gut Microbial Dysbiosis: a Review of Applied Indexes for Assessment of Intestinal Microbiota Imbalances. Appl Environ Microbiol. 2021.

Workflow of -omics data for dysbiosis tracking



Clinical decision support system
CDSS



doi: 10.1097/MIB.0000000000000602. PMID: 26588090.

doi:10.7150/thno.47289. https://www.thno.org/v10p11278.htm

BIOBANK

BBMRI.it
Biobanking and
BioMolecular Resources
Research Infrastructure
of Italy

Sample and data collection

Processing

Storage

Shipment

Users
R&D, Academia, Industries

SCALE UP

RETE IDEA

Mission

- To protect health in childhood and adolescence
- To promote scientific and technological research
- To promote a net amongst all Scientific Hospital and Care Institutes (IRCCS) belonging to the Network

«The human microbiota in systems medicine applied to diagnosis and therapy in pediatrics»

COME RIPRISTINARE LA COMPOSIZIONE DEL MICROBIOTA

Dalla disbiosi all'eubiosi

- **PROBIOTICI**
- **PREBIOTICI**
- **SIMBIOTICI**
- **ALIMENTI FUNZIONALI**
- **Fecal microbita transplantation**

PROBIOTICI

- Microrganismi vivi che, se somministrati in quantità adeguate, conferiscono un beneficio alla salute dell'ospite

CARATTERISTICHE

- Essere attivi e vitali
- Essere sicuri
- Sopravvivere nel tratto gastrointestinale
- Colonizzare l'intestino
- Possedere caratteristiche di probioticità (conferire un beneficio fisiologico dimostrato secondo criteri fissati)

Probiotic effects

Randomized Controlled Trial

Nutrients. 2020 Dec 29;13(1):87. doi: 10.3390/nu13010087.

Effects of a Synbiotic Formula on Functional Bowel Disorders and Gut Microbiota Profile during Long-Term Home Enteral Nutrition (LTHEN): A Pilot Study

Valentina D'Onofrio [1], Federica Del Chierico [2], Paola Belci [1], Pamela Vernocchi [2], Andrea Quagliariello [2], Sofia Reddel [2], Giorgia Conta [3 4], Maria Vittoria Mancino [1], Maurizio Fadda [1], Maria Carmine Scigliano [1], Roberta Morelli [1], Antonella De Francesco [1], Fabio Guagnini [5], Filippo Fassio [6], Rosalba Galletti [1], Lorenza Putignani [7]

Sci Rep. 2019 Mar 21;9(1):4996. doi: 10.1038/s41598-019-41149-6.

Gut microbiota profile in children affected by atopic dermatitis and evaluation of intestinal persistence of a probiotic mixture

Sofia Reddel [1], Federica Del Chierico [1], Andrea Quagliariello [1], Simona Giancristoforo [2], Pamela Vernocchi [1], Alessandra Russo [1], Alessandro Fiocchi [3], Paolo Rossi [4], Lorenza Putignani [5], May El Hachem [2]

Arch Med Sci. 2018 Jan;14(1):81-87. doi: 10.5114/aoms.2016.62150. Epub 2016 Sep 6.

Bifidobacteria and lactobacilli in the gut microbiome of children with non-alcoholic fatty liver disease: which strains act as health players?

Valerio Nobili [1 2], Lorenza Putignani [3 4], Antonella Mosca [1], Federica Del Chierico [4], Pamela Vernocchi [4], Anna Alisi [2], Laura Stronati [5], Salvatore Cucchiara [6], Marco Toscano [7], Lorenzo Drago [7]

Int J Mol Sci. 2021 Feb 6;22(4):1649. doi: 10.3390/ijms22041649.

Gut Microbiota Profile in Children with IgE-Mediated Cow's Milk Allergy and Cow's Milk Sensitization and Probiotic Intestinal Persistence Evaluation

Maurizio Mennini [1], Sofia Reddel [2], Federica Del Chierico [2], Simone Gardini [3], Andrea Quagliariello [2], Pamela Vernocchi [2], Rocco Luigi Valluzzi [1], Vincenzo Fierro [1], Carla Riccardi [1], Tania Napolitano [1], Alessandro Giovanni Fiocchi [1], Lorenza Putignani [4]

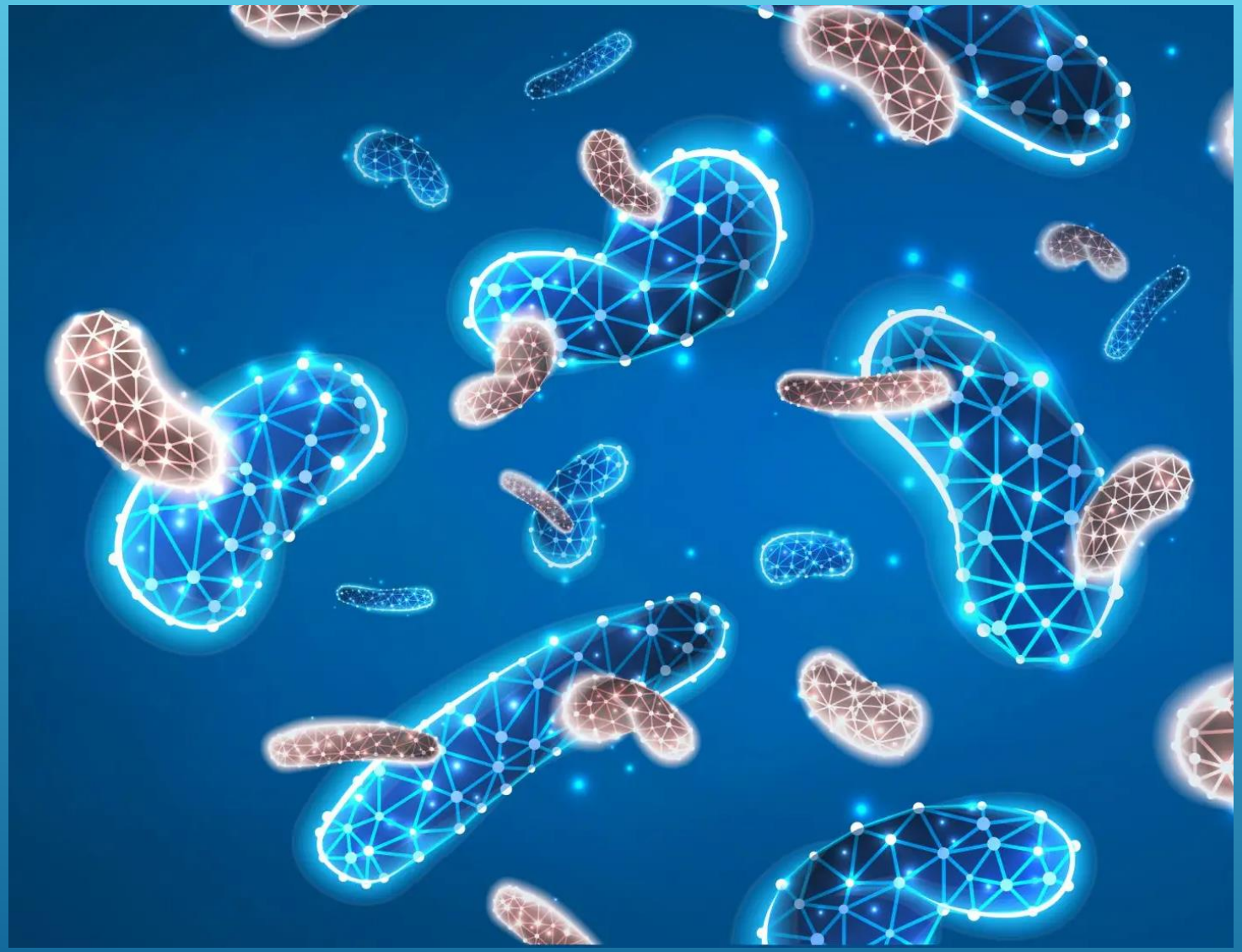
Nutrients. 2017 Dec 9;9(12):1342. doi: 10.3390/nu9121342.

A Metagenomic and in Silico Functional Prediction of Gut Microbiota Profiles May Concur in Discovering New Cystic Fibrosis Patient-Targeted Probiotics

Pamela Vernocchi [1], Federica Del Chierico [2], Andrea Quagliariello [3], Danilo Ercolini [4], Vincenzina Lucidi [5], Lorenza Putignani [6 7]

PREBIOTICI

- Fibre alimentari solubili e non digeribili
- Naturalmente presenti nella frutta e verdura
- Negli integratori alimentari (sorbitolo, pectine, xilitolo)
- Favoriscono la crescita dei batteri probiotici nel colon
- Migliorano le funzioni intestinali (attraverso il richiamo di H_2O nel colon e idratando il materiale fecale)



SIMBIOTICI

- Integratori alimentari che contengono simultaneamente ceppi probiotici e sostanze prebiotiche.

