



ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ

Δημοκρίτειο Πανεπιστήμιο Θράκης
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**Προγράμματος Μεταπτυχιακών
Σπουδών «Λοιμωξιολογία»
Αθήνα, 4 Απριλίου 2022**

-Μικροβιακή χλωρίδα- Από την συμβίωση στην παθογένεια

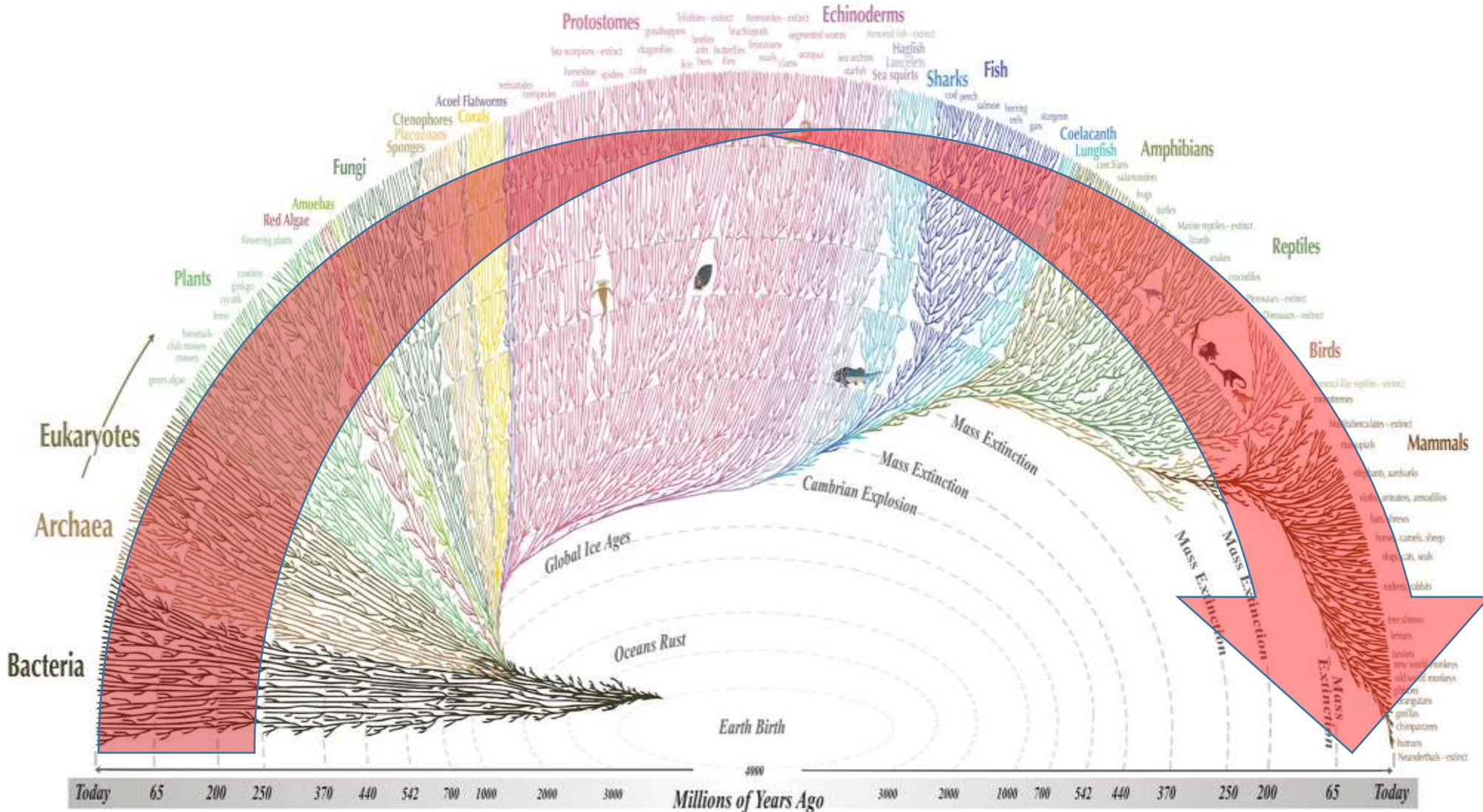
Γεώργιος Κολιός, MD PhD
Γαστρεντερολόγος - Κλινικός Φαρμακολόγος
Καθηγητής Φαρμακολογίας
Ιατρικό Τμήμα Δ.Π.Θ.

**Μικροβιακή χλωρίδα
vs
Μικροβίωμα**

Μικροβίωμα

- Το σύνολο του γενετικού υλικού (DNA) των μικροοργανισμών αποτελεί το **Μικροβίωμα**
- Όπως το σύνολο του γενετικού υλικού των κυττάρων μας αποτελεί το **Γονιδίωμα** μας

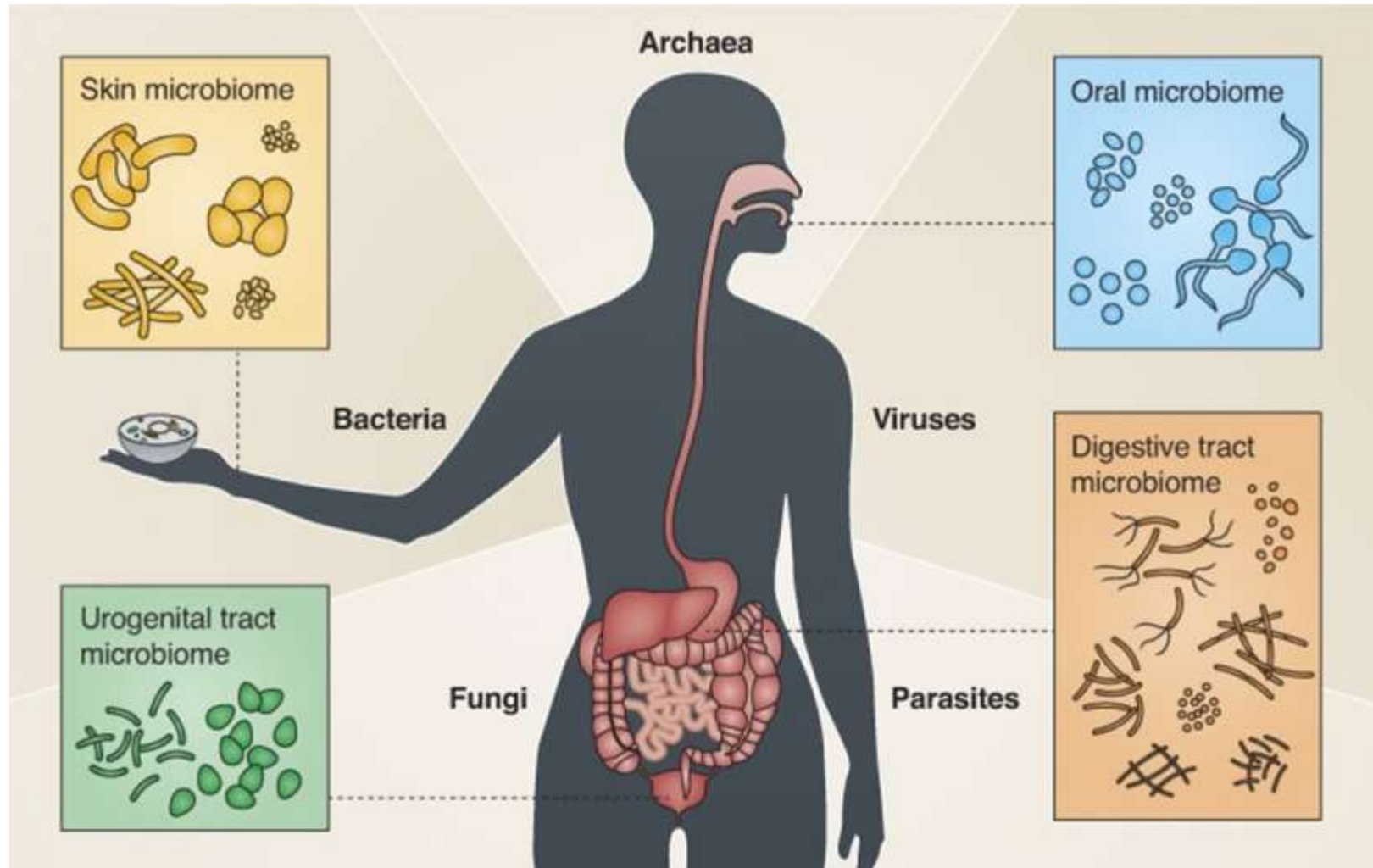
Το δέντρο της Ζωής. The tree of Life



All the major and many of the minor living branches of life are shown on this diagram, but only a few of those that have gone extinct are shown. Example: Dinosaurs - extinct



Μικροβιακή Χλωρίδα



(Wendy S. Garrett. J Cell Biol. 2015;210:919-920)

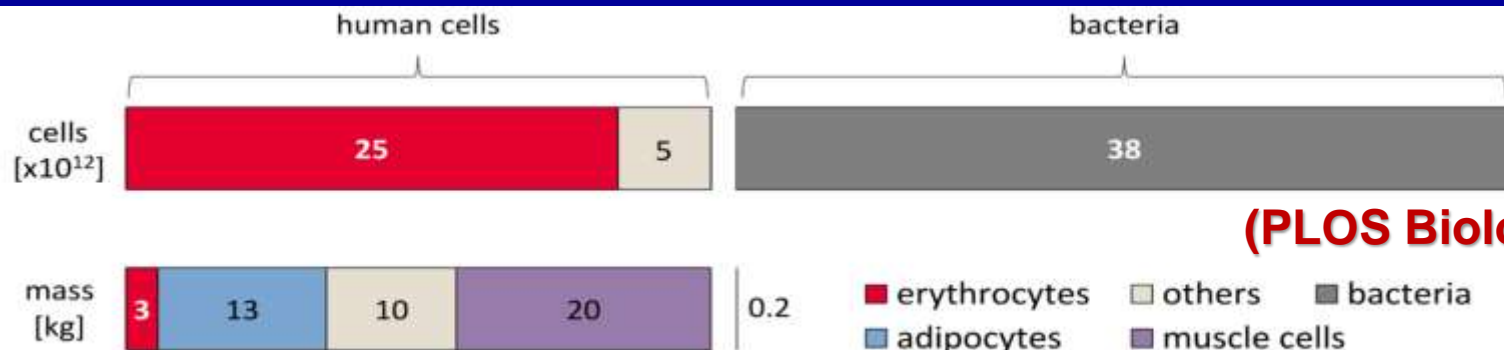
Μικροβιακή Χλωρίδα

- 10^{12} βακτήρια του σώματός μας (3% του σωματικού βάρους) $> 10 \times 10^{11}$ ανθρώπεια κύτταρα
- Βακτηριακά γονίδια (3,000,000) $> 100 \times$ ανθρώπια γονίδια (23,000)

(Costello et al Science. 2009)

...more recent research estimates that to 3:1...

Nature 2016



(PLOS Biology 2016)

Fig 3. Distribution of cell number and mass for different cell types in the human body (for a 70 kg adult man). The upper bar displays the

Μικροβιακή Χλωρίδα

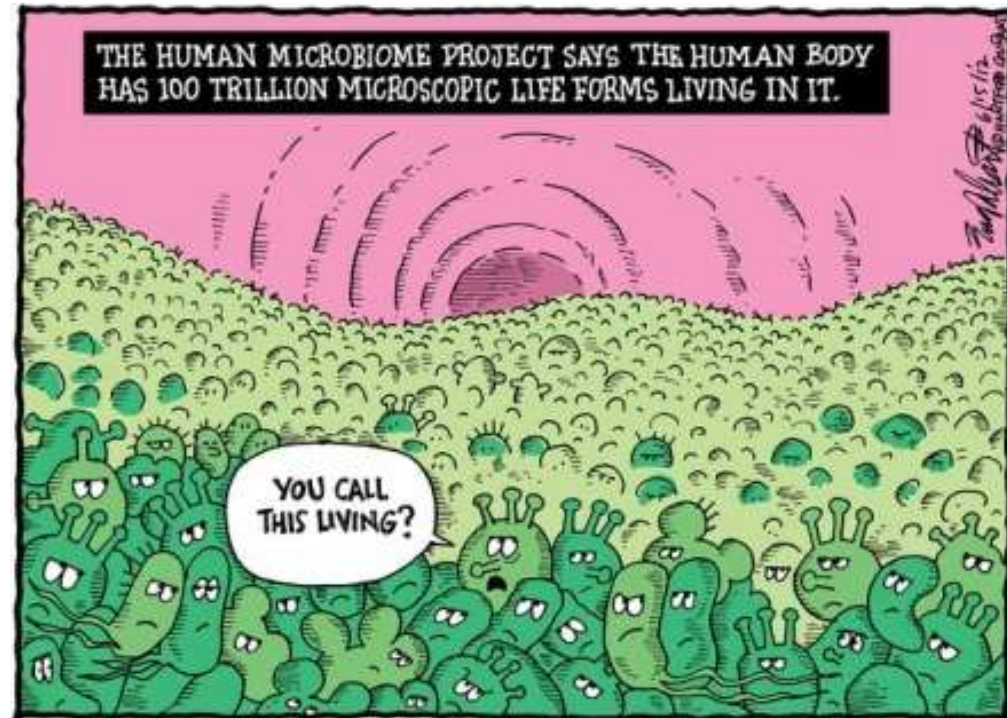
- Υπάρχουν 100 τρισεκατομμύρια μικροοργανισμοί στο σώμα του ανθρώπου... 10 φορές περισσότερα από ανθρώπινα κύτταρα.....

BACTERIA are 10 to 50 times



SMALLER
than human cells

THE HUMAN MICROBIOME PROJECT SAYS THE HUMAN BODY HAS 100 TRILLION MICROSCOPIC LIFE FORMS LIVING IN IT.




70% των μικροοργανισμών της χλωρίδας μας παραμένουν “μη καλλιεργήσιμα”

cultivated fraction

Tannock 2000 21-37%

Suau 1999 21-32%

An iceberg floating in the ocean. The tip of the iceberg is visible above the water surface, while the much larger, submerged part is below the surface. This visualizes the concept that only a small fraction of microorganisms are culturable.

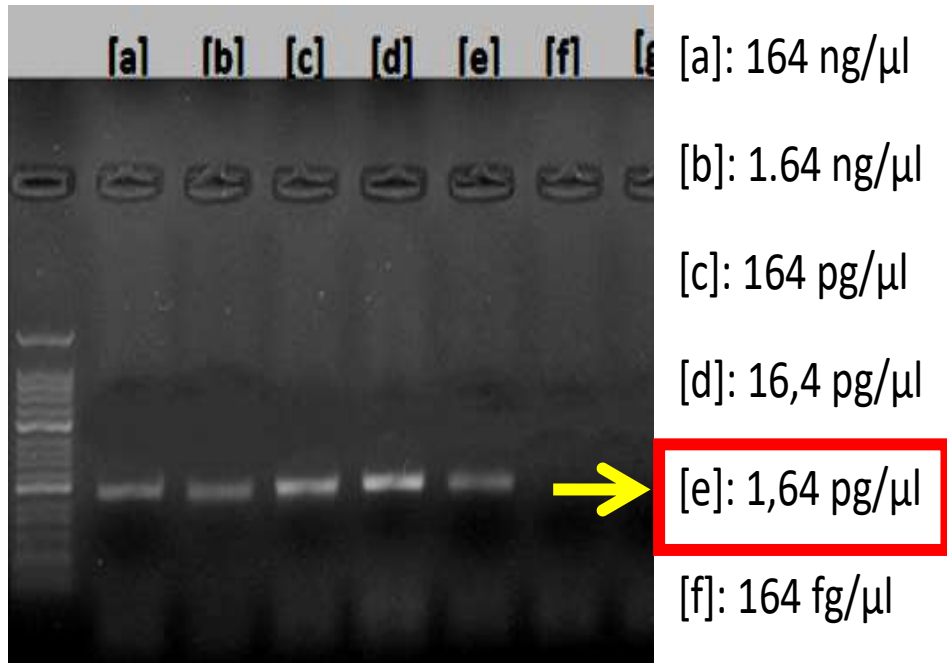
→ Ανάγκη για την ανάπτυξη νέων ανεξάρτητων τεχνικών

Ανίχνευση μικροβιώματος

- Σήμερα η ανάπτυξη των τεχνικών της **Μοριακής Ιατρικής** μας δίνει την δυνατότητα να ξεπεράσουμε τα προβλήματα των καλλιεργειών.
- Όμως ακόμη η **μη ταυτοποίηση του μικροβιώματος** όλων των μικροοργανισμών παραμένει ένα μεγάλο ζήτημα.