La loro funzione viene svolta dalla attività sinergica di entrambi nell'intestino

ALIMENTI FUNZIONALI

Qualsiasi alimento modificato che fornisce un beneficio oltre a quello attribuito a ogni specifico nutriente in esso contenuto.

Deve rimanere un alimento e dimostrare il suo effetto in quantità normalmente consumate in una dieta.

Qualsiasi alimento contenente probiotici e prebiotici è un alimento funzionale (yogurt che contengono colture viventi di batteri probiotici, prebiotici e nutrienti della dieta...)

IL TRAPIANTO DI MICROBIOTA FECALE

Il trapianto di microbiota fecale umano (FMT) consiste nella somministrazione del materiale fecale di un donatore sano nell'intestino di un soggetto malato per il trattamento di specifiche patologie correlate a uno squilibrio del microbiota intestinale.

INDICAZIONI TERAPEUTICHE

L'unica indicazione terapeutica riconosciuta e approvata ad oggi:

- **Infezioni ricorrenti da Clostridioides difficile MDR nel paziente ADULTO**

Indicazioni sperimentali e trial clinici :

- Inflammatory bowel diseases
- gastro-intestinal acute graft-versus-Host disease after Allogeneic hematopoietic stem cell transplantation
- Colonizzazione intestinali in pazienti in attesa di trapianto di cellule staminali
- Primary Sclerosing Cholangitis
- Cirrhosis
- Obesity
- autism

Linee guida europee Gennaio 2017

OPEN ACCESS

European consensus conference on faecal microbiota transplantation in clinical practice

Giovanni Cammarota,[1] Gianluca Ianiro,[1] Herbert Tilg,[2] Mirjana Rajilić-Stojanović,[3] Patrizia Kump,[4] Reetta Satokari,[5] Harry Sokol,[6] Perttu Arkkila,[7] Cristina Pintus,[8] Ailsa Hart,[9] Jonathan Segal,[9] Marina Aloi,[10] Luca Masucci,[11] Antonio Molinaro,[12] Franco Scaldaferri,[1] Giovanni Gasbarrini,[1] Antonio Lopez-Sanroman,[13] Alexander Link,[14] Pieter de Groot,[15] Willem M de Vos,[5,16] Christoph Högenauer,[4] Peter Malfertheiner,[14] Eero Mattila,[17] Tomica Milosavljević,[18] Max Nieuwdorp,[12,15,19] Maurizio Sanguinetti,[11] Magnus Simren,[20] Antonio Gasbarrini,[1] The European FMT Working Group

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2016-313017)

For numbered affiliations see end of article.

Correspondence to
Professor G Cammarota, Gastroenterological Area, Fondazione Policlinico Universitario Gemelli, Università Cattolica del Sacro Cuore, Largo A. Gemelli 8, Rome 00168, Italy; giovanni.cammarota@unicatt.it

Received 7 September 2016
Revised 1 December 2016
Accepted 4 December 2016
Published Online First 13 January 2017

ABSTRACT

Faecal microbiota transplantation (FMT) is an important therapeutic option for *Clostridium difficile* infection. Promising findings suggest that FMT may play a role also in the management of other disorders associated with the alteration of gut microbiota. Although the health community is assessing FMT with renewed interest and patients are becoming more aware, there are technical and logistical issues in establishing such a non-standardised treatment into the clinical practice with safety and proper governance. In view of this, an evidence-based recommendation is needed to drive the practical implementation of FMT. In this European Consensus Conference, 28 experts from 10 countries collaborated, in separate working groups and through an evidence-based process, to provide statements on the following key issues: FMT indications; donor selection; preparation of faecal material; clinical management and faecal delivery and basic requirements for implementing an FMT centre. Statements developed by each working group were evaluated and voted by all members, first through an electronic Delphi process, and then in a plenary consensus conference. The recommendations were released according to best available evidence, in order to act as guidance for physicians who plan to implement FMT, aiming at supporting the broad

of this infection, the therapeutic role played by FMT is therefore important to save human lives and to decrease the economic burden on healthcare systems.[8–11] Based on these data, both the European Society for Microbiology and Infectious Disease and the American College of Gastroenterology recommend FMT as a treatment for rCDI.[12 13]

Beyond the treatment of CDI, FMT has also been investigated in other disorders associated with the alteration of gut microbiota. In particular, studies in humans include RCTs in patients with UC and metabolic syndrome (MS).[14–16]

The global interest in FMT is increasing, and both doctors and patients are increasingly aware and informed. Although the dissemination of FMT in the clinical practice is restricted by regulatory and bureaucratic issues (principally related to costs, donor programme, safety control),[17–19] the FMT practice is booming, ranging from highly organised stool banking programmes to individual treatments with patient-identified directed donors, and even to individual and harmful do-it-yourself practices. Working groups (WGs) from the USA, Austria and France released recommendations on indications and methods of FMT.[20–22] Authoritative published guidelines and recommendations have been released

Linee guida internazionali Settembre 2019

OPEN ACCESS

International consensus conference on stool banking for faecal microbiota transplantation in clinical practice

Giovanni Cammarota ,[1] Gianluca Ianiro,[2] Colleen R Kelly,[3] Benjamin H Mullish ,[4] Jessica R Allegretti,[5] Zain Kassam,[6,7] Lorenza Putignani,[8] Monika Fischer,[9] Josbert J Keller,[10,11] Samuel Paul Costello,[12] Harry Sokol,[13,14,15] Patrizia Kump,[16] Reetta Satokari,[17] Stacy A Kahn,[18] Dina Kao,[19] Perttu Arkkila,[20] Ed J Kuijper,[21] Maria J GT Vehreschild,[22] Cristina Pintus,[23] Loris Lopetuso,[24] Luca Masucci,[25] Franco Scaldaferri,[24] E M Terveer,[11,21] Max Nieuwdorp,[26] Antonio López-Sanromán,[27] Juozas Kupcinskas,[28] Ailsa Hart,[29] Herbert Tilg,[30] Antonio Gasbarrini[31]

For numbered affiliations see end of article.

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giovanni.cammarota@unicatt.it

GC and GI are joint first authors.

Received 26 July 2019
Revised 10 September 2019
Accepted 22 September 2019
Published Online First 28 September 2019

Watch Video
gut.bmj.com

ABSTRACT

Although faecal microbiota transplantation (FMT) has a well-established role in the treatment of recurrent *Clostridioides difficile* infection (CDI), its widespread dissemination is limited by several obstacles, including lack of dedicated centres, difficulties with donor recruitment and complexities related to regulation and safety monitoring. Given the considerable burden of CDI on global healthcare systems, FMT should be widely available to most centres.

Stool banks may guarantee reliable, timely and equitable access to FMT for patients and a traceable workflow that ensures safety and quality of procedures. In this consensus project, FMT experts from Europe, North America and Australia gathered and released statements on the following issues related to the stool banking: general principles, objectives and organisation of the stool bank; selection and screening of donors; collection, preparation and storage of faeces; services and clients; registries, monitoring of outcomes and ethical issues; and

Administration (FDA), FMT requires strict quality control to prevent harmful consequences.[20]

Stool banks can provide reliable, timely and equitable access to FMT for CDI, and also facilitate a standardised, cost-effective and traceable workflow that ensures safety and quality of procedures[21] compared with single FMT centres. Stool banks are currently unevenly distributed and differ considerably in legislation, organisation and structure.[22–24]

The aim of this consensus report is to provide guidance on the general organisation and the criteria required to establish a stool bank.

METHODS

Consensus development process

The consensus process was developed according to the following steps: selection of expert panel members, identification of key issues and related working group (WG), development of statements

Necessità di un aggiornamento delle linee guida

- COVID-19
- Nuove indicazioni terapeutiche
- Aggiornamenti per screening donatore e preparazione emulsione

Screening of faecal microbiota transplant donors during the COVID-19 outbreak: suggestions for urgent updates from an international expert panel

Published Online
March 16, 2020
https://doi.org/10.1016/
S2468-1253(20)30082-0

This online publication has been corrected. The corrected version first appeared at thelancet.com/gastrohep on May 14, 2020

As the outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread from China to other countries, governments and the medical community are taking steps to prevent transmission, from common sense recommendations to radical quarantine measures.[1]

In that context, timely recommendations concerning the screening of donors of human cells, tissues, or cellular or tissue-based products have been released, as the potential for transmission of COVID-19 through transplant is not yet known. Several institutions have recommended interim precautions to screen new donors. The US Food and Drug Administration has suggested considering a donor's history of travel to areas of outbreak, cohabitation with infected individuals, or diagnosis or suspicion of COVID-19 within the 28 days before recovery of donor tissue.[2] Similar measures have been taken by the Global Alliance of Eye Bank Associations and by the Joint United Kingdom Blood Transfusion Services Professional Advisory Committee to rule out potential donors.[3,4] The European Society for Blood and Marrow Transplantation has recommended excluding potential donors who have been diagnosed with COVID-19, and waiting at least 21 days before donation in those with a history of high-risk travel or contact.[5] In Italy, where the COVID-19 outbreak is spreading rapidly, the real-time RT-PCR assays of nasopharyngeal swab samples (or bronchoalveolar lavage in deceased individuals).[6]

Faecal microbiota transplantation is a novel treatment that has rapidly earned a major role in the management of recurrent *Clostridioides difficile* infection because of its clear advantages over antibiotics.[7] It is becoming increasingly more widespread and standardised around the world. Last year, an international expert panel, including several authors of this Comment, released recommendations on how to screen faecal microbiota transplant donors, including a medical history and blood and stool examinations.[8]

Given the global COVID-19 outbreak, we, as an international group of experts in faecal microbiota transplantation and stool banking, believe that recommendations to update (at least temporarily) the screening of stool donors are urgently needed, as the risk of transmitting SARS-CoV-2 by faecal microbiota transplantation might be higher than that in other tissue transplants. Evidence has shown that the SARS-CoV-2 can be found in faeces, and that stool samples can remain positive for the virus even when it is no longer detectable in the respiratory tract, suggesting the possibility of a faecal–oral route of transmission.[9] This concept is supported by the presence of gastrointestinal symptoms in some patients affected by COVID-19.[10] Another relevant issue is that faecal microbiota

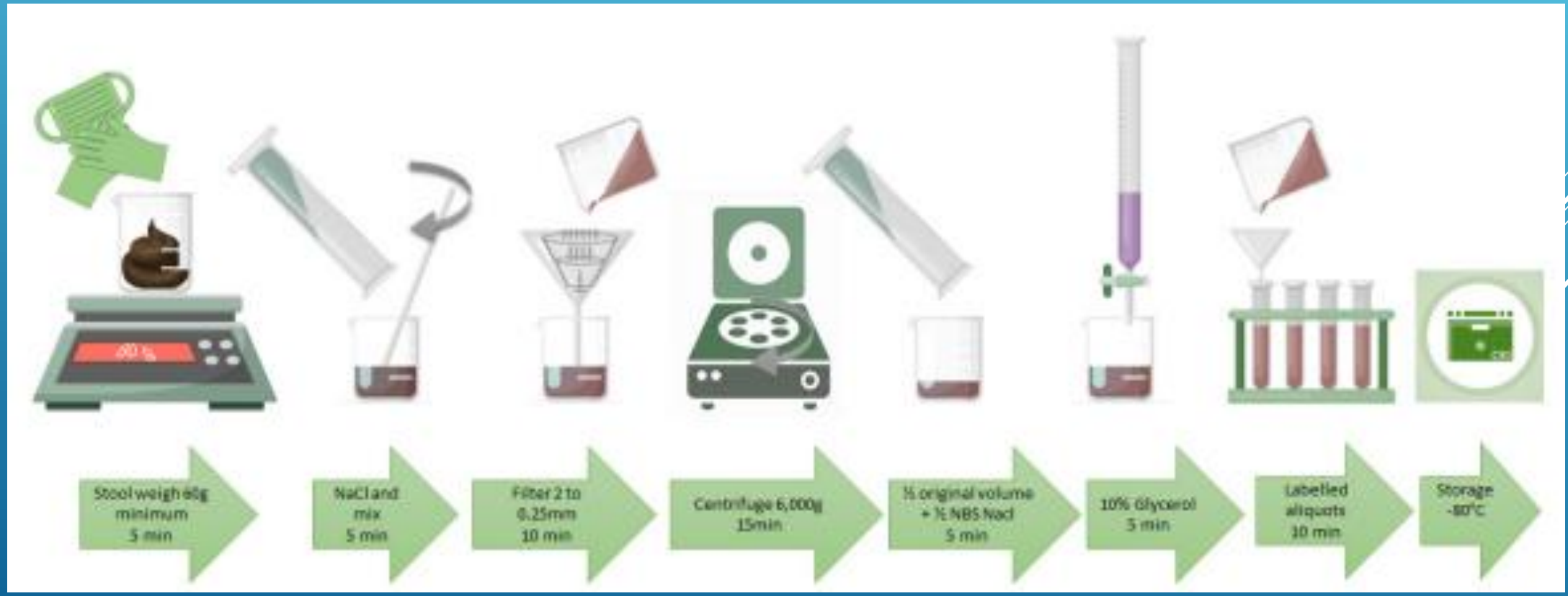
- ***Il donatore verrà sottoposto a interviste per escludere la presenza di malattie croniche o familiarità per esse.***
- ***Inoltre il donatore è sottoposto a:***
- ***Esami batteriologici*:**
- (*Clostridium difficile*, patogeni gastrointestinali, batteri farmaco-resistenti, *Vibrio cholera* e *Listeria monocytogenes etc.)*
- ***Esami parassitologici:***
- (*Giardia intestinalis, Cryptosporidium, Entamoeba histolytica* etc.);
- ***Esami virologici :***
- (CMV, EPATITE A , HBV, HCV, SIFILIDE, HIV, etc.)
- ***Esami chimico-clinici :***
- Emocromo completo, PCR, Albumina, Creatinina, Transaminasi etc.)

SCREENING DONATORE

How is the FMT Administered?

- Small bowel upper endoscopy to the jejunum
- Nasojejunal tube placement
- Colonoscopy
- Retention enemas
- Oral capsules

FECAL MICROBIOTA TRANSPLANTATION



SICUREZZA

IMPATTO DI STRUTURA
Aree classificate per la preparazione delle emulsioni fecali per FMT

N°	NOME LOCALE	Area (mq.)	H (m.)	Vol. (mc)	Cl. di Contam. (at rest)	Vol/h
1-019	Air lock / Spogliatoio	2,70	2,40	6,48	D (ISO 8)	> 20
1-019A	Laboratorio manipolazione Campioni	15,28	2,70	41,26	D (ISO 8)	> 20
CAPPA FLV (*)	Area manipolazione campioni (c.f.l.)	1,045	0,74	0,7734	A (ISO 3)	> 1.500
Pass box	Passamateriali	0,45	0,80	0,36	D (ISO 8)	> 20

NOTA (*): Cappa a flusso laminare marca FASTER modello CytoFAST Elite 218, dimensioni utili interne mm 1802x740x580 (WxHxD), portata aria in estrazione 728 mc/h

LOCALE	Valore Δp (in mm H_2O)
Spogliatoio (locale 1-019)	1.4±0.4
Lab manipolaz. campioni (locale 1-019A)	3.2±0.4
Pass-box	1.6±0.4

IMPATTO DI PROCESSI

Controlli microbiologici periodici

CRYOLAB

VERBALE CRYOLAB Numero 200129
DATA 10/08/2020

1 CLIENTE
SPETTABILE OSPEDALE PEDIATRICO BAMBINO GESU', Unità del Microbioma Umano, Polo di Ricerca San Paolo

2 INDIRIZZO
Viale di San Paolo, 15 00146 Roma

3 SERVIZIO/ATTIVITA'
Analisi microbiologiche, presso il LAB FMT

4 PREMESSA
Nell'ambito dell'accordo esistente con il Vostro Spettabile Ente (contratto prot. N.191/19 AB/FB del 21 marzo 2019) "Contratto per l'affidamento del servizio di controllo della contaminazione microbiologica all'interno degli ambienti classificati del Laboratorio FMT dell'Ospedale Pediatrico Bambino Gesù",

siamo con la presente a formalizzare i report del controllo effettuato in data 24 luglio 2020 previsti per il servizio di cui sopra.

5 ESAMI
Il protocollo utilizzato per i controlli indicati è il seguente

1 Validazione microbiologica ambientale.

5.1 VALIDAZIONE MICROBIOLOGICA AMBIENTALE
Questo tipo di controllo è eseguito in accordo con quanto stabilità e previsto nell'Annex 1 "Manifacture of Sterile Medicinal Products" del Volume 4 EU Guidelines to Good Manufacturing Practice (GMP) "Medicinal Products for Human and Veterinary Use".
Ricordiamo che gli ambienti e I dispositivi di condizionamento aria, dovrebbero essere monitorati regolarmente durante il loro funzionamento; il monitoraggio delle posizioni più critiche per il campionamento dovrebbe essere stabilito sulla base di uno studio formale di analisi dei rischi e dei risultati ottenuti durante la classificazione di camere e/o dispositivi di condizionamento aria.