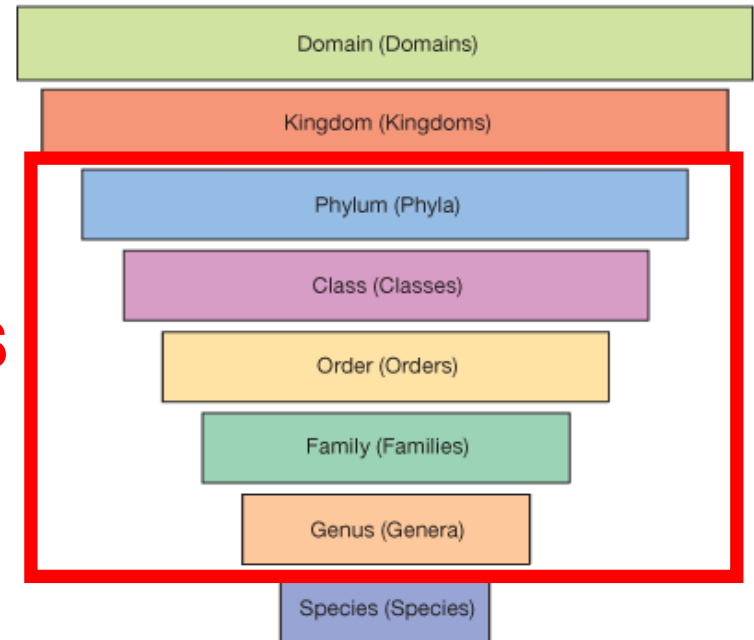
Ενίσχυση βακτηριδιακού DNA

- Τιτλοδότηση (διαδοχικές αραιώσεις DNA κοπράνων)

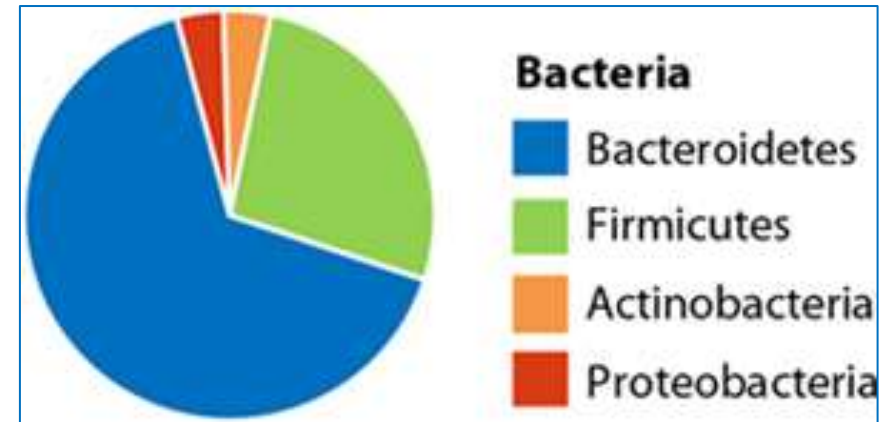
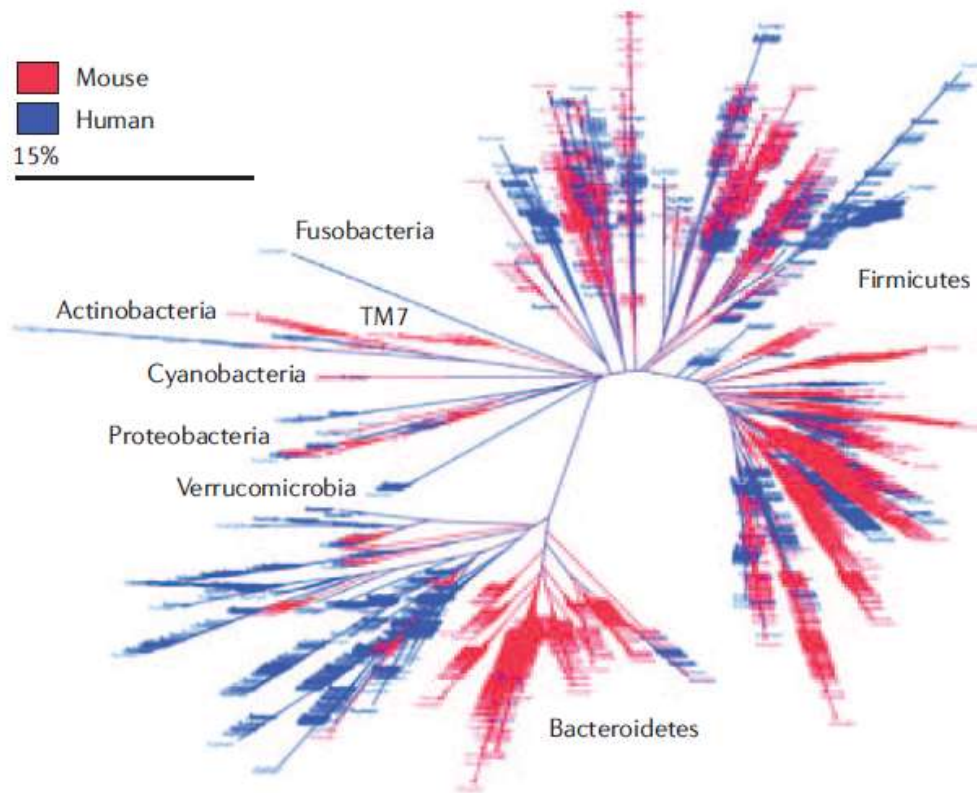


- Και με τις πρόσφατες μεθόδους **γονοτύπησης του γονιδίου 16S** (μικρή ριβοσωμική υπομονάδα), φτάνουμε μέχρι διακριτικής ικανότητας ταυτοποίησης του γένους, αλλά όχι του είδους.
- Και αν θέλουμε μεγαλύτερη ανάλυση, μπορούμε να γονοτυπήσουμε όλο το μικροβιακό γενετικό υλικό με **shotgun sequencing**.

How animals are classified



Η σύνθεση του «φυσιολογικού» ανθρώπινου εντερικού μικροβιώματος



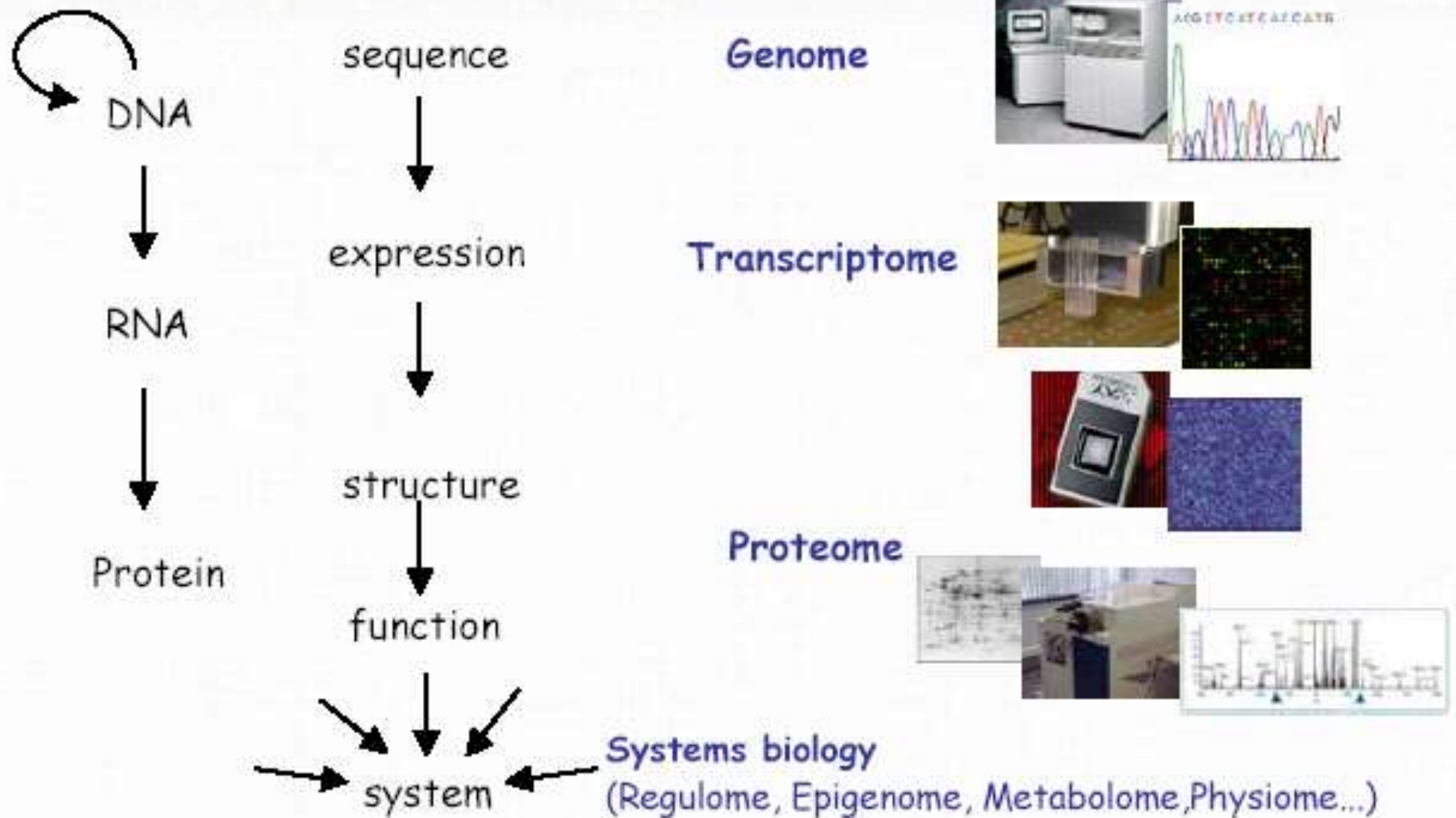
Παθοβιωτικά (pathobionts)

- Proteobacteria
- Actinobacteria

Συμβιωτικά - αντιφλεγμονώδη

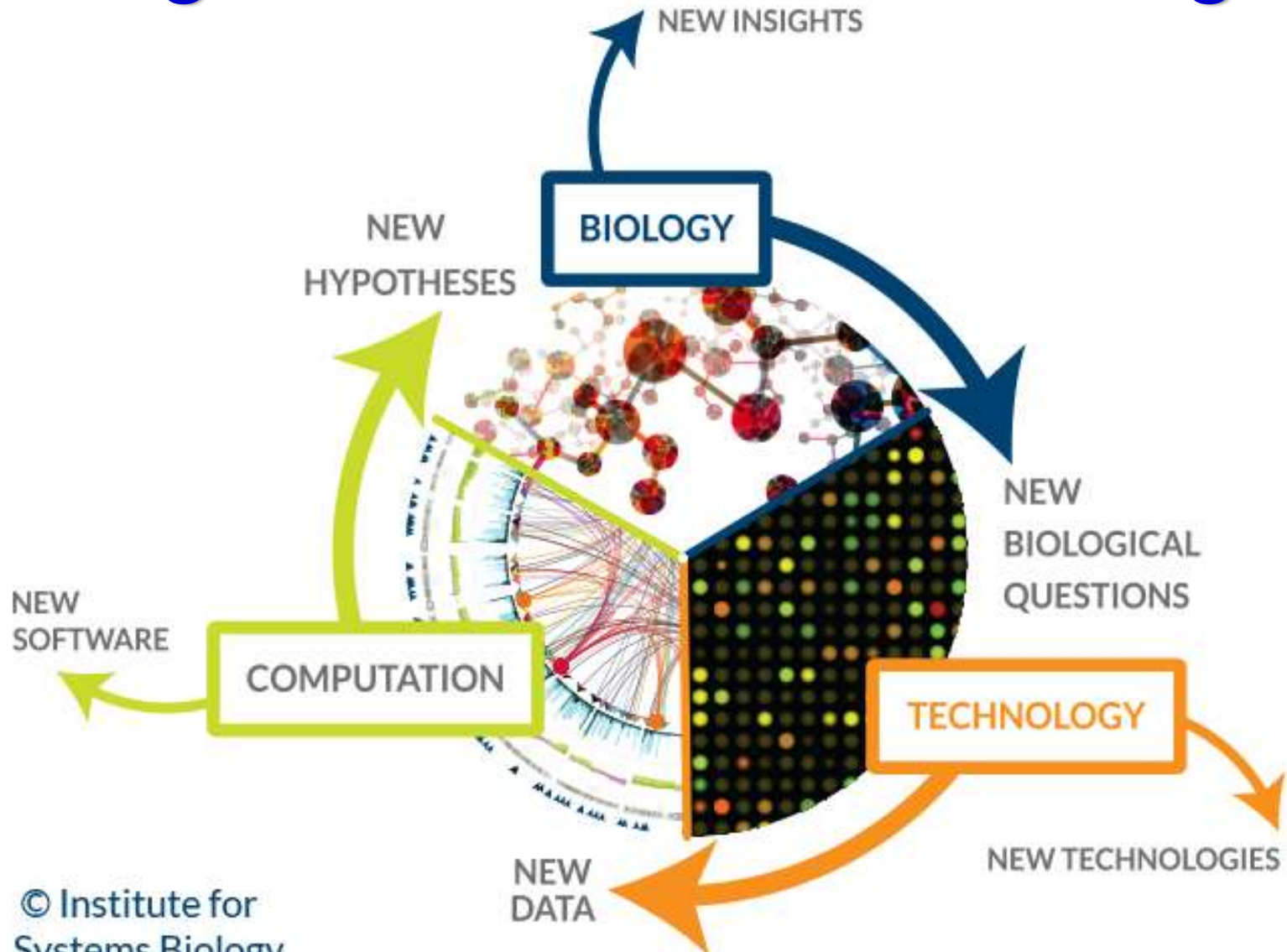
- Firmicutes
- Bacteroidetes

Biology as Information Science: in Large Scale!



Bioinformatics:

Transforming bio-information to knowledge



Commentary

Personomics: The Missing Link in the Evolution from Precision Medicine to Personalized Medicine

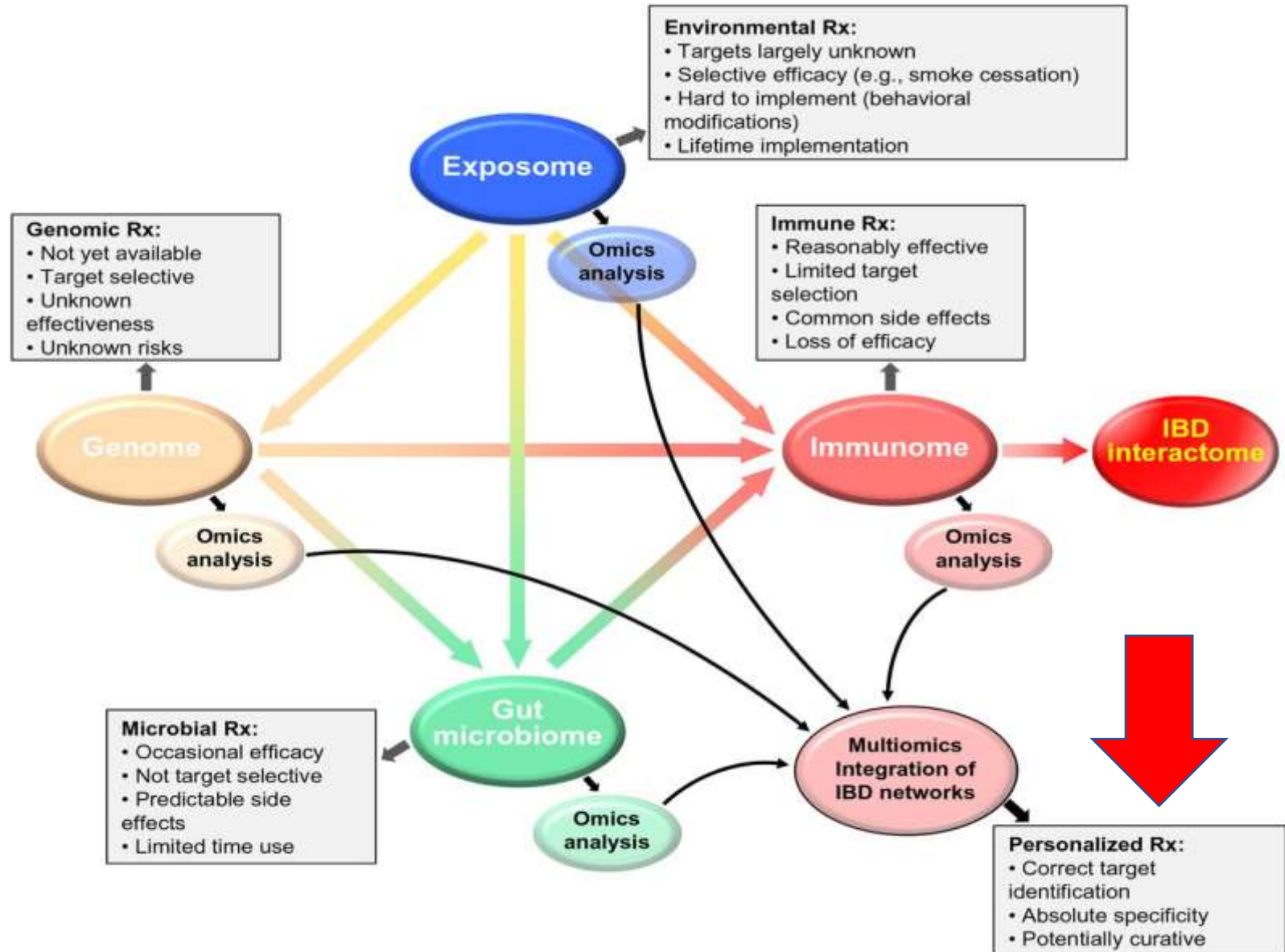
Roy C. Ziegelstein

Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; rziegel2@jhmi.edu;
Tel.: +1 410-955-8401

derived from knowing the patient as a person. These unique personal characteristics are defined by the tools of personalized medicine—personomics—which take into account an individual's personality, preferences, values, goals, health beliefs, social support network, financial resources, and unique life circumstances that affect how and when a given health condition will manifest in that person and how that condition will respond to treatment. In this paradigm, precision medicine may be considered a necessary step in the evolution of medical care to personalized medicine, with personomics as the missing link.

(J. Pers. Med. 2017)

The concept of the IBD interactome



(Danese et al. *Gut* 2016)

PERSPECTIVE

Big Data: Astronomical or Genomical?

Zachary D. Stephens¹, Skylar Y. Lee¹, Faraz Faghri², Roy H. Campbell², Chengxiang Zhai³, Miles J. Efron⁴, Ravishankar Iyer¹, Michael C. Schatz^{5*}, Saurabh Sinha^{3*}, Gene E. Robinson^{6*}

Table 1. Four domains of Big Data in 2025. In each of the four domains, the projected annual storage and computing needs are presented across the data lifecycle.

<u>Data Phase</u>	<u>Astronomy</u>	<u>Twitter</u>	<u>YouTube</u>	<u>Genomics</u>
Acquisition	25 zetta-bytes/year	0.5–15 billion tweets/year	500–900 million hours/year	1 zetta-bases/year
Storage	1 EB/year	1–17 PB/year	1–2 EB/year	2–40 EB/year
Analysis	In situ data reduction	Topic and sentiment mining	Limited requirements	Heterogeneous data and analysis
	Real-time processing	Metadata analysis		Variant calling, ~2 trillion central processing unit (CPU) hours
	Massive volumes			All-pairs genome alignments, ~10,000 trillion CPU hours
Distribution	Dedicated lines from antennae to server (600 TB/s)	Small units of distribution	Major component of modern user's bandwidth (10 MB/s)	Many small (10 MB/s) and fewer massive (10 TB/s) data movement

(Stephens et al PLoS Biol. 2015)

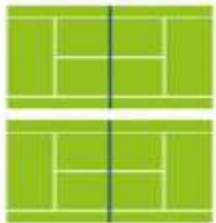
Εντερική χλωρίδα του ανθρώπου



GUT MICROBIOTA

A huge quantity (**hundreds of trillions**) of **bacteria** and other microorganisms inhabit your intestines; they are key for your **health and wellbeing**.

The gastrointestinal



TRACT SURFACE

is as big as
2 TENNIS COURTS

400m²

95%

of your bacteria
is located in the

GASTROINTESTINAL
TRACT



Gut microbiota's
WEIGHT



can reach up to
1 to 2 Kg



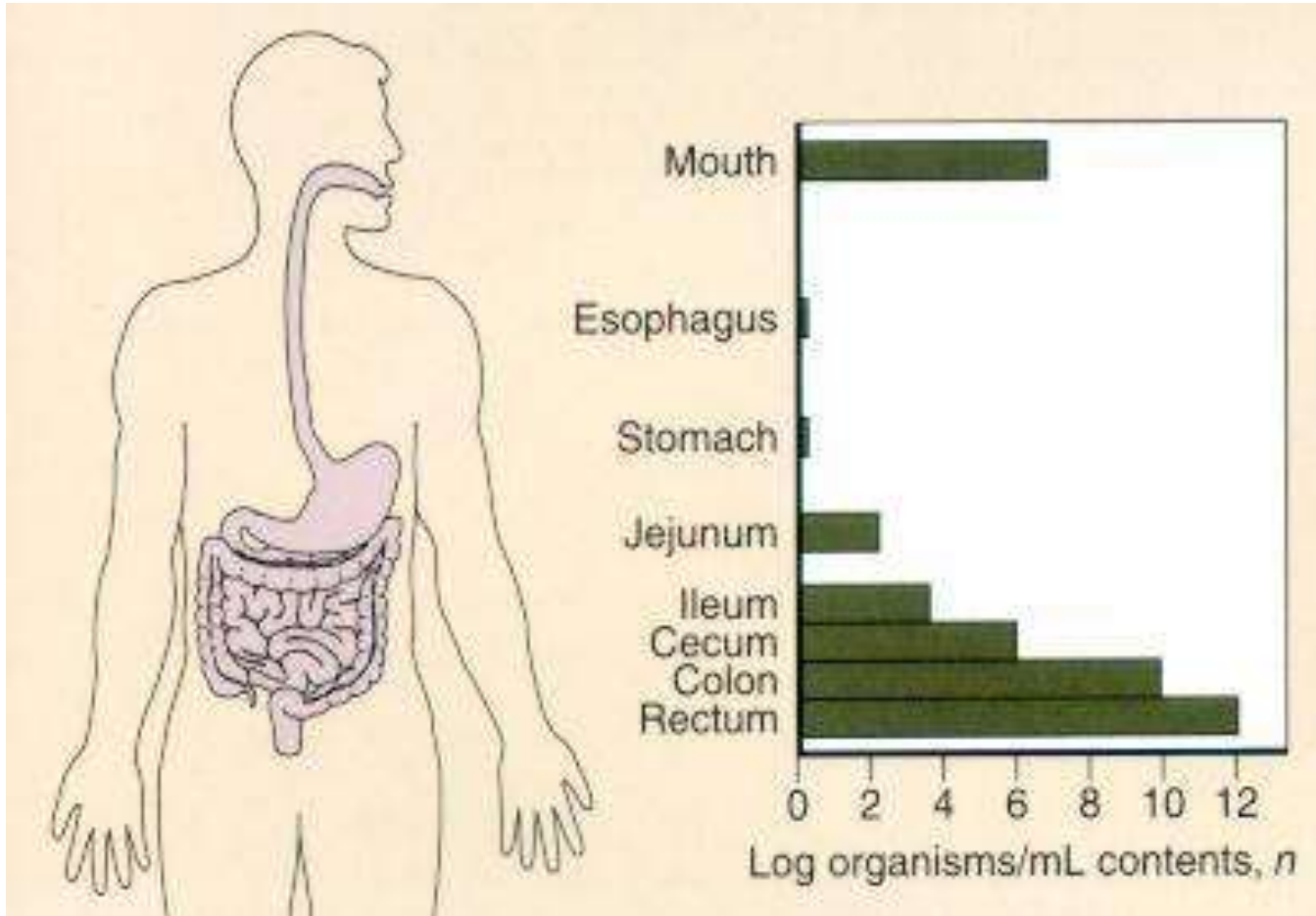
HALF OF YOUR STOOL
IS **NOT** LEFTOVER FOOD.

IT'S

MICROBIAL BIOMASS

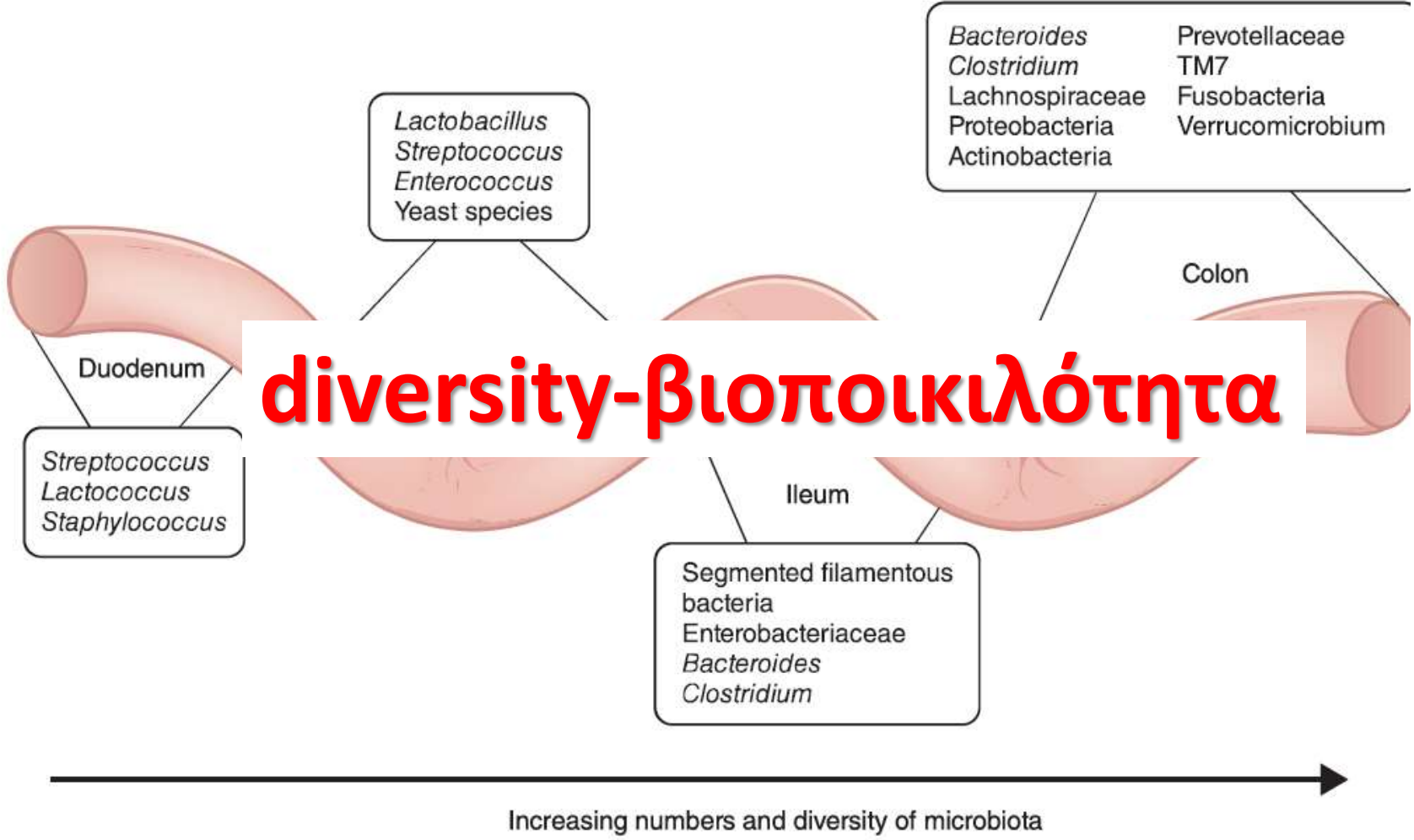
PRI 2012

Φυσιολογική χλωρίδα του πεπτικού σωλήνα



(Laissue & Gebbers. *Curr Stud Hematol Blood Transfus* 1992)

Φυσιολογική χλωρίδα του πεπτικού σωλήνα



(Brown et al. Nature Immunol 2013)

Ο ρόλος της Εντερικής Χλωρίδας

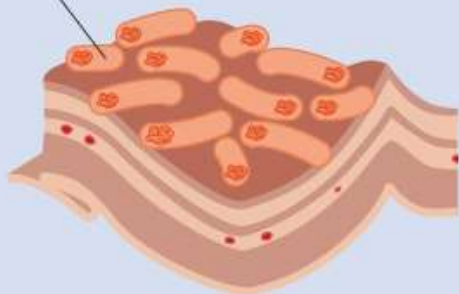
- Η εντερική χλωρίδα και ο εντερικός βλεννογόνος είναι δύο συμβιωτικοί οργανισμοί.
- **Που αναπτύχθηκαν παράλληλα μέσα στην εξελικτική πορεία.**
- Ο εντερικός αυλός παρέχει ευνοϊκές συνθήκες ανάπτυξης και θρεπτικά υλικά.
- Η χλωρίδα τροφικό/μεταβολικό ρόλο και προστασία από παθογόνους παράγοντες.

Ο ρόλος της Φυσιολογικής Εντερικής Χλωρίδας

Protective functions

Pathogen displacement
Nutrient competition
Receptor competition
Production of anti-microbial factors e.g., bacteriocins, lactic acids

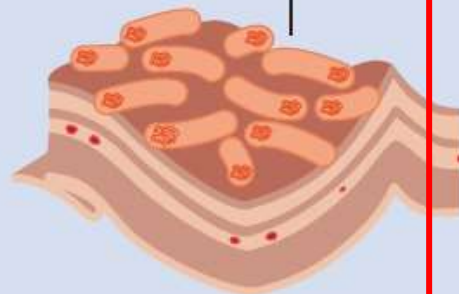
Commensal bacteria



Structural functions

Barrier fortification
Induction of IgA
Apical tightening of tight junctions
Immune system development

IgA



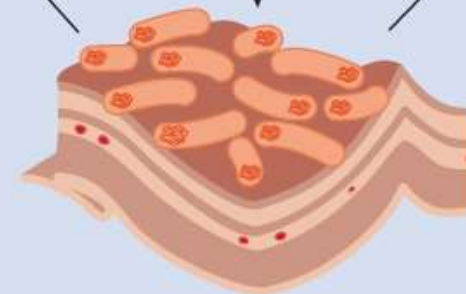
Metabolic functions

Control IEC differentiation and proliferation
Metabolize dietary carcinogens
Synthesize vitamins e.g., biotin, folate
Ferment non-digestible dietary residue and endogenous epithelial-derived mucus
Ion absorption
Salvage of energy