LA NOSTRA ESPERIENZA DA MAGGIO 2017 AD OGGI

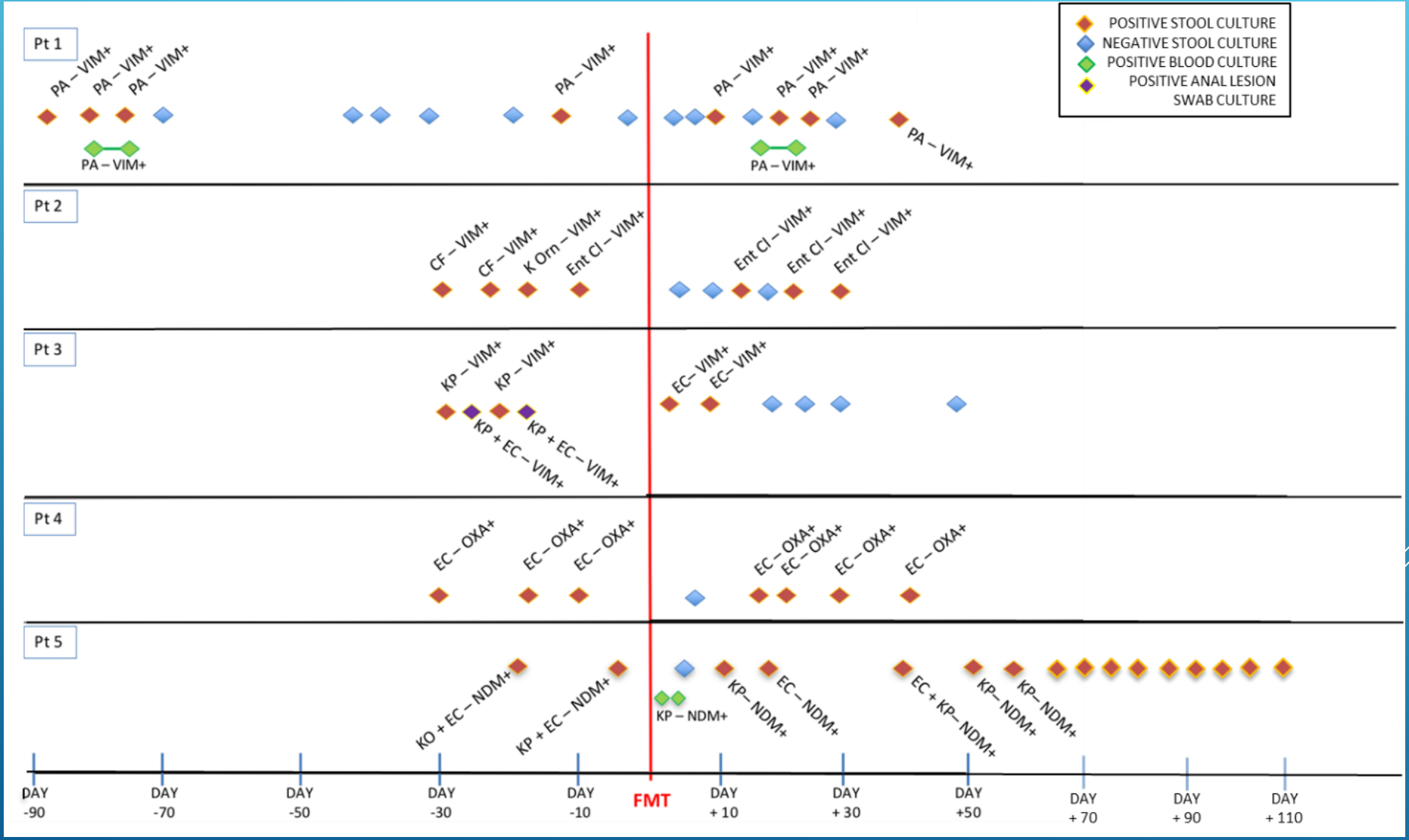
24 pazienti trattati con FMT

5 pazienti trattati con FMT da donatore familiare

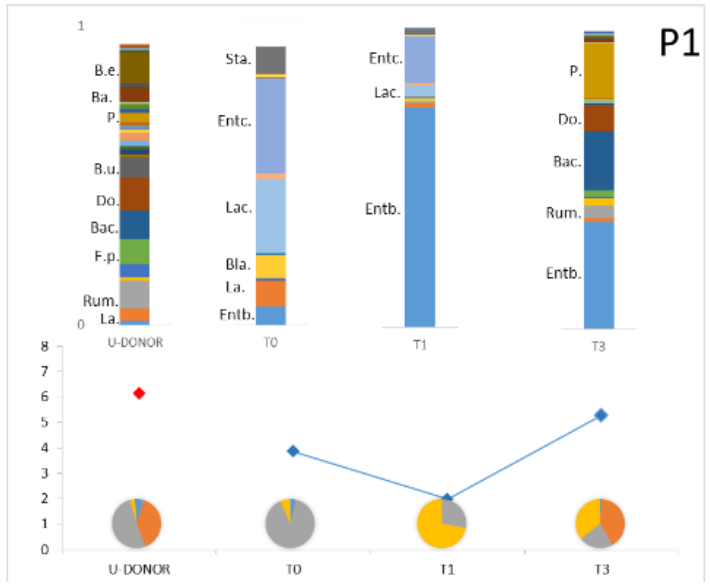
19 pazienti trattati con FMT da donatore universale

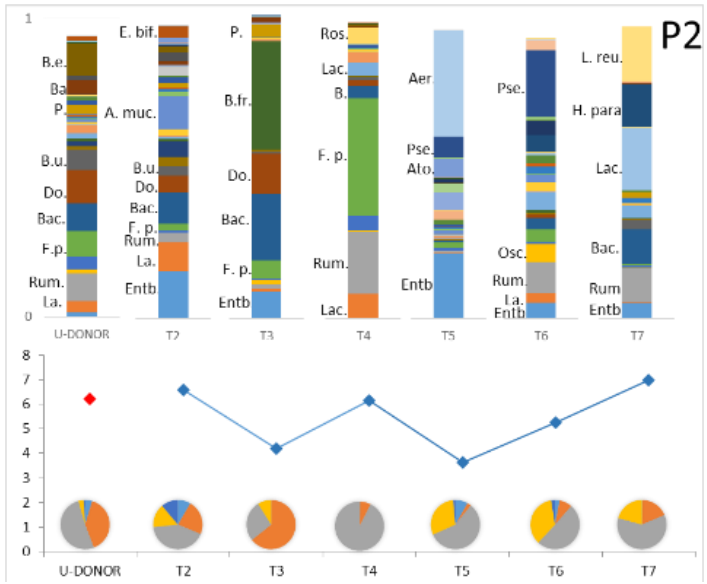
Fecal Microbiota Transplantation 1

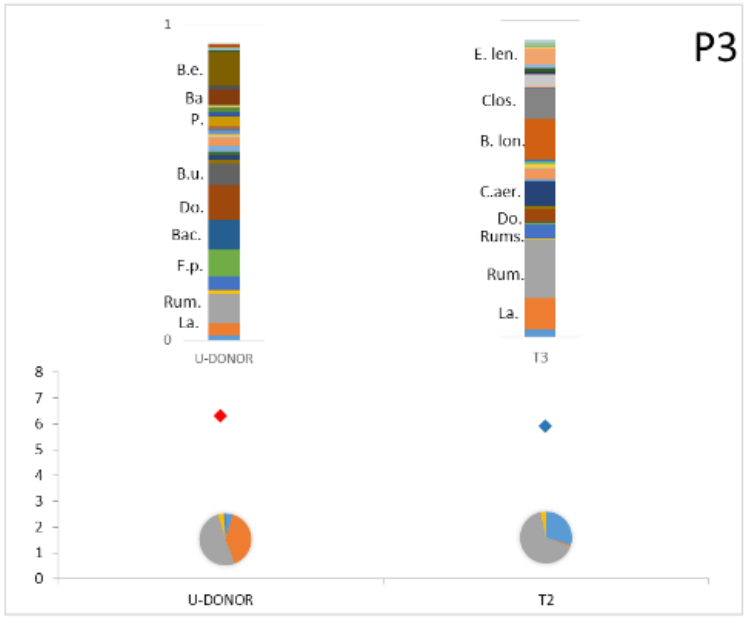
Decolonization of multi-drug resistant bacteria by fecal microbiota transplantation in 5 pediatric patients before allogeneic hematopoietic stem cell transplantation: gut microbiota profiling, infectious and clinical outcomes. Haematologica. 2020