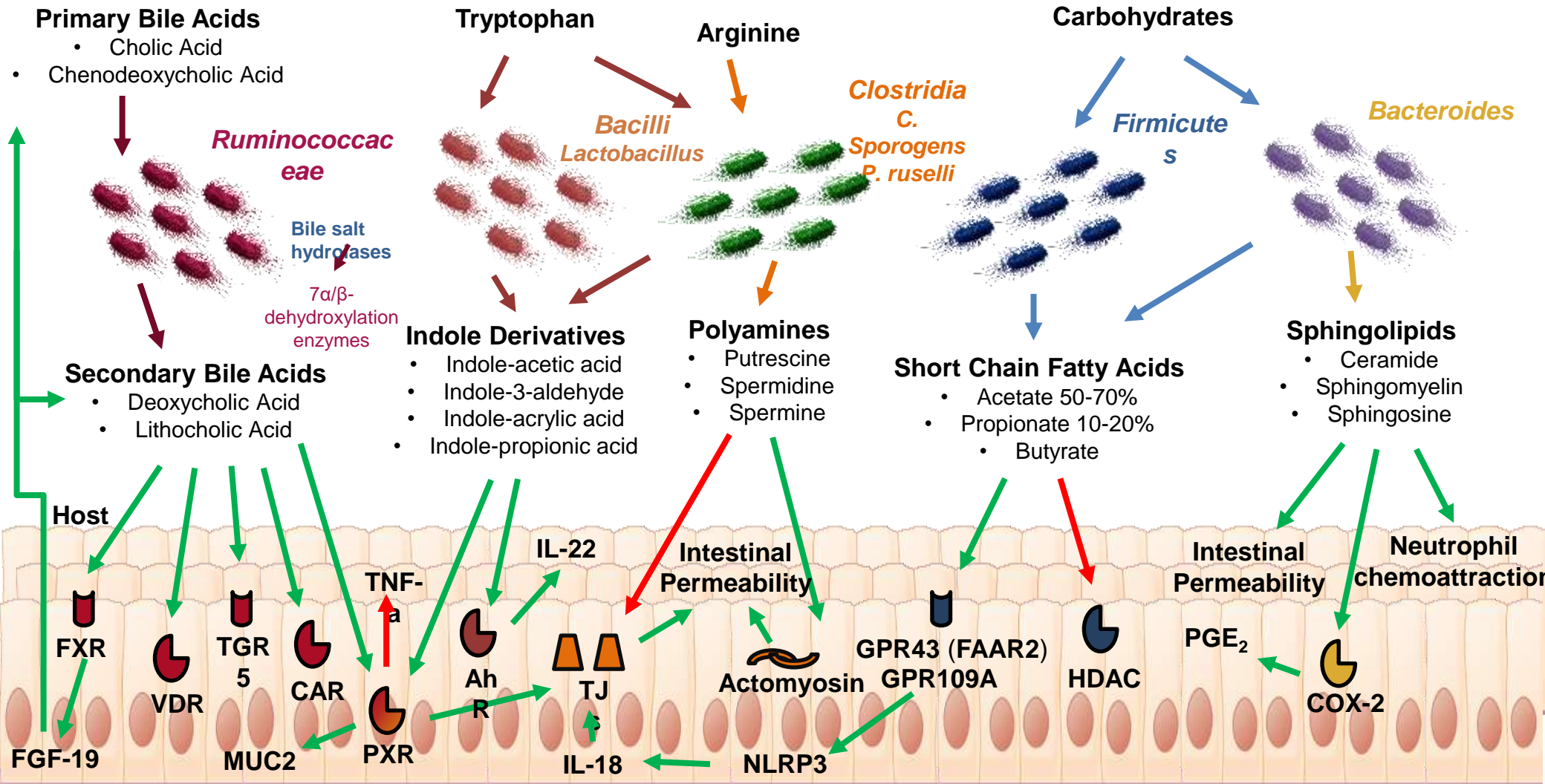
Short-chain fatty acids

Mg²⁺
Ca²⁺
Fe²⁺

Vitamin K
Biotin
Folate



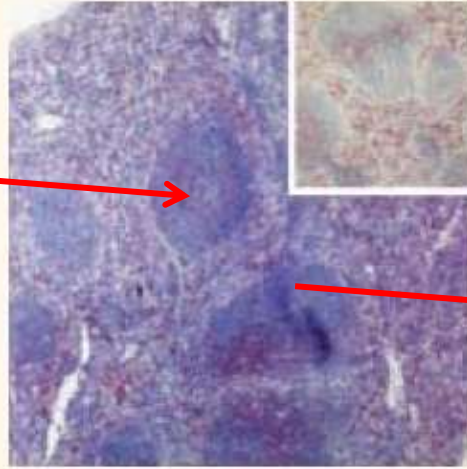
Microbiota Metabolite Map



(Unpublished data)

Ανάπτυξη του βλεννογονίου ανοσολογικού συστήματος

a Splenic CD4
(inset: splenic CD8)

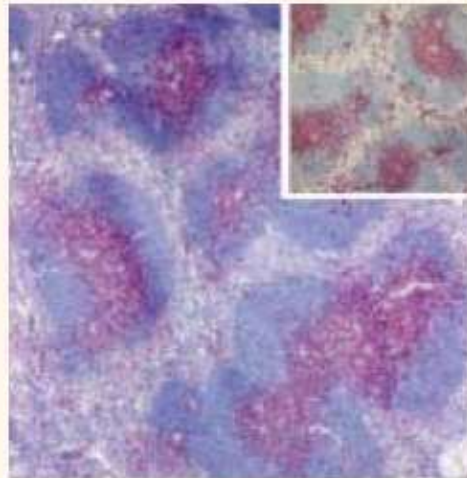
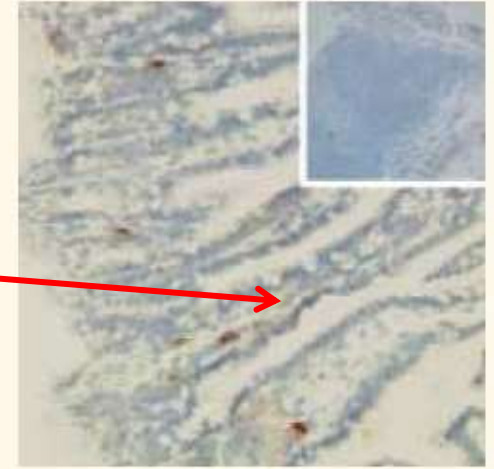


Germ-free mouse

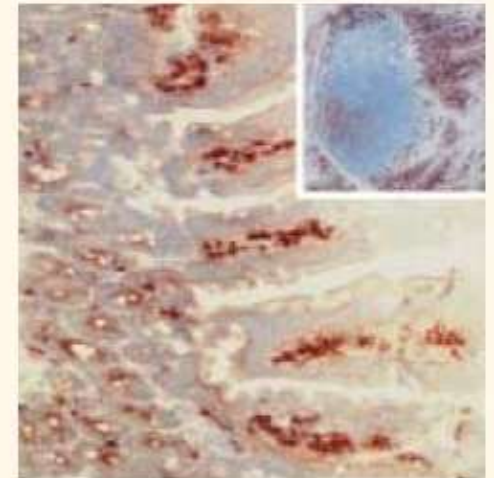
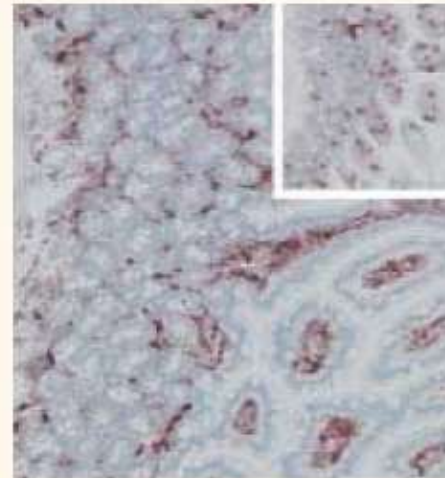
b Intestinal CD4
(inset: intestinal CD8)



c Intestinal IgA
(inset: Peyer's-patch IgA)



Mouse colonized
with intestinal bacteria



(Macpherson & Harris. Nature Reviews in Immunol 2004)

Η τελική διαφοροποίηση των βλεννογονικών λεμφοκυττάρων επηρεάζεται από την μικροχλωρίδα

segmentous filamentous bacteria *type 17 T helper (TH17) cells*

Clostridia

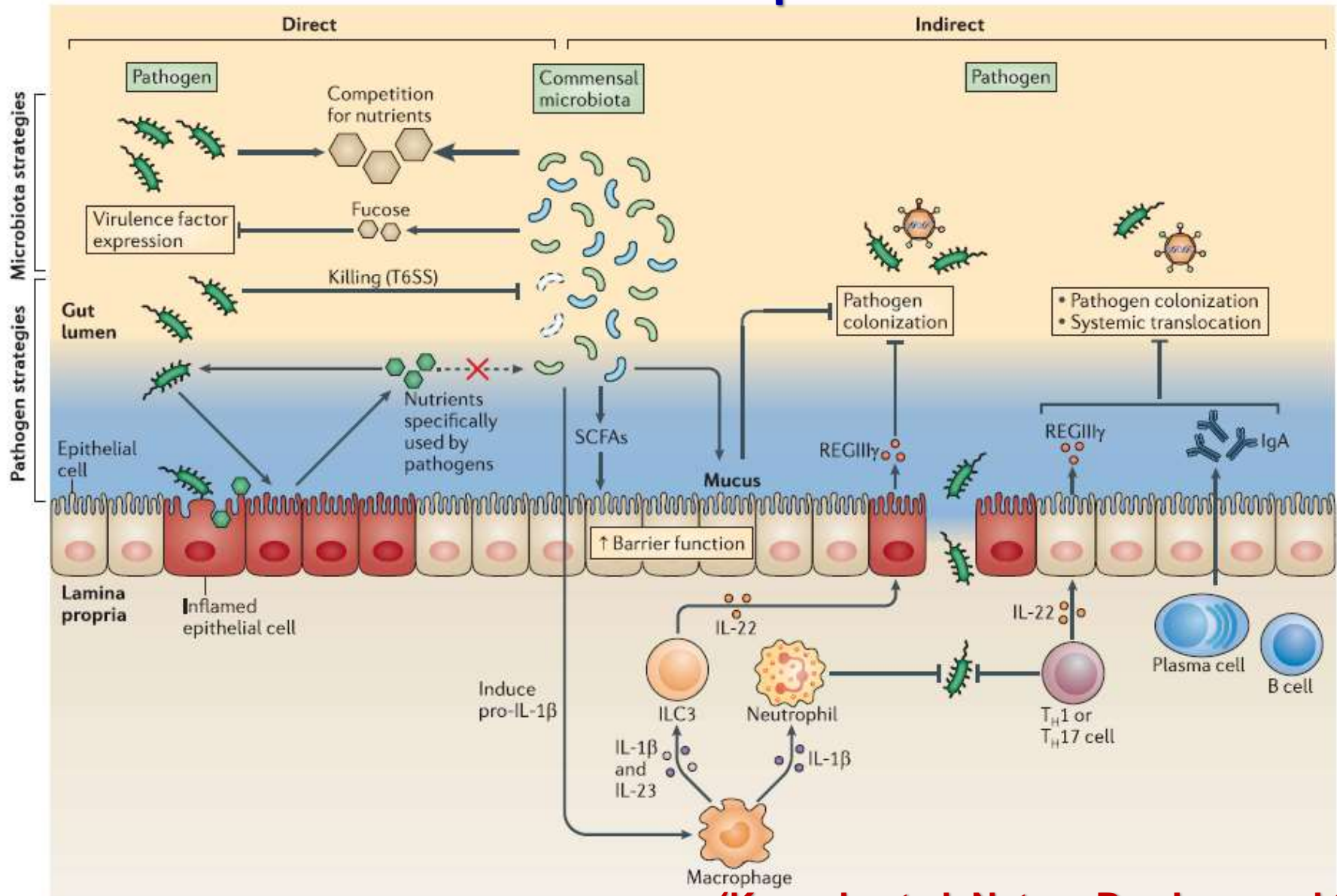
Treg cells

Bacteroides fragilis

type 1 T helper (TH1) cells

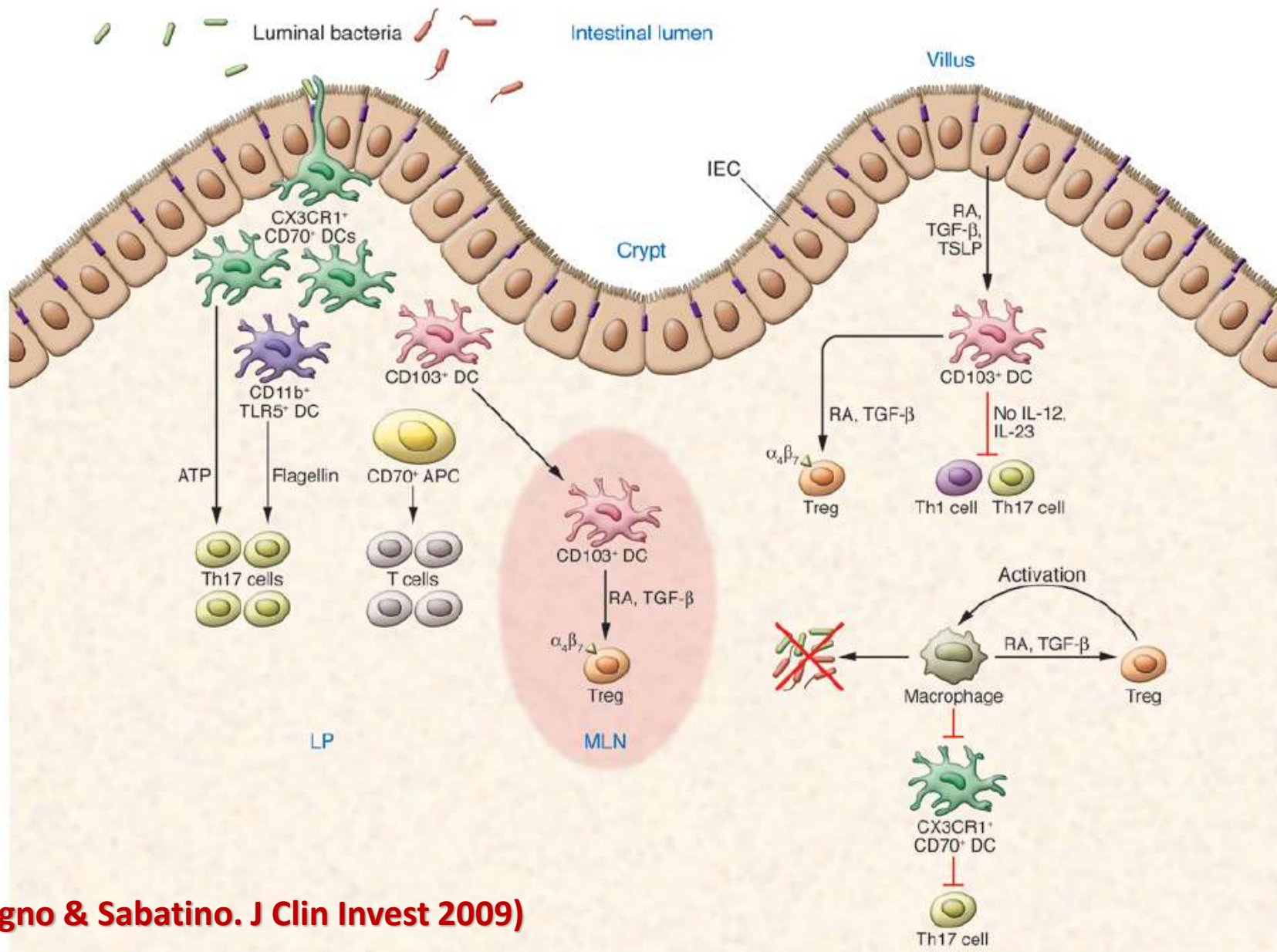
Hooper, L. V., *Science* 336, 1268–1273 (2012)

Έμμεση και άμεση συμβολή της χλωρίδας στην αντίσταση κατά των παθογόνων



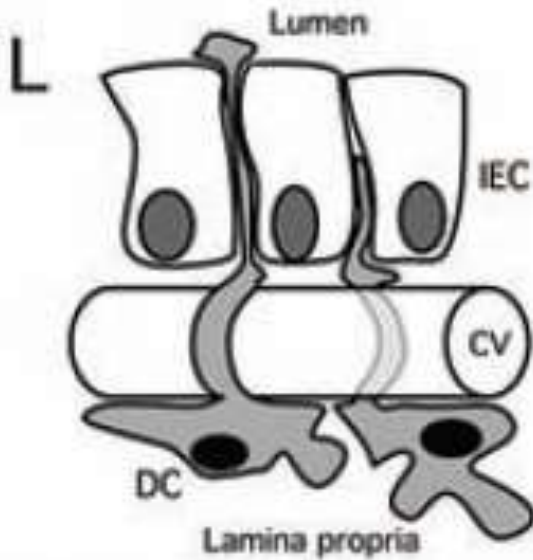
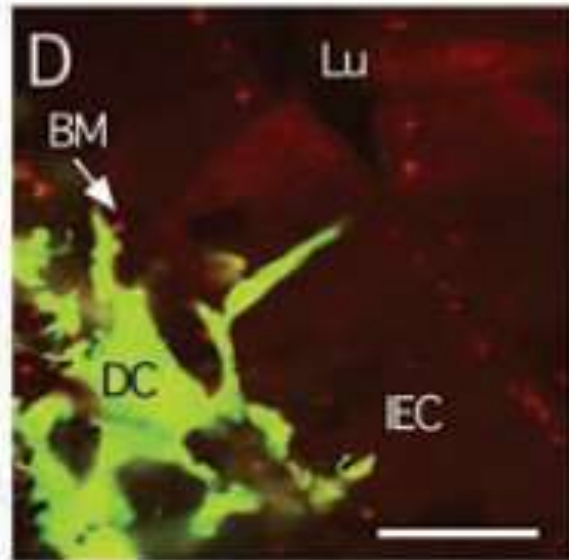
(Kamada et al. Nature Rev Immunol 2013)