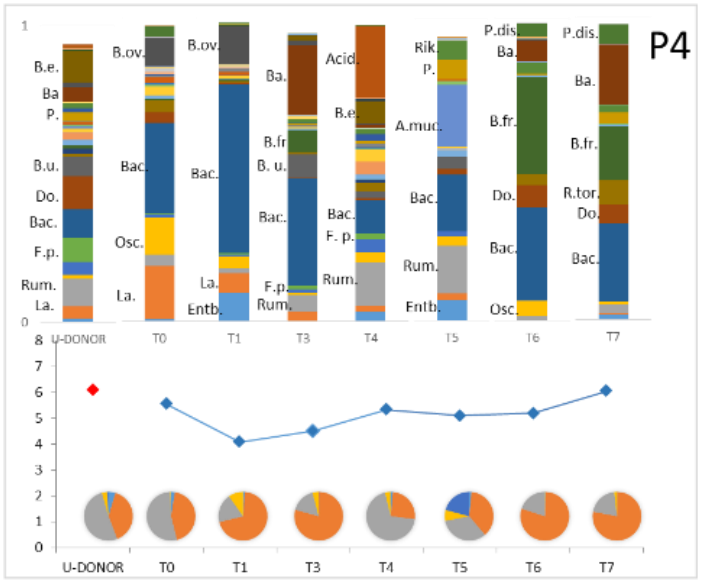


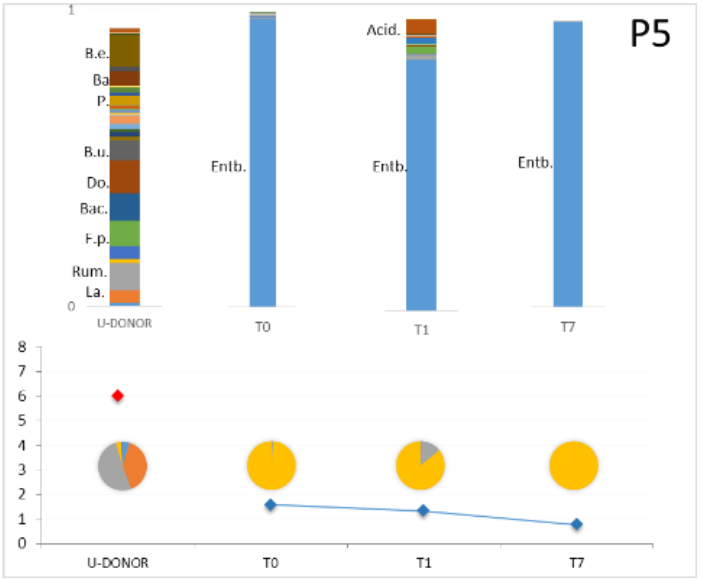
gut microbiota profiling











Actinobacteria Bacteroidetes Firmicutes Proteobacteria Verrucomicrobia

Conclusion

FMT **safety and feasibility** in pediatric patients with hematologic disorders immediately before the aplastic phase of HSCT

Patient 5 experienced a sepsis few hours after FMT; however, after careful clinical revision, this event was attributed to contamination of the central venous line by the caregiver

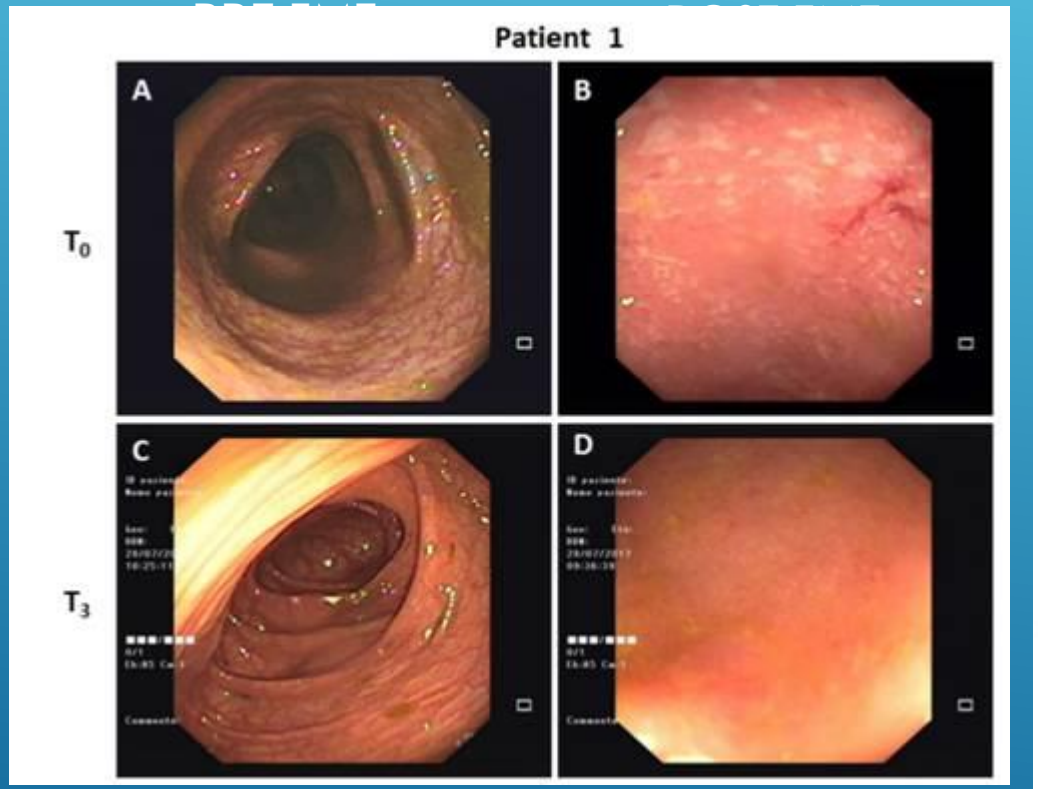
In all patients, only few symptoms related to the FMT procedure were recorded (nausea, bloating and abdominal pain), all being transient and easily controlled by symptomatic drugs

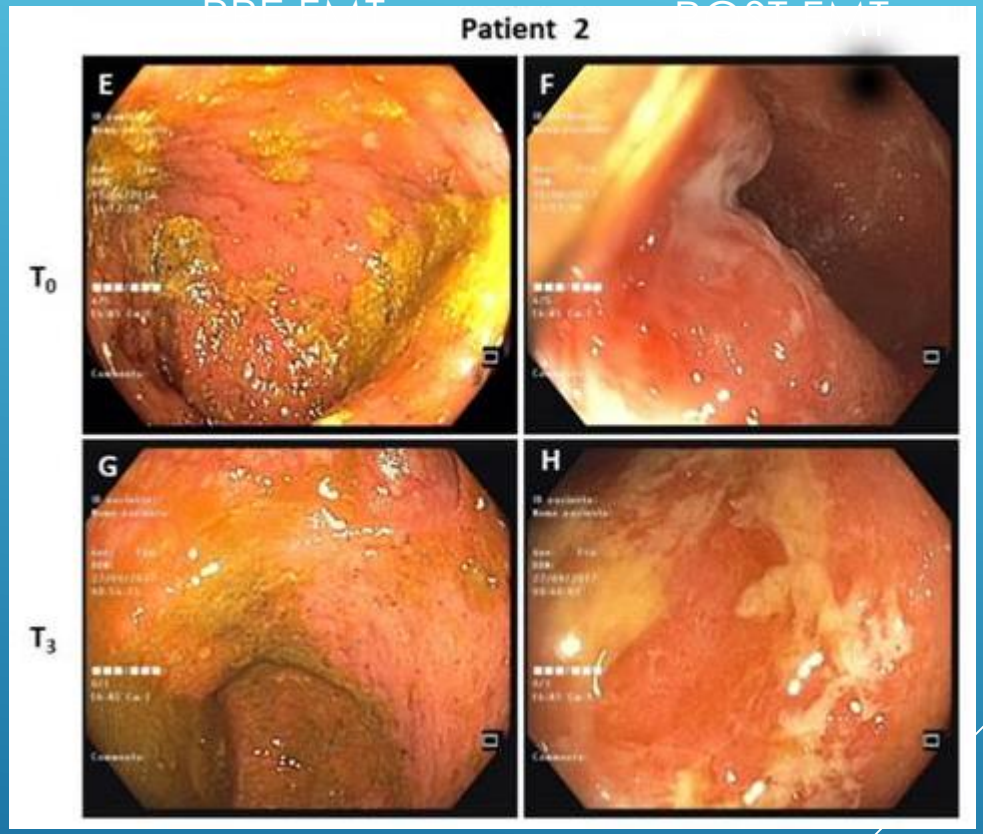
The recipients' microbiota seems to be colonized by donor bacteria starting from one week after the procedure

1 day after FMT we recorded the overgrowth of facultative anaerobes and aerobes, as Enterobacteriaceae, probably promoted by the O_2 conditions generated during the FMT emulsion

Afterward, the slow growth of strictly anaerobes from donor (*e.g.*, *Bacteroides*, *Parabacteroides*, *Dorea*, *F. prausnitzii*, *Ruminococcaceae*) reduced the O_2 conditions suppressing the Enterobacteriaceae relative amount

Fecal microbiota transplant in two ulcerative colitis pediatric cases: gut microbiota and clinical course correlations





doi: 10.3390/microorganisms8101486.

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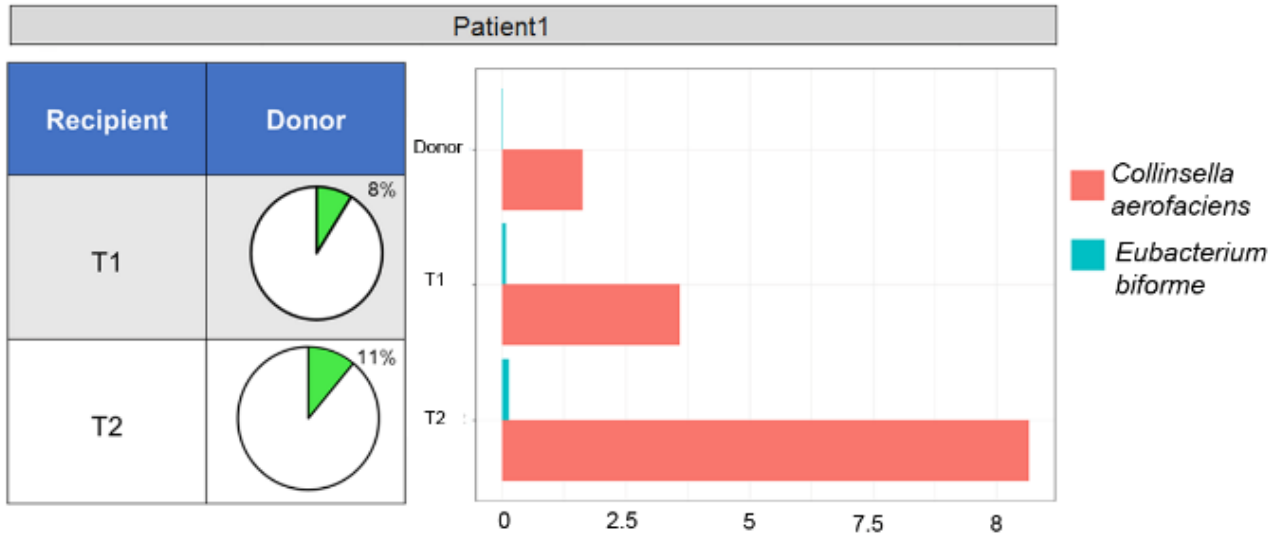
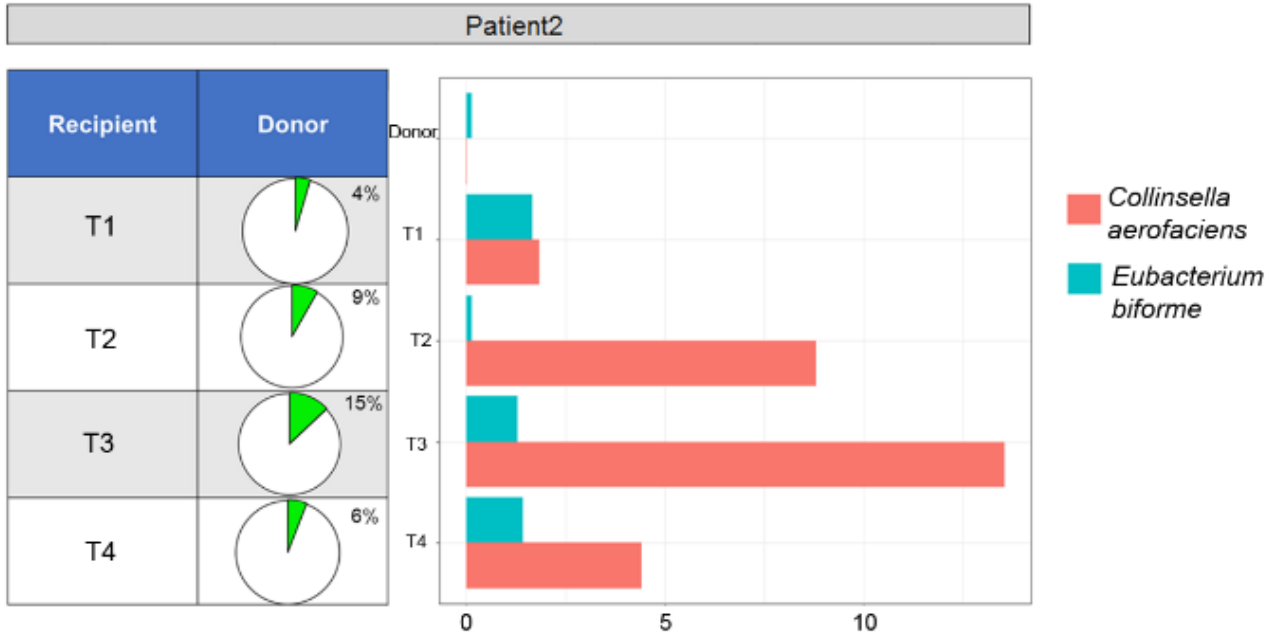
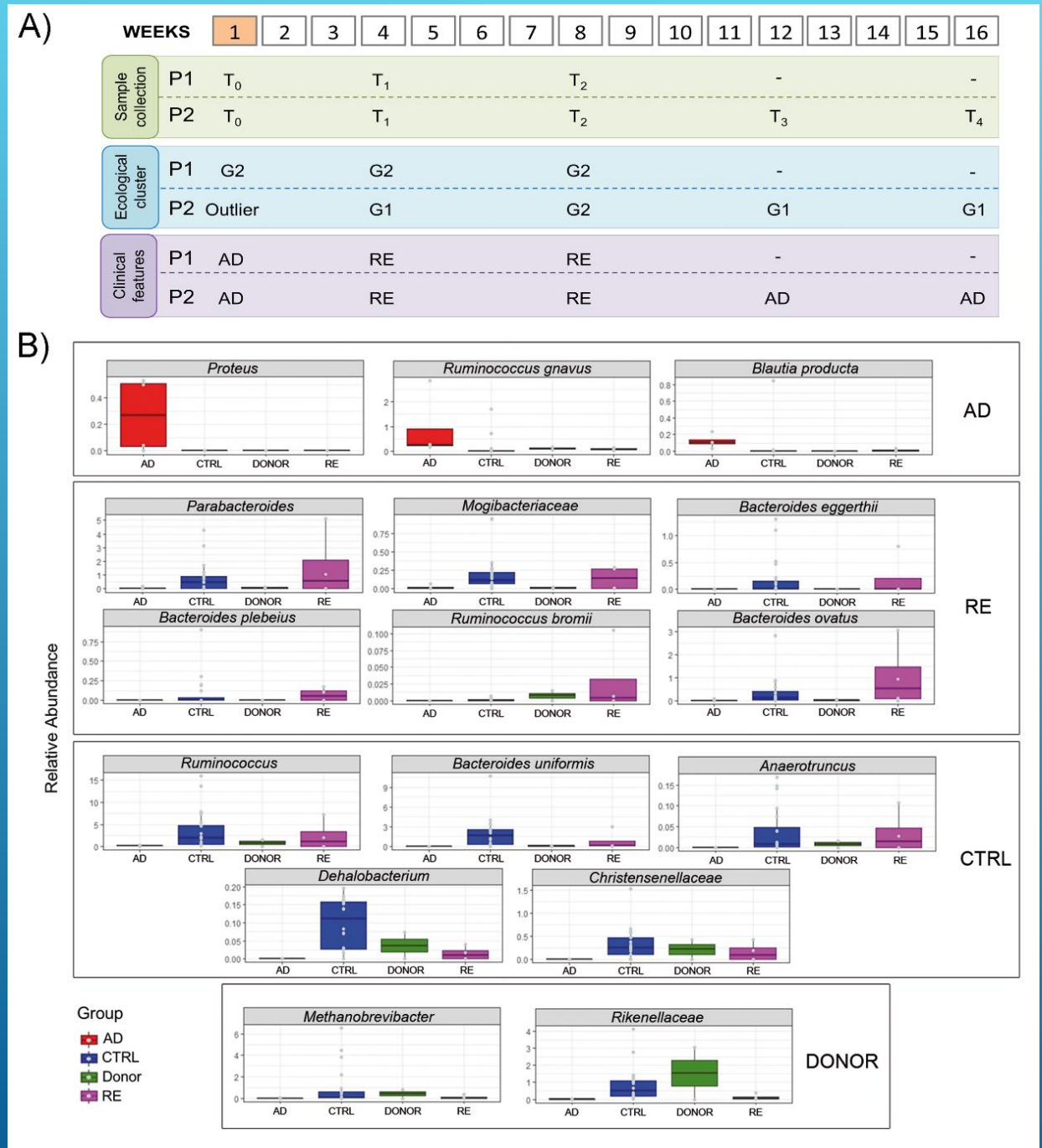

Patient1
Recipient
Donor
T1
T2
8%
11%
Donor
T1
T2
0
2.5
5
7.5
8
Collinsella aerofaciens
Eubacterium biforme

B)


Patient2
Recipient
Donor
T1
T2
T3
T4
4%
9%
15%
6%
Donor
T1
T2
T3
T4
0
5
10
Collinsella aerofaciens
Eubacterium biforme