Μηχανισμοί ανοχής

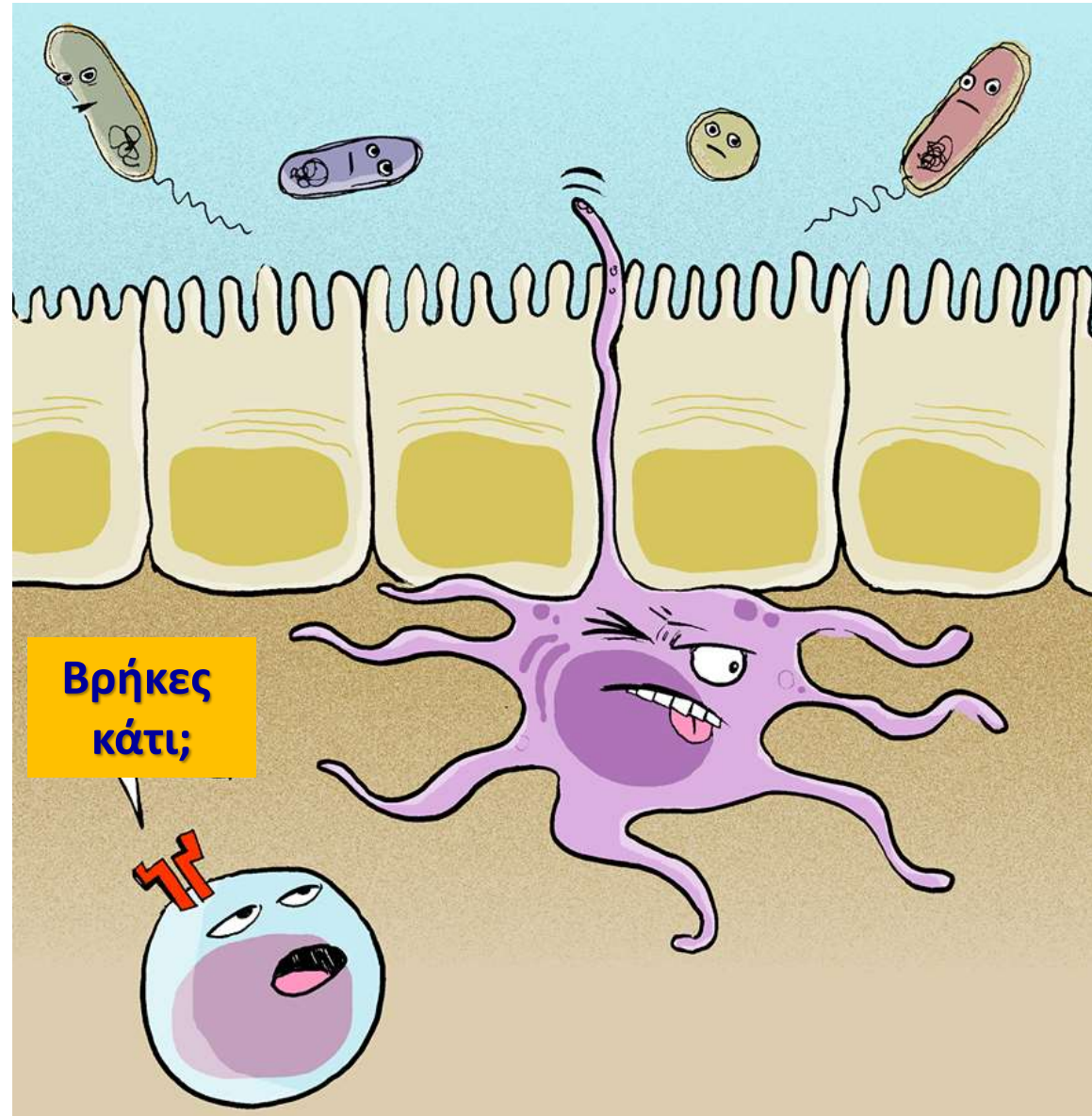


(Rescigno & Sabatino. J Clin Invest 2009)


Ανοχή (tolerance)



(Niess et al. Science 2005)



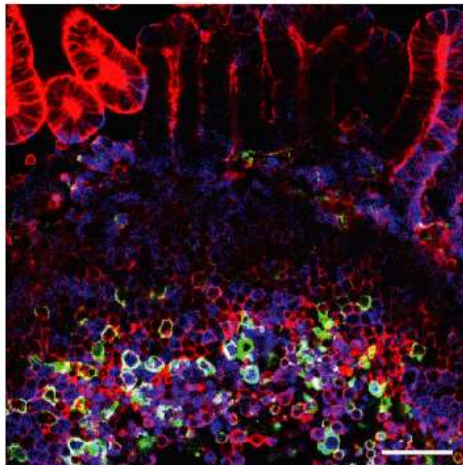
Identification of subepithelial mesenchymal cells that induce IgA and diversify gut microbiota

Kazuki Nagashima¹, Shinichiro Sawa^{1,2}, Takeshi Nitta¹, Masanori Tsutsumi¹, Tadashi Okamura^{3,4}, Josef M Penninger⁵ , Tomoki Nakashima⁶⁻⁸ & Hiroshi Takayanagi¹

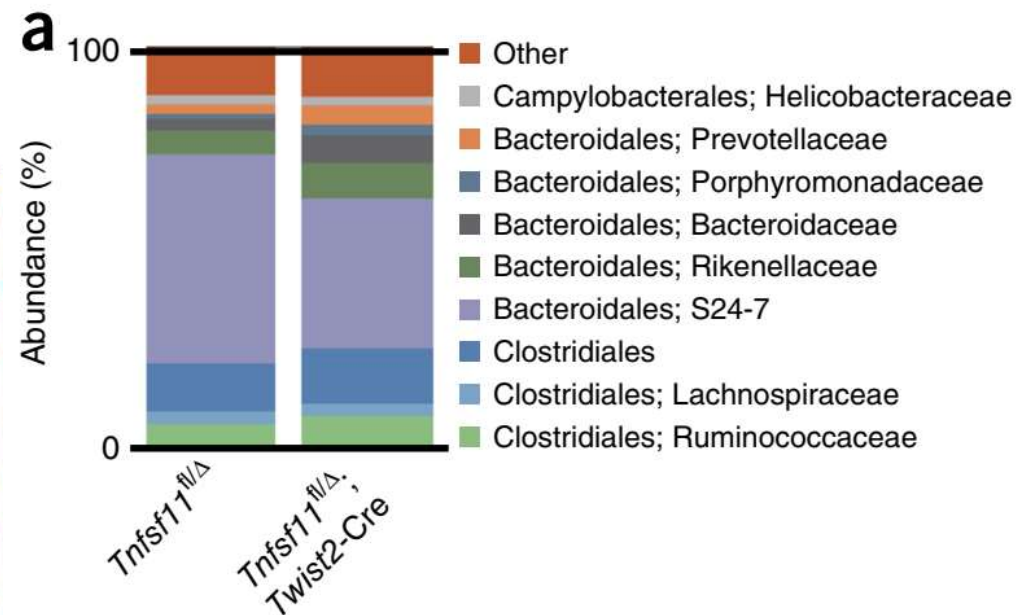
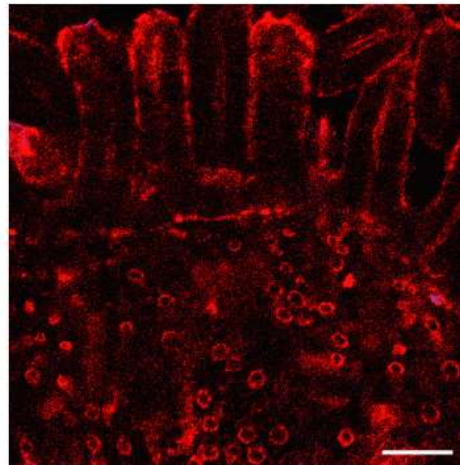
FAE of PP

Tnfsf11^{fl/Δ}

GP2 CCL20 Phalloidin



Tnfsf11^{fl/Δ}; *Twist2*-Cre



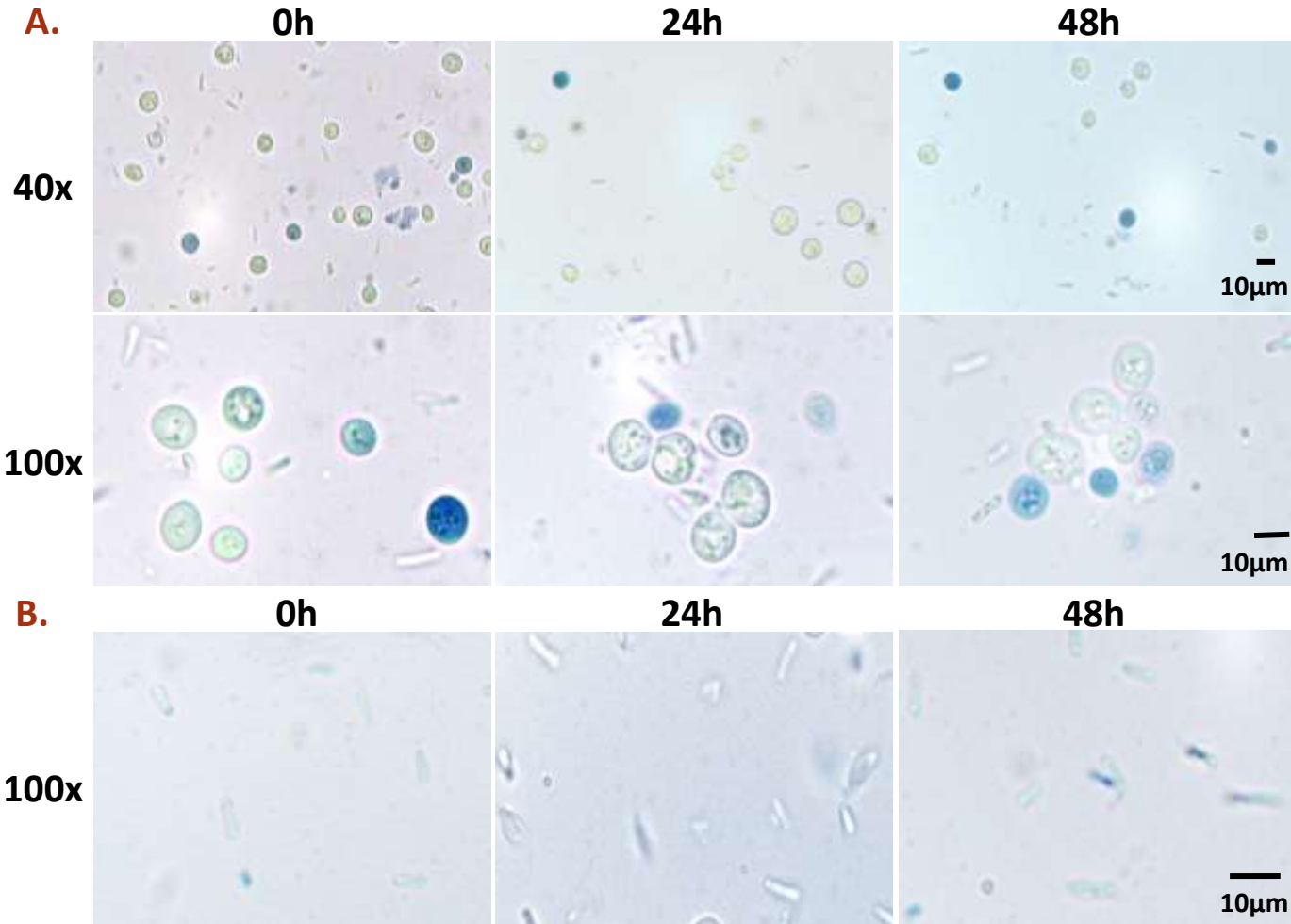
(Nature Immunology 2017)

Έλεγχος βιωσιμότητας με Trypan Blue

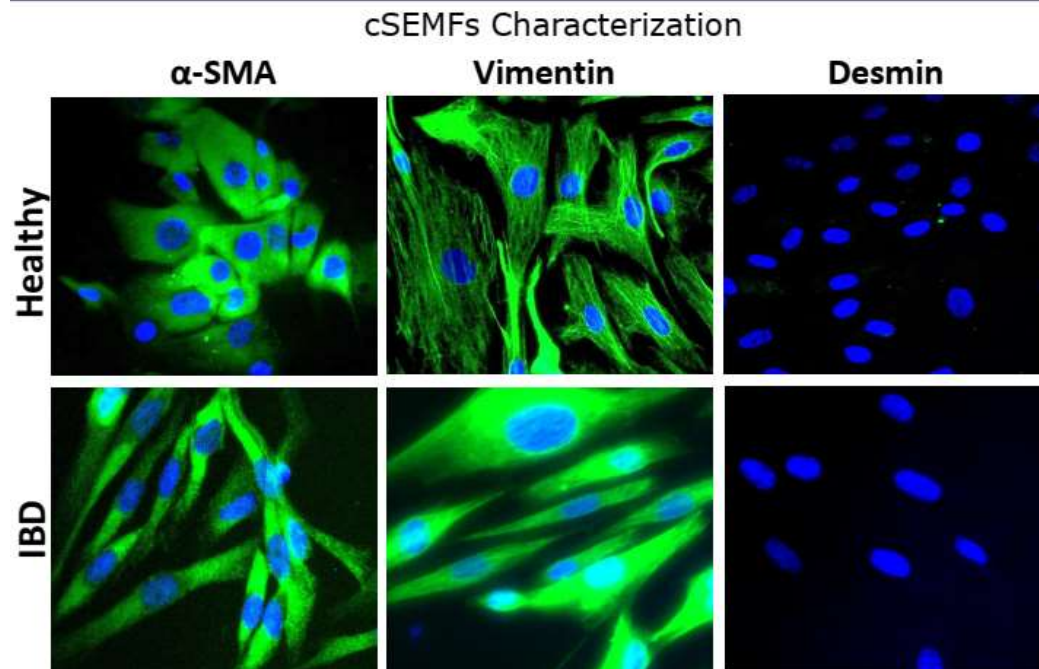
Σχεδόν όλα τα κύτταρα παρέμειναν ζωντανά, καθώς δεν βάφτηκαν με τη χρώση με Trypan Blue:

A. μείγμα των στελεχών *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium lactis*, *Saccharomyces boulardii*

B. *Lactobacillus plantarum*.



- **Methods** Primary SEMFs were isolated from endoscopically-obtained colonic biopsies from healthy individuals and were found **α -SMA** and **vimentin** positive and desmin negative by immunofluorescence.
- Healthy SEMFs were stimulated with 10^2 , 10^4 , 10^8 BU/ml of a mix of ***Lactobacillus Plantarum***, ***Saccharomyces Boulardii***, ***Bifidobacterium Lactis***, ***Lactobacillus Acidophilus*** for 6 or 48 hours. Total RNA was collected at 6h and mRNA transcripts for profibrotic factors were measured by reverse transcription quantitative PCR. At 48h, supernatants were collected, and total collagen protein secreted was measured by Sircol Assay.



(Unpublished data)

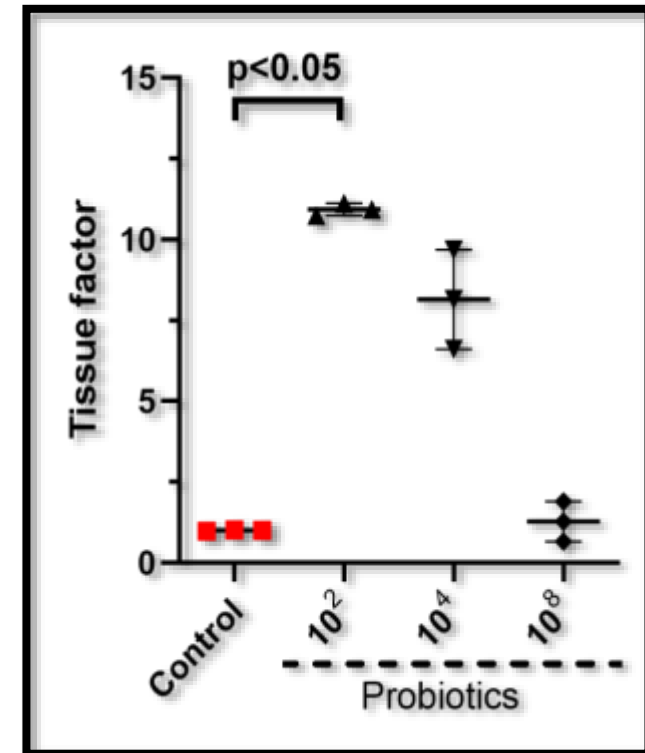
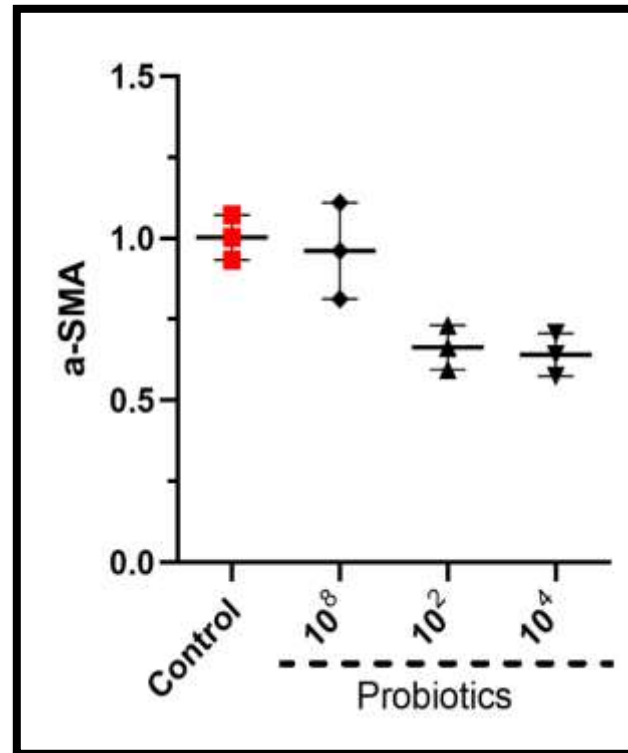
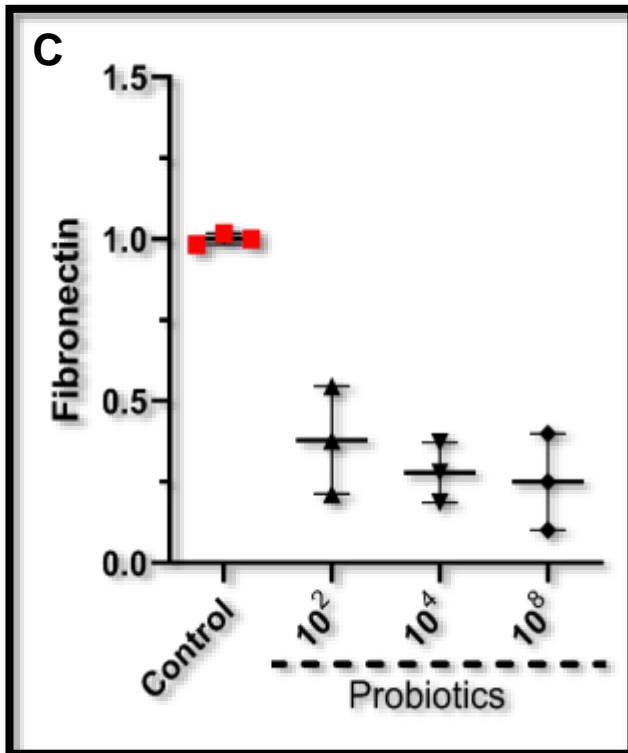
Fibronectin, tissue factor, α -Smooth Muscle Actin

The mix of *Lactobacillus Plantarum*, *Saccharomyces Boulardii*, *Bifidobacterium Lactis* and *Lactobacillus Acidophillus* resulted in **decreased**:

- mRNA Fibronectin and α -SMA expression (although not statistically significant)

And in **increased**:

- mRNA Tissue Factor expression (10^2 BU/ml: 2.717-fold, 10.74-11.12, $p < 0.05$)

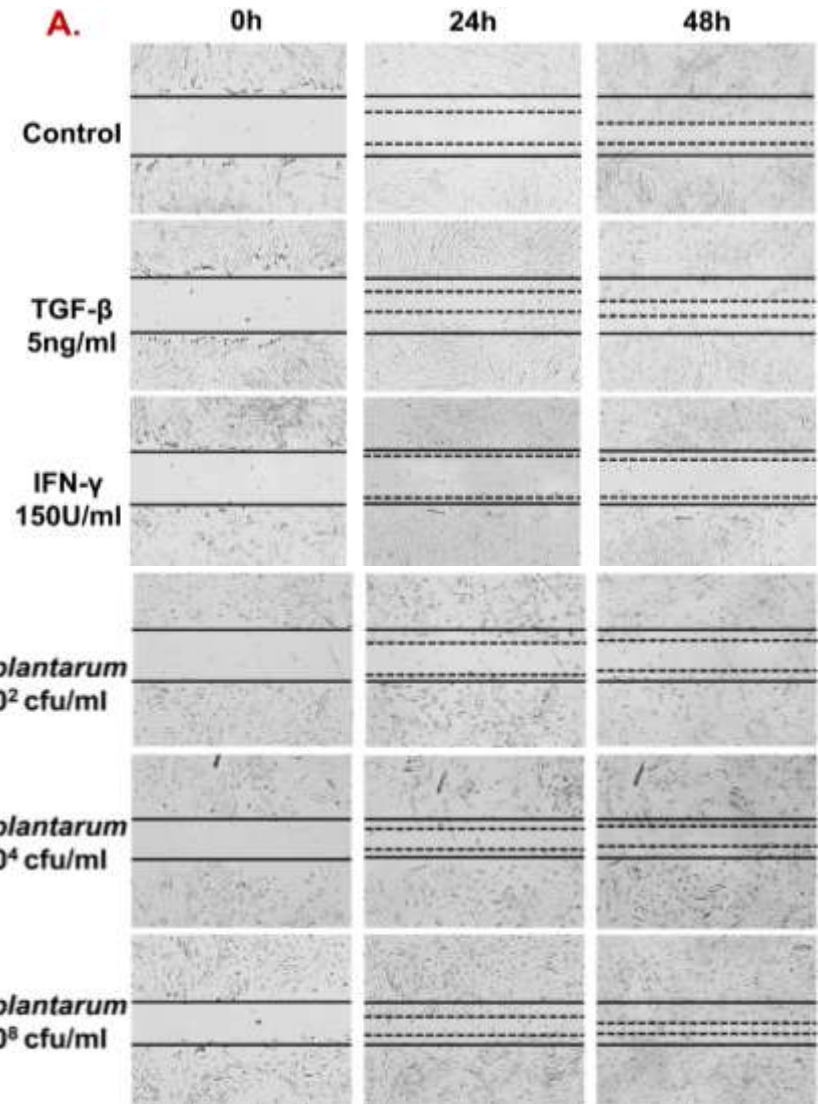
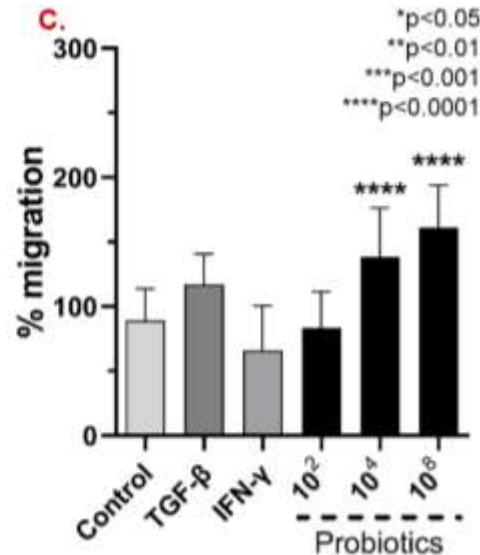
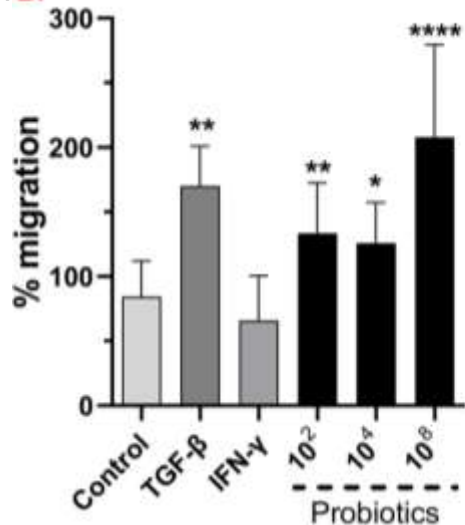


(Unpublished data)

Επίδραση του *L. plantarum* στο μεταναστευτικό ρυθμό των ΕΥΜ

Το μεμονωμένο στέλεχος *Lactobacillus plantarum* οδήγησε στη **δοσοεξαρτώμενη αύξηση** του ρυθμού μετανάστευσης των ΕΥΜ:

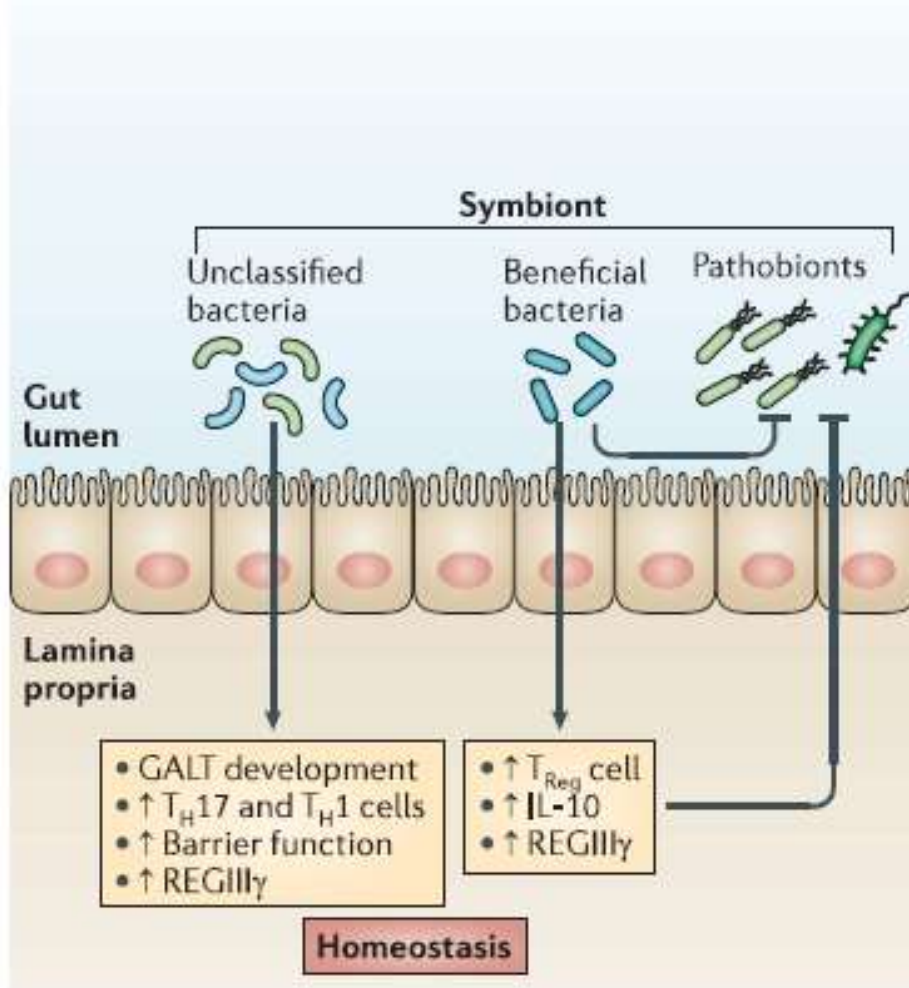
- **10² cfu/ml**: μετά από 24 ώρες κατά **128.30%**, IQR: 100.60 – 161.80, $p < 0.05$,
- **10⁴ cfu/ml**: μετά από 24 ώρες κατά **124.10%**, IQR: 96.09 – 156.10, $p < 0.05$ και μετά από 48 ώρες κατά **146.90%**, IQR: 101.80 – 175.20, $p < 0.0001$,
- **10⁸ cfu/ml**: μετά από 24 ώρες κατά **222.20%**, IQR: 153.10 – 271.00, $p < 0.0001$ και μετά από 48 ώρες κατά **162.70%**, IQR: 146.30 – 179.90, $p < 0.0001$.



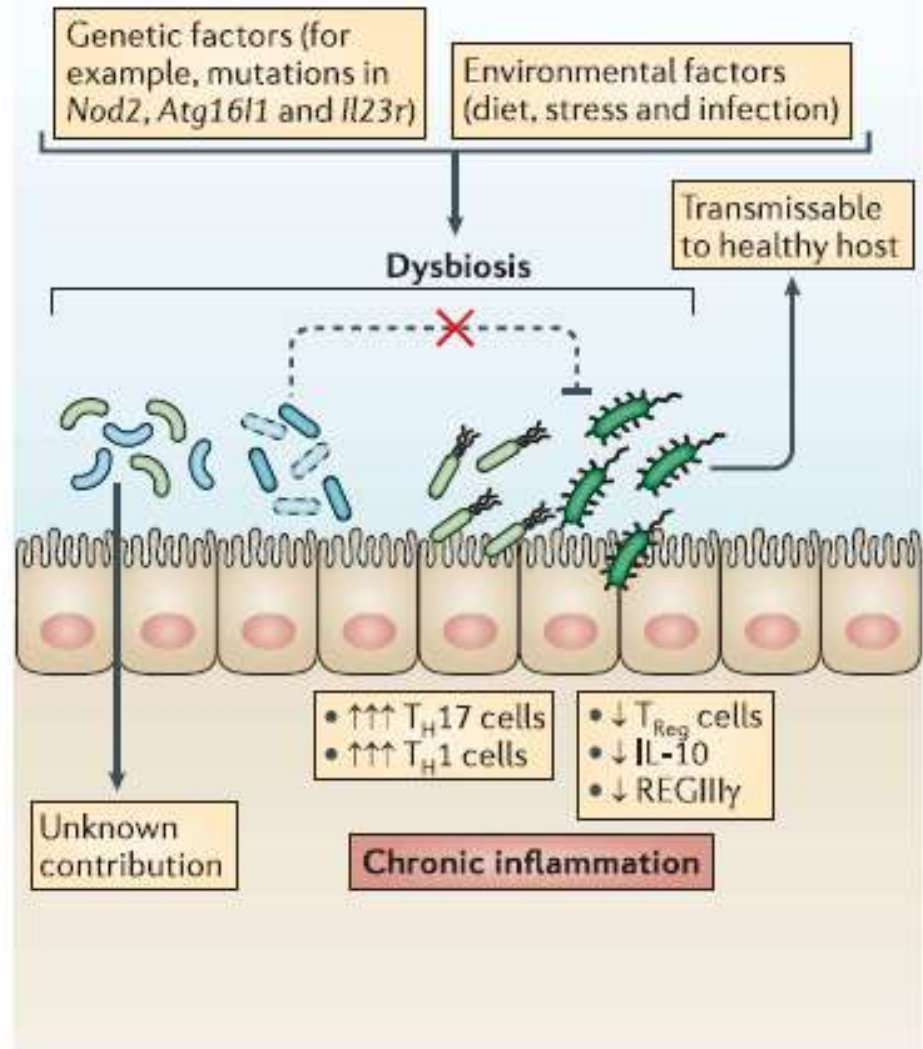
(Unpublished data)