A)
WEEKS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Sample collection
P1 T0 T1 T2 - -
P2 T0 T1 T2 T3 T4
Ecological cluster
P1 G2 G2 G2 - -
P2 Outlier G1 G2 G1 G1
Clinical features
P1 AD RE RE - -
P2 AD RE RE AD AD
B)
Relative Abundance
Proteus
Ruminococcus gnavus
Blautia producta
AD
Parabacteroides
Mogibacteriaceae
Bacteroides eggerthii
Bacteroides plebeius
Ruminococcus bromii
Bacteroides ovatus
RE
Ruminococcus
Bacteroides uniformis
Anaerotruncus
Dehalobacterium
Christensenellaceae
CTRL
Methanobrevibacter
Rikenellaceae
DONOR
AD
CTRL
DONOR
RE
Group
AD
CTRL
Donor
RE

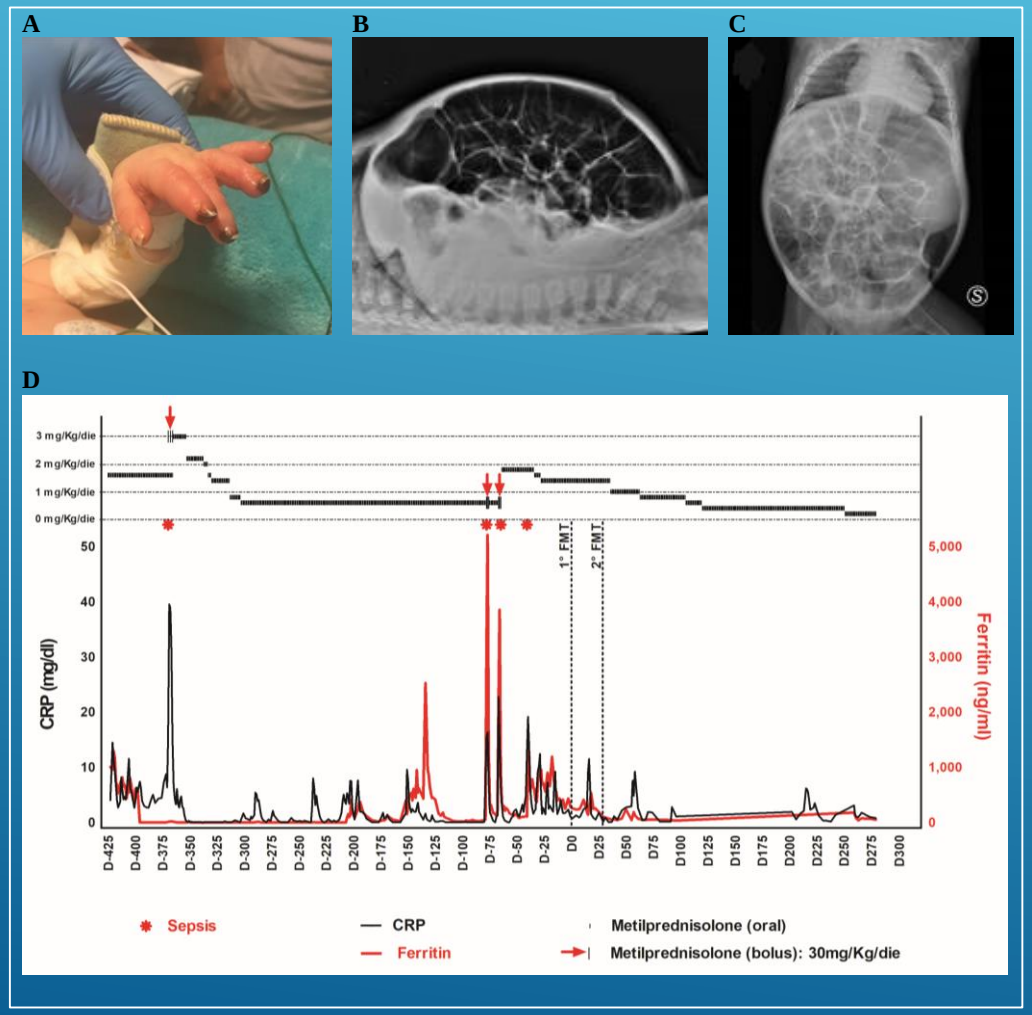
CONCLUSION

- After FMT, the improvement of clinical conditions was recorded for both patients.
- After 12 months, the mild UC patient was in clinical remission, while the moderate UC patient, after 12 weeks, had a clinical worsening.
- Increase of *Collinsella aerofaciens* and *Eubacterium biforme*, inherited by respective donors.
- Decrease of *Proteus* and *Blautia producta*, and the increment of *Parabacteroides*, *Mogibacteriaceae*, *Bacteroides eggerthi*, *Bacteroides plebeius*, *Ruminococcus bromii*, and *B Bacteroides ovatus* were associated to patient's remission condition.
- FMT results in a long-term response in mild UC, while in the moderate form probably there is the need of multiple FMT administrations.
- FMT leads in decreasing of potentially pathogens and in the increasing of microorganisms correlated to remission status.

MICROBIOTA TRANSPLANT TO CONTROL INFLAMMATION IN A *NLRC4*-RELATED DISEASE PATIENT WITH RECURRENT HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH)

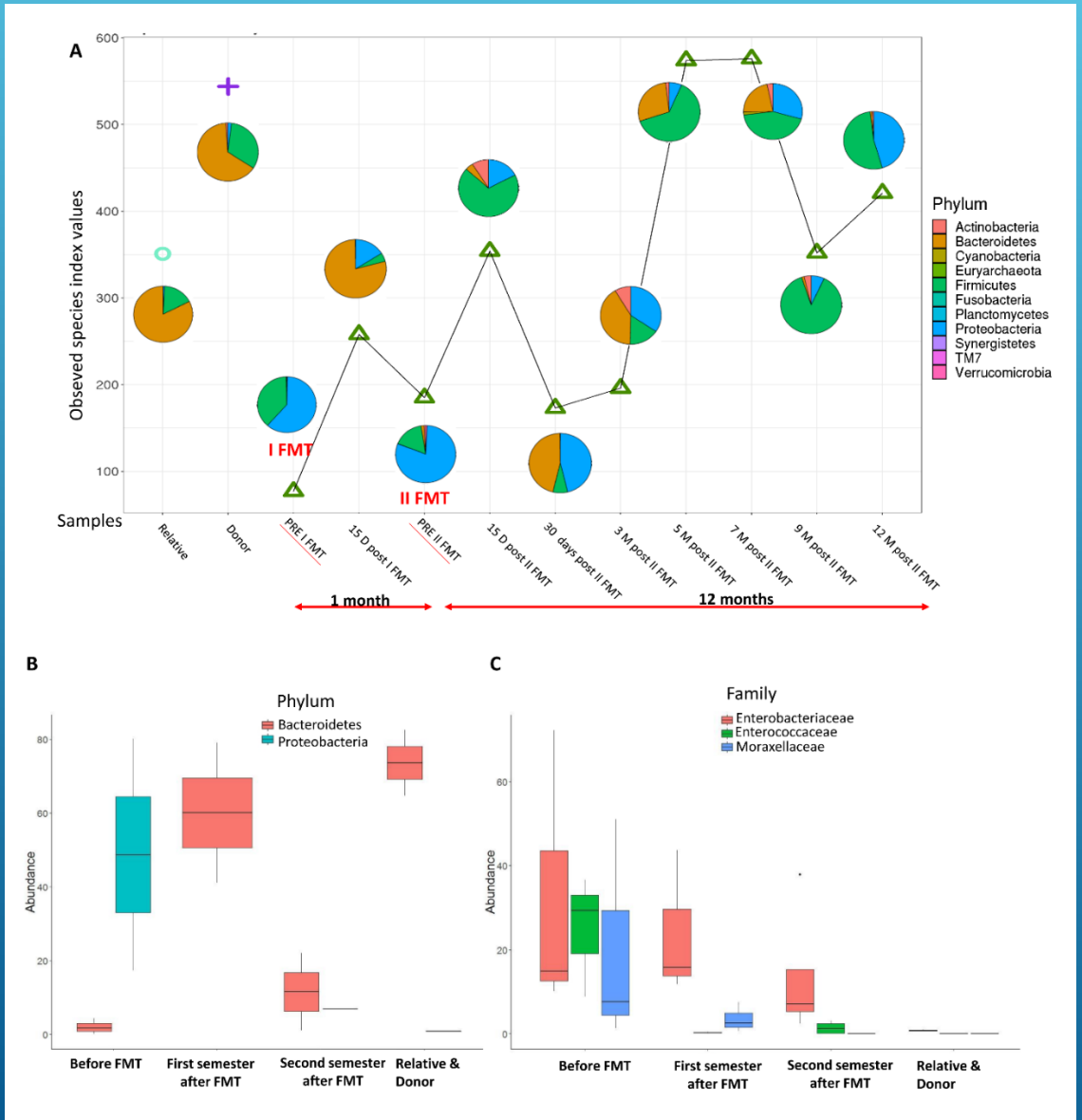
- Gain of function (GOF) mutations in *NLRC4*, encoding for a cytoplasmic NOD-like receptor, are associated with a distinct autoinflammatory syndrome characterized by early-onset **enterocolitis and recurrent hemophagocytic lymphohistiocytosis**
- Inflammasomes are innate immune sensors that respond to pathogen- and damage-associated signals with caspase-1 activation and subsequent production and release of inflammatory cytokines, interleukin 1β and IL-18. *NLRC4* GOF mutations cause constitutive caspase-1 cleavage and increased production of IL-18.
- In murine models has been demonstrated that the NLRC4 inflammasome is essential in host defence against enteric pathogens.
- The intricate relationship between the gut microbiota and inflammasome activation suggests the relevance of intestinal dysbiosis in maintain the persistent gut inflammation related to the *NLRC4*-related disease.

Case report



- A Caucasian 16-month-old presented, from 1 month of life, with recurrent HLH, diarrhoea and vasculitic skin lesions caused by a de novo missense mutation in *NLRC4* (I343N) gene. As expected IL-18 levels were persistently and markedly elevated.
- His inflammatory flares and episodes of HLH were partially controlled with various immunomodulatory treatments
- The patient presented persistent diarrhoea with gut inflammation and colonization by MDR pathogens (*Enterobacter cloacae* and *Enterococcus faecalis*); both of them translocated and caused four sepsis episodes.

Results



- Presence of intestinal dysbiosis before FMT with a very poor microbiome mostly characterized by proteobacteria that include the most pro-inflammatory species.
- He received a first FMT of fresh preparation by endoscopy and ileoscopy, of both ileostomic ansae. This was followed by rapid clinical improvement allowing, ileal anastomosis with uneventful recanalization after 72 hours.
- A month later, a second FMT of frozen preparation was performed by upper endoscopy.

Conclusion

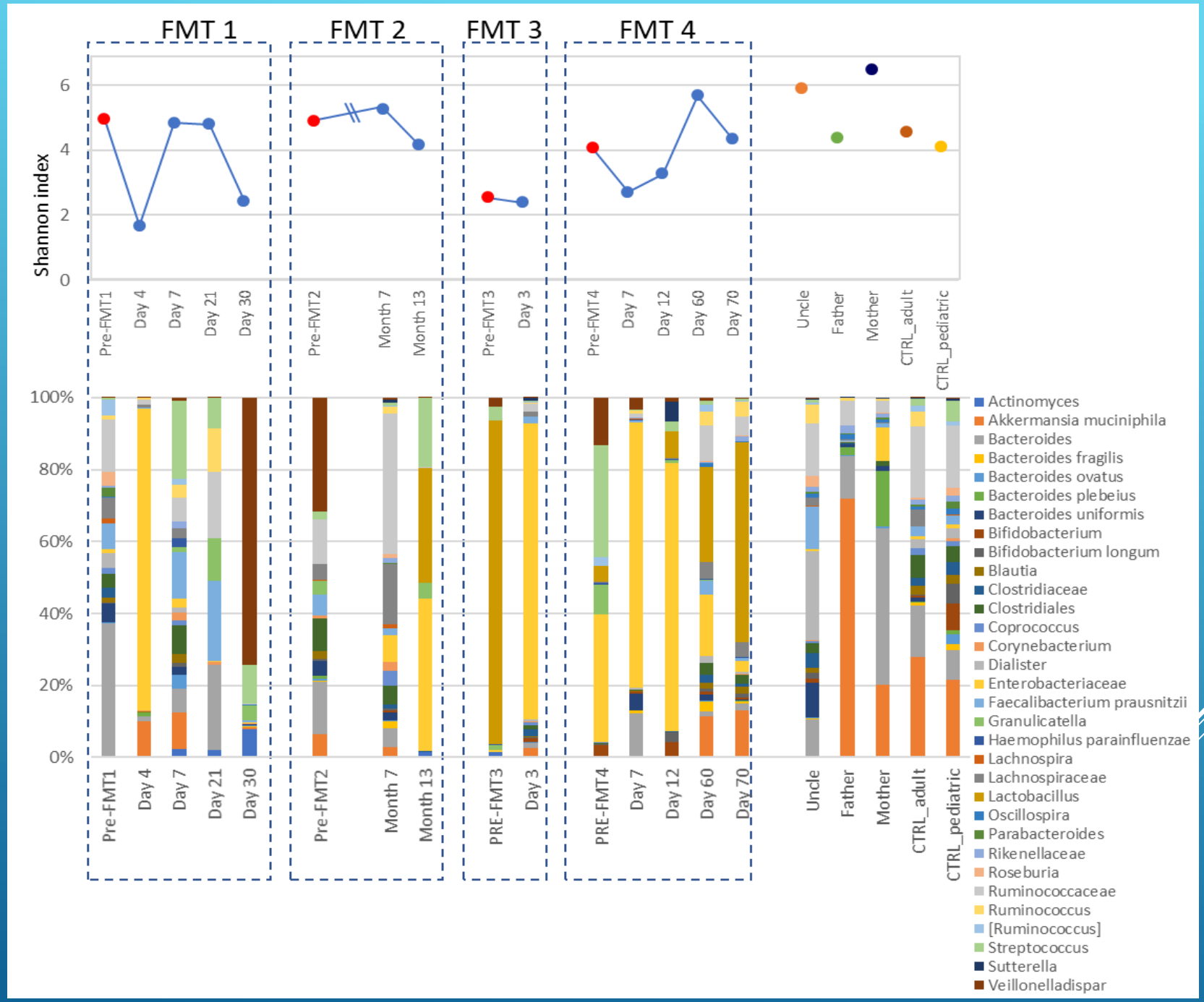
- 7 days after the first FMT the patient had a donor-like microbiota profile
- During the first semester after the second FMT, the proportion of Bacteroidetes and the bacterial richness increased, the latter reaching the highest level.
- Before FMT, we observed the increase of Proteobacteria while after the FMT the decrease
- After 24 months from FMT, the patient showed a gut microbiota profile completely established
- After the two FMT, the patient did not develop any complication.
- Stool cultures did not show presence of enteropathogens, in particular the **two MDR pathogens detected before FMT were not found**.
- **His inflammatory disease was more easily controlled. No additional HLH flares occurred, even though, as expected IL-18 remained persistently elevated**

FECAL MICROBIOTA TRANSPLANTATION IN A PEDIATRIC PATIENT FOR THE TREATMENT OF STEROID-REFRACTORY INTESTINAL GRAFT-VERSUS-HOST DISEASE

Case report

- A 5-year old boy, affected by high-risk M6 acute myelogenous leukemia in 1st complete remission, developed overall grade III GVHD (skin stage 2 and gut stage 3) 27 days after an allogeneic HSCT
- The patient did not respond to full-dose steroid treatment, he progressed to grade IV GVHD. He was then treated with infliximab, again without response.
- A compassionate treatment with the anti-CD26 monoclonal antibody (moAb) begelomab was started with partial response.
- Given the severity and poor-responsiveness of GVHD, based on preliminary findings of other groups, FMT was considered.
- The parents were screened. The father was deemed ineligible for positivity to *Dientamoeba fragilis* and the mother was chosen as the donor.

Results


FMT 1
FMT 2
FMT 3
FMT 4
Shannon index
0
2
4
6
Pre-FMT1
Day 4
Day 7
Day 21
Day 30
Pre-FMT2
Month 7
Month 13
Pre-FMT3
Day 3
Pre-FMT4
Day 7
Day 12
Day 60
Day 70
Uncle
Father
Mother
CTRL_adult
CTRL_pediatric
100%
80%
60%
40%
20%
0%
Actinomyces
Akkermansia muciniphila
Bacteroides
Bacteroides fragilis
Bacteroides ovatus
Bacteroides plebeius
Bacteroides uniformis
Bifidobacterium
Bifidobacterium longum
Blautia
Clostridiaceae
Clostridiales
Coprococcus
Corynebacterium
Dialister
Enterobacteriaceae
Faecalibacterium prausnitzii
Granulicatella
Haemophilus parainfluenzae
Lachnospira
Lachnospiraceae
Lactobacillus
Oscillospira
Parabacteroides
Rikenellaceae
Roseburia
Ruminococcaceae
Ruminococcus
[Ruminococcus]
Streptococcus
Sutterella
Veillonelladispar

Results

- After the **first FMT**, gut microbiota composition showed a large expansion of the Enterobacteriaceae with the reduction of microbial richness, followed by a gradual substitution by different taxa such as *Streptococcus*, *Ruminococcus* and Ruminococcaceae, *Faecalibacterium prausnitzii*, Clostridiales, *Bacteroides* and *Akkermansia muciniphila*, between the 7 and 21 days after FMT, resulting into the increase of Shannon index.
- After 30 days of FMT *Veillonella dispar* increased, reaching the 63% of the entire content of microbiota, whit the consequent decline of microbial richness index.

- After the **third FMT** the expansion of Enterobacteriaceae was observed,
- After **the fourth** we assisted to the reduction of Enterobacteriaceae and the increase of beneficial bacteria such as *Akkermansia muciniphila*, Ruminococcaceae/*Ruminococcus* and *Faecalibacterium prausnitzii*.

Thanks for the attention