Ευβίωση και δυσβίωση

Homeostasis



Inflammatory bowel disease

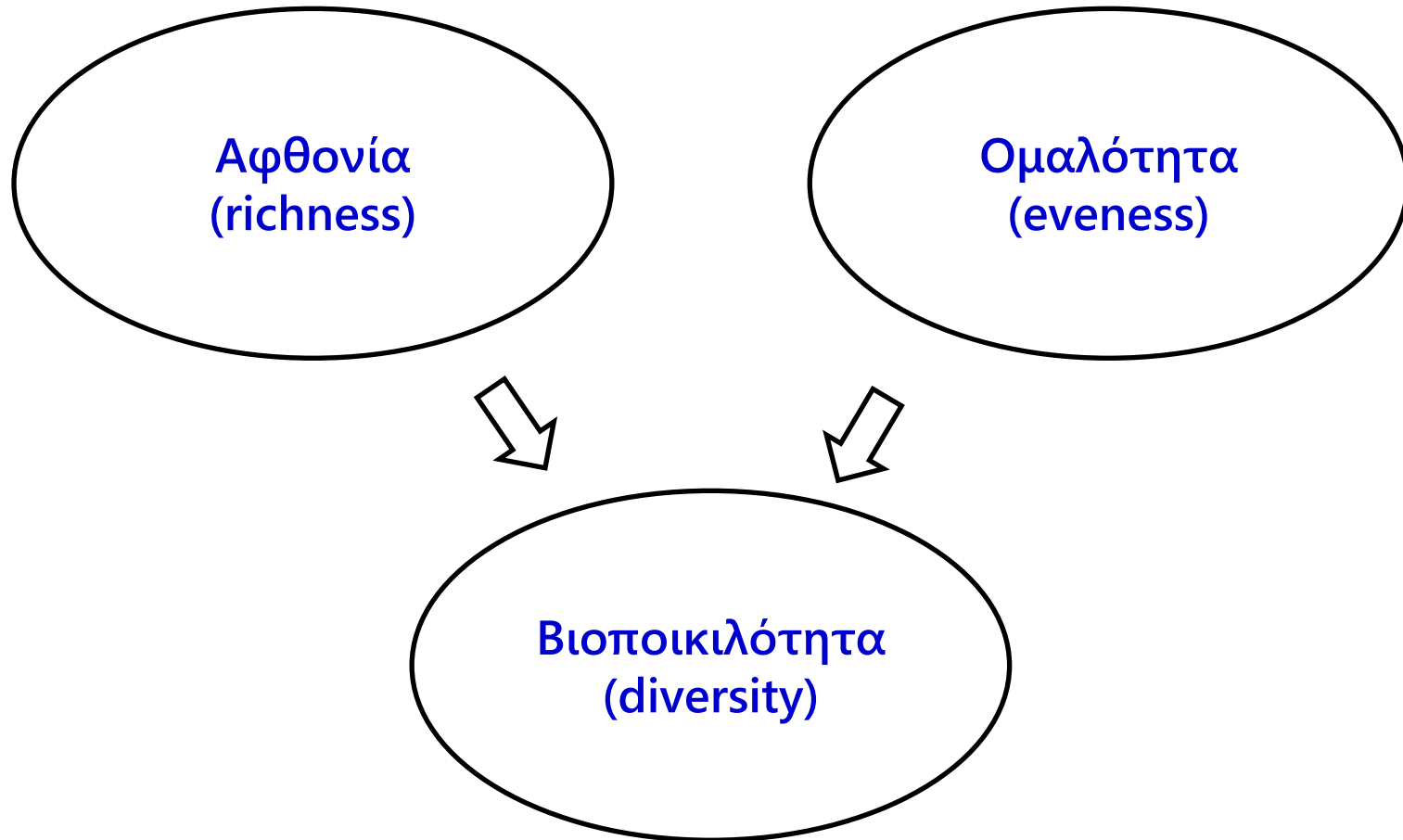


(Kamada et al. Nature Rev Immunol 2013)

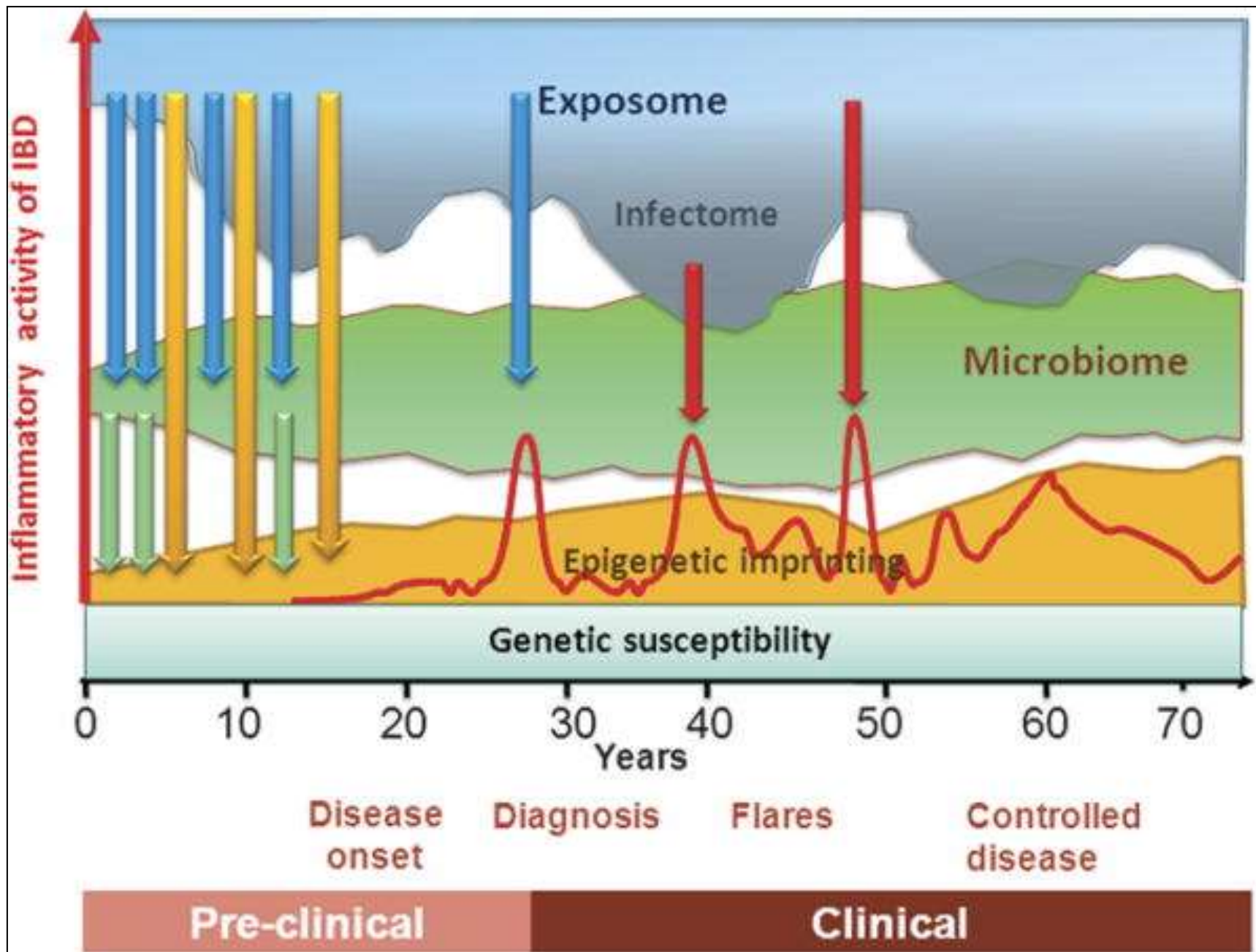
Τύποι δυσβίωσης

- Ⓢ Ελάττωση βιο-ποικιλότητας εντερικής χλωρίδας
- Ⓢ Μεταβολή της σχετικής σύστασης της χλωρίδας (αναλογική συμμετοχή φύλων)
- Ⓢ Υπερανάπτυξη δυνητικών παθογόνων/εξαφάνιση προστατευτικών μικροοργανισμών
- Ⓢ Μεταβολές στη λειτουργική σύνθεση της χλωρίδας
- Ⓢ Διαταραγμένη αναγνώριση της χλωρίδας από το βλεννογονικό ανοσολογικό σύστημα

Βασικά χαρακτηριστικά του μικροβιώματος



Εντερική χλωρίδα του ανθρώπου και περιβαλλοντικές επιδράσεις



(Rogler & Vavricka Inflamm Bowel Dis. 2015;21:400-408)

Εξωγενείς επιδράσεις στην εντερική χλωρίδα

- Δίαιτα
- Ηλικία
- Φάρμακα
- Κανόνες Υγιεινής
- Επάγγελμα
- Περιβάλλον
 - *E. Coli* serogroups
- Κινητικότητα του εντέρου
- Κύηση
- Φλεγμονές
- Ανοσολογικό σύστημα
- Χρόνια νοσήματα
 - Αλκοολισμός
 - Διαβήτης

Effects of Psychological, Environmental and Physical Stressors on the Gut Microbiota

J. Philip Karl^{1*}, Adrienne M. Hatch¹, Steven M. Arcidiacono², Sarah C. Pearce³,
Ida G. Pantoja-Feliciano², Laurel A. Doherty² and Jason W. Soares²

¹ Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA, United States,

Military training

Karl et al., 2017a

Psychological stress

Knowles et al., 2008

Circadian disruption

Benedict et al., 2016

Kato-Kataoka et al., 2016

High Altitude

Kleessen et al., 2005

Travelers' diarrhea

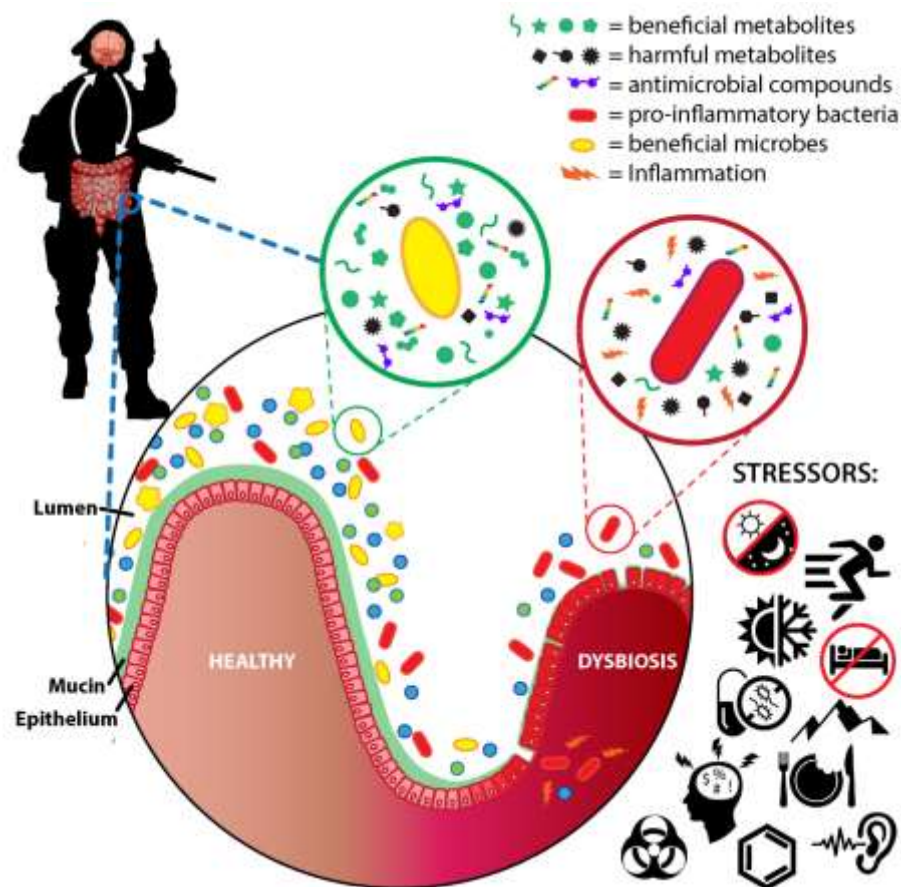
Kampmann et al., 2016

Noise

Diet

Food Restriction

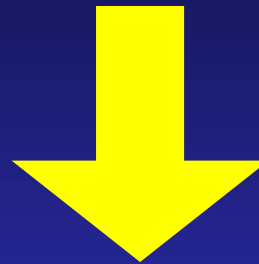
Diet Composition





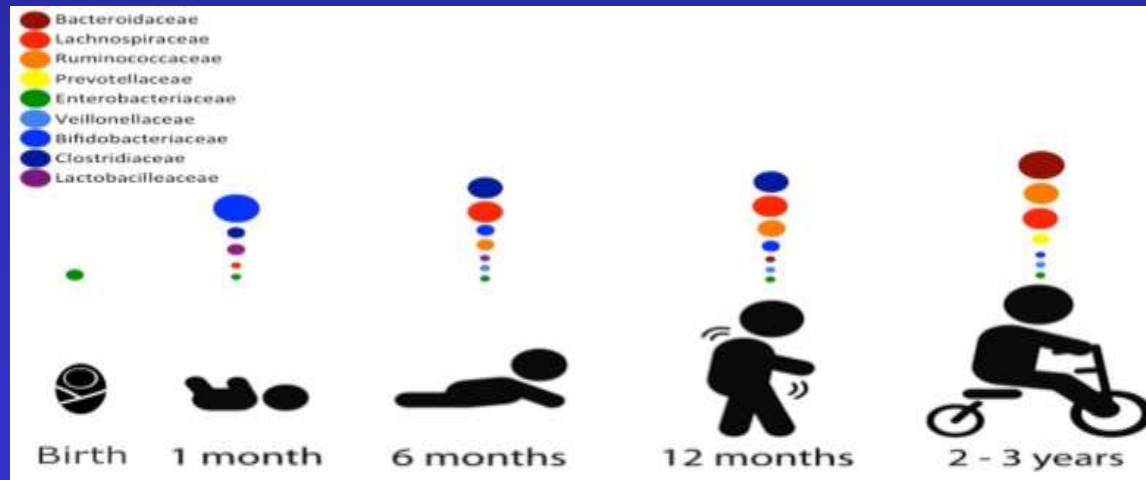
Γέννηση: το έντερο είναι “στείρο”

Εποικισμός από
βακτήρια



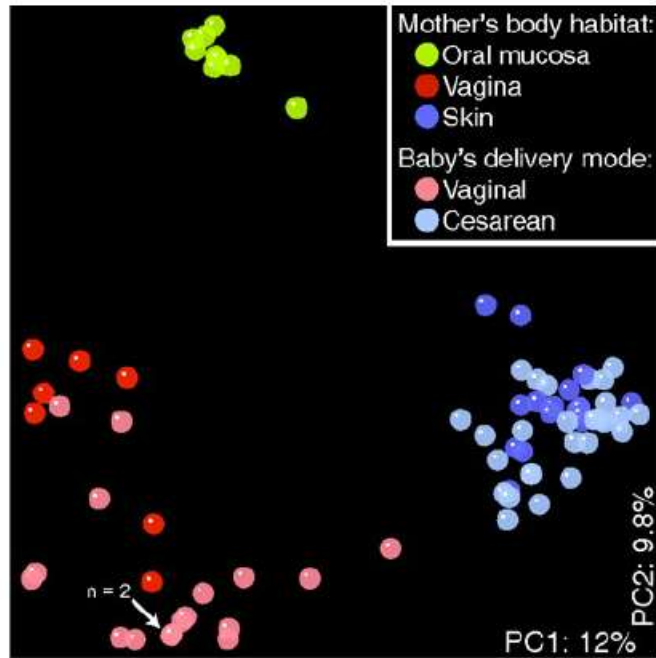
Περιβάλλον και
παράγοντες
ξενιστή

Απόκτηση χλωρίδας του ενηλίκου (2 έτη)



(Marie-Claire Arrieta Front Immunol. 2014; 5: 427)

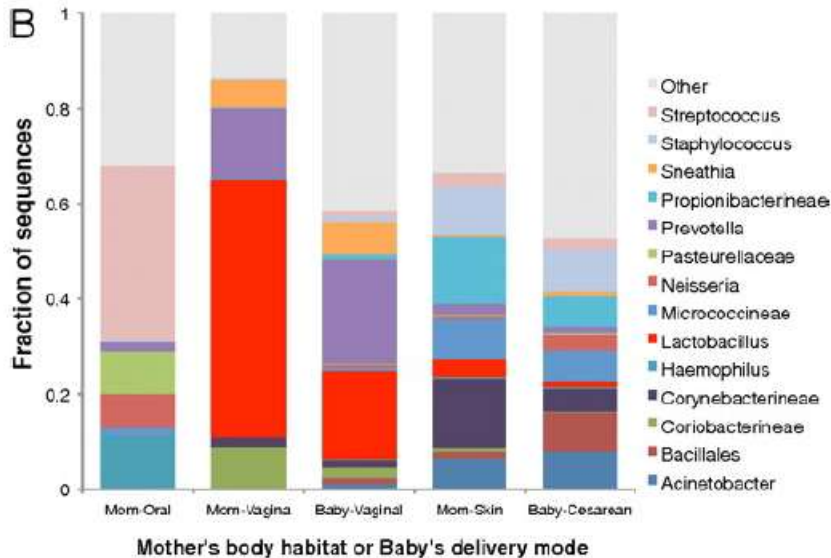
A



- Vaginal delivery:
 - ✓ intestinal colonization reflective of maternal vaginal flora
 - ✓ Lactobacillus
 - ✓ Prevotella

- Cesarean delivery:

- ✓ Epidermal colonization
- ✓ Clostridium
- ✓ Staphylococcus
- ✓ Propionibacterium
- ✓ Corynebacterium
- ✓ ↓ anaerobes: lower numbers of Bacteroides and Bifidobacterium



(Gritz & Bhandari. Front Pediatr. 2015)

Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

Maria G. Dominguez-Bello^{1,2,*}, Kassandra M. De Jesus-Laboy², Nan Shen⁸, Laura M. Cox¹, Amnon Amir^{3,7}, Antonio Gonzalez^{3,7}, Nicholas A. Bokulich¹, Se Jin Song^{3,4}, Marina Hoashi⁵, Juana I. Rivera-Vina⁶, Keimari Mendez⁶, Rob Knight^{3,7}, and Jose C. Clemente^{8,9,*}

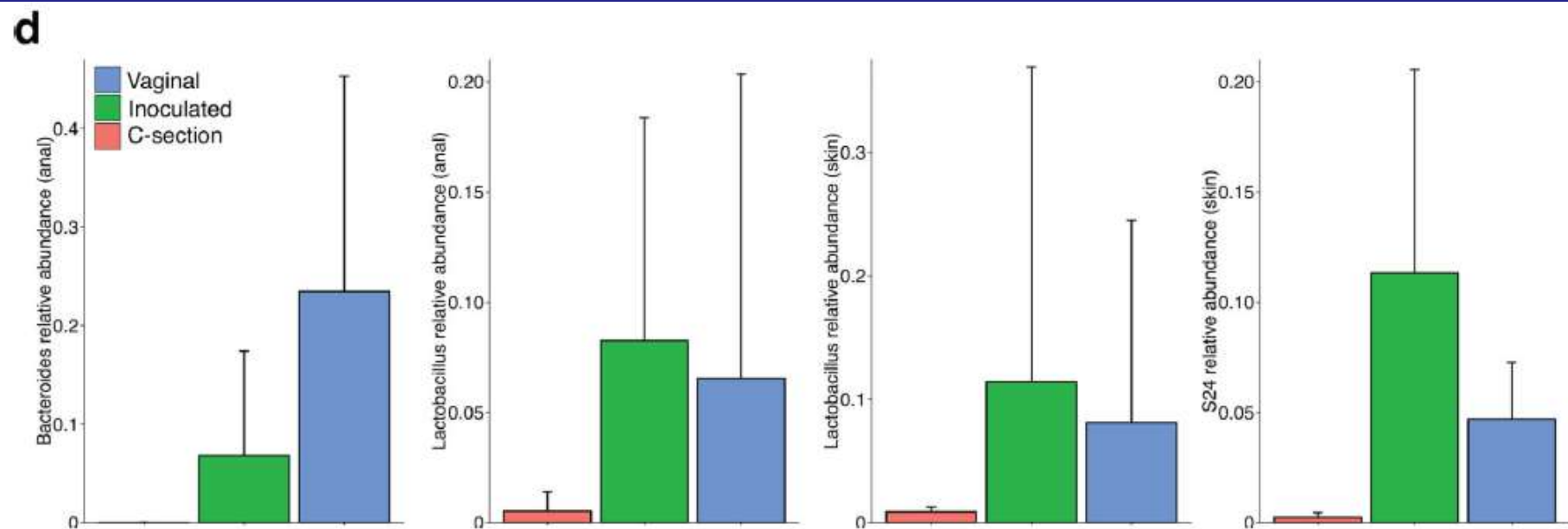


Figure 1. Restoring the maternal microbiota in infants born by C-section

Μητρικό γάλα

Praveen et al. *Microbiome* (2015) 3:41
DOI 10.1186/s40168-015-0104-7



RESEARCH

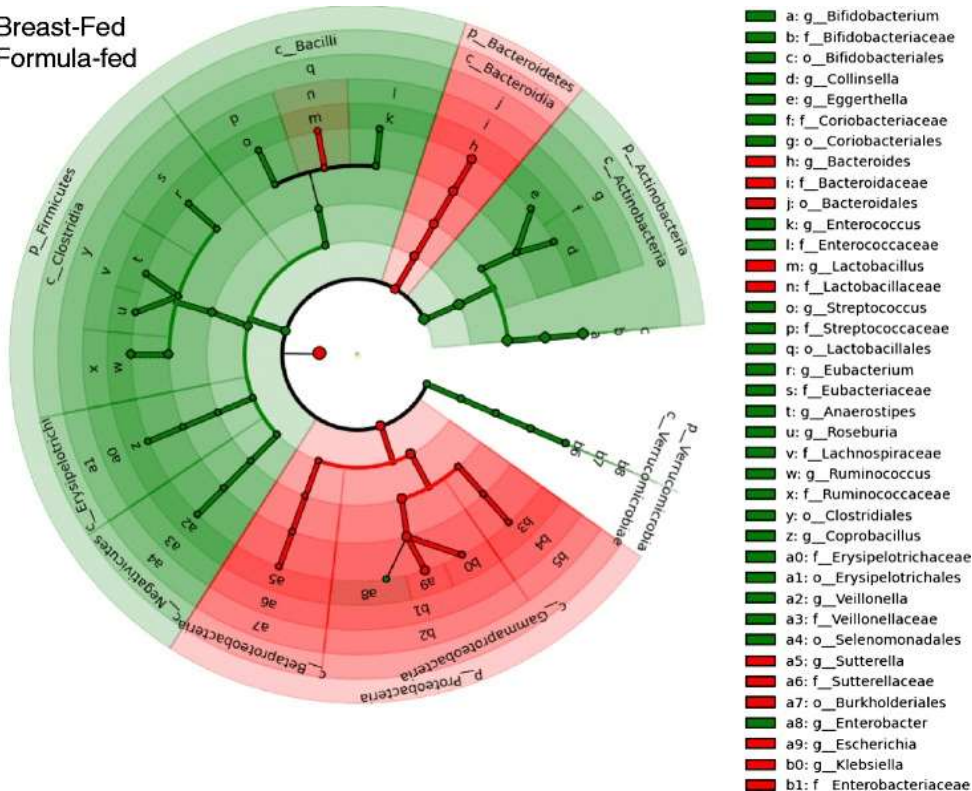
Open Access



The role of breast-feeding in infant immune system: a systems perspective on the intestinal microbiome

Paurush Praveen^{1*}, Ferenc Jordan¹, Corrado Priami^{1,2} and Melissa J. Morine^{1,2}

■ Breast-Fed
■ Formula-fed

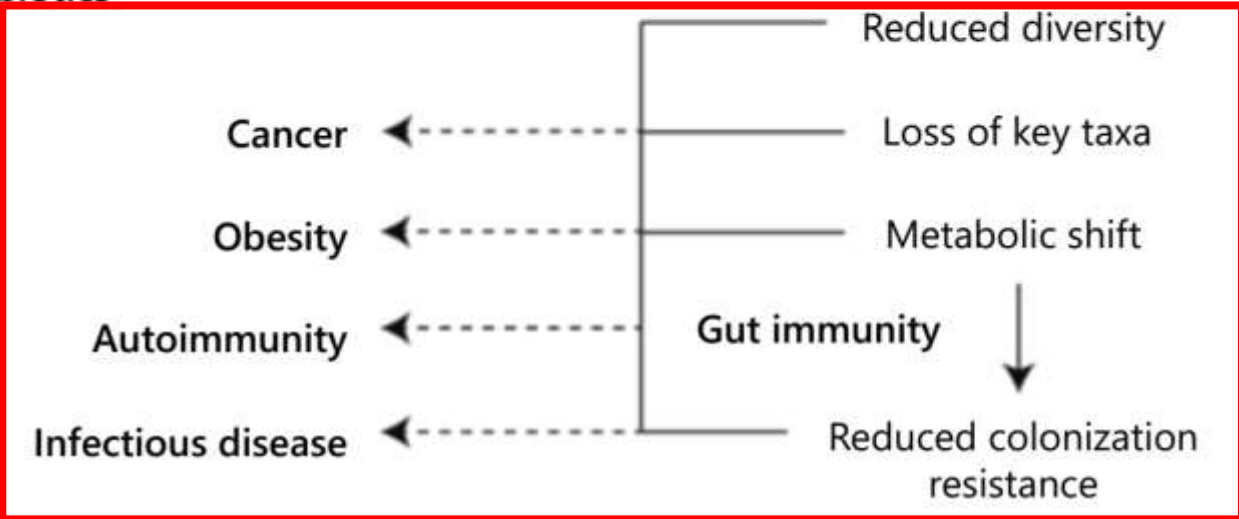
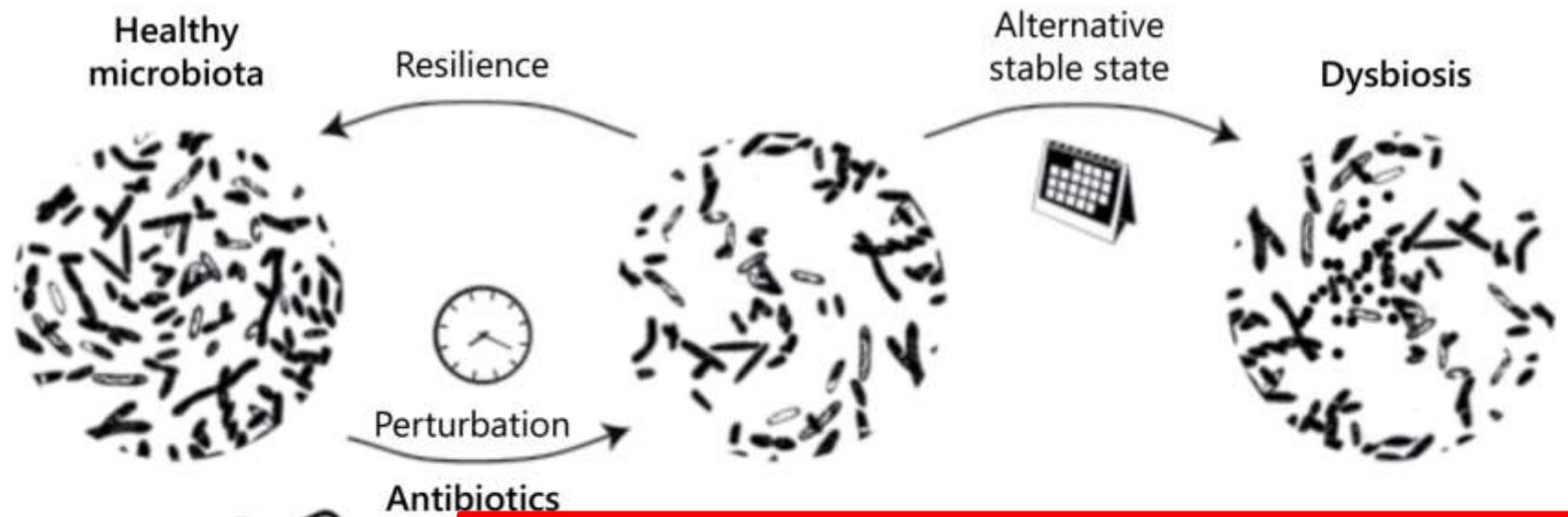


Conclusions: Our findings revealed that there is co-expression of more genes in breast-fed samples but lower microbial diversity compared to formula-fed.

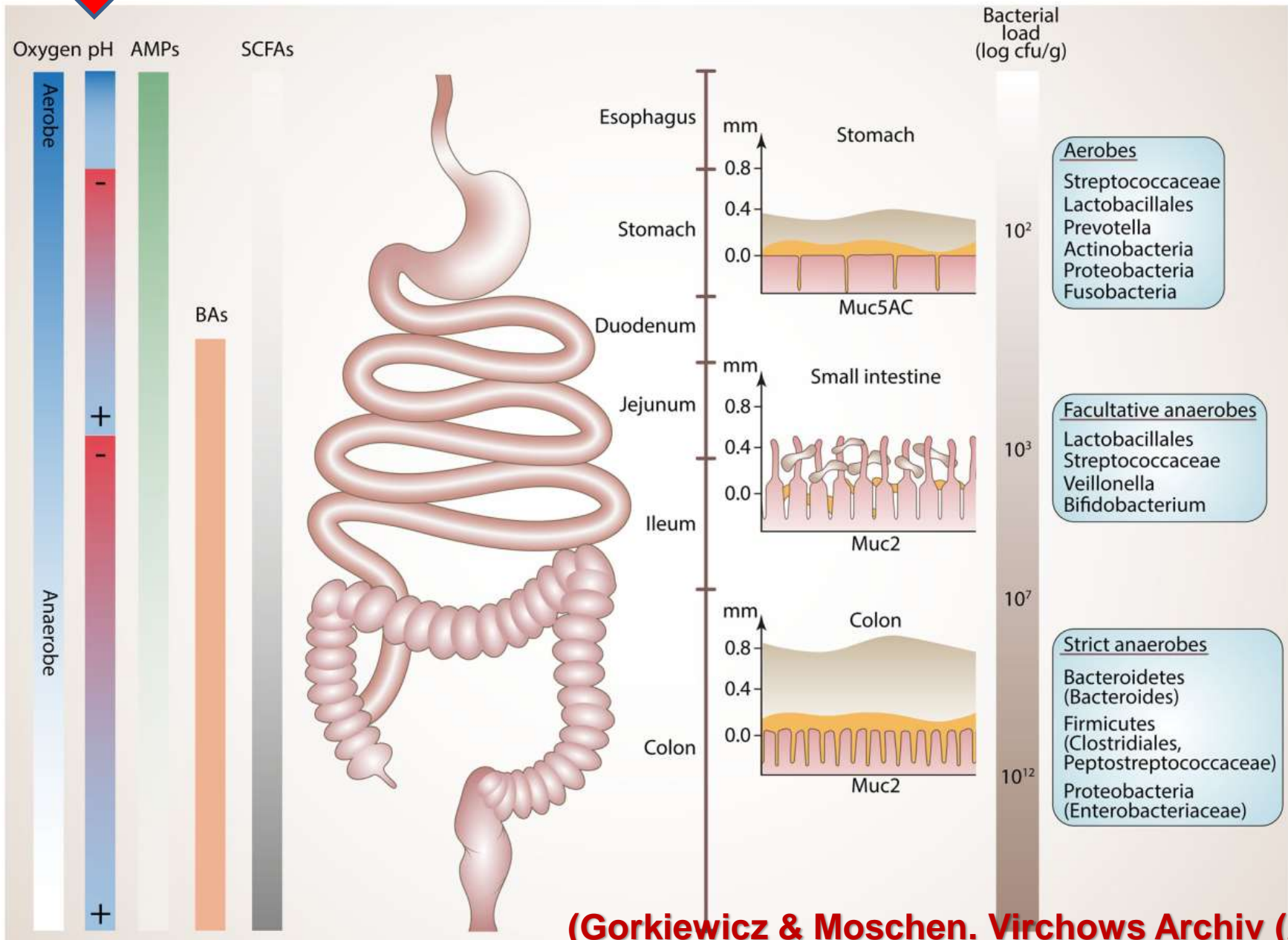
Effects of Antibiotics on Gut Microbiota

Kathleen Lange^a Martin Buerger^a Andreas Stallmach^{a,b} Tony Bruns^a, **Dig Dis 2016;34:260–268**

^aDepartment of Internal Medicine IV (Gastroenterology, Hepatology, and Infectious Diseases), and ^bThe Integrated Research and Treatment Center for Sepsis Control and Care (CSCC), Jena University Hospital, Jena, Germany



Stomach microflora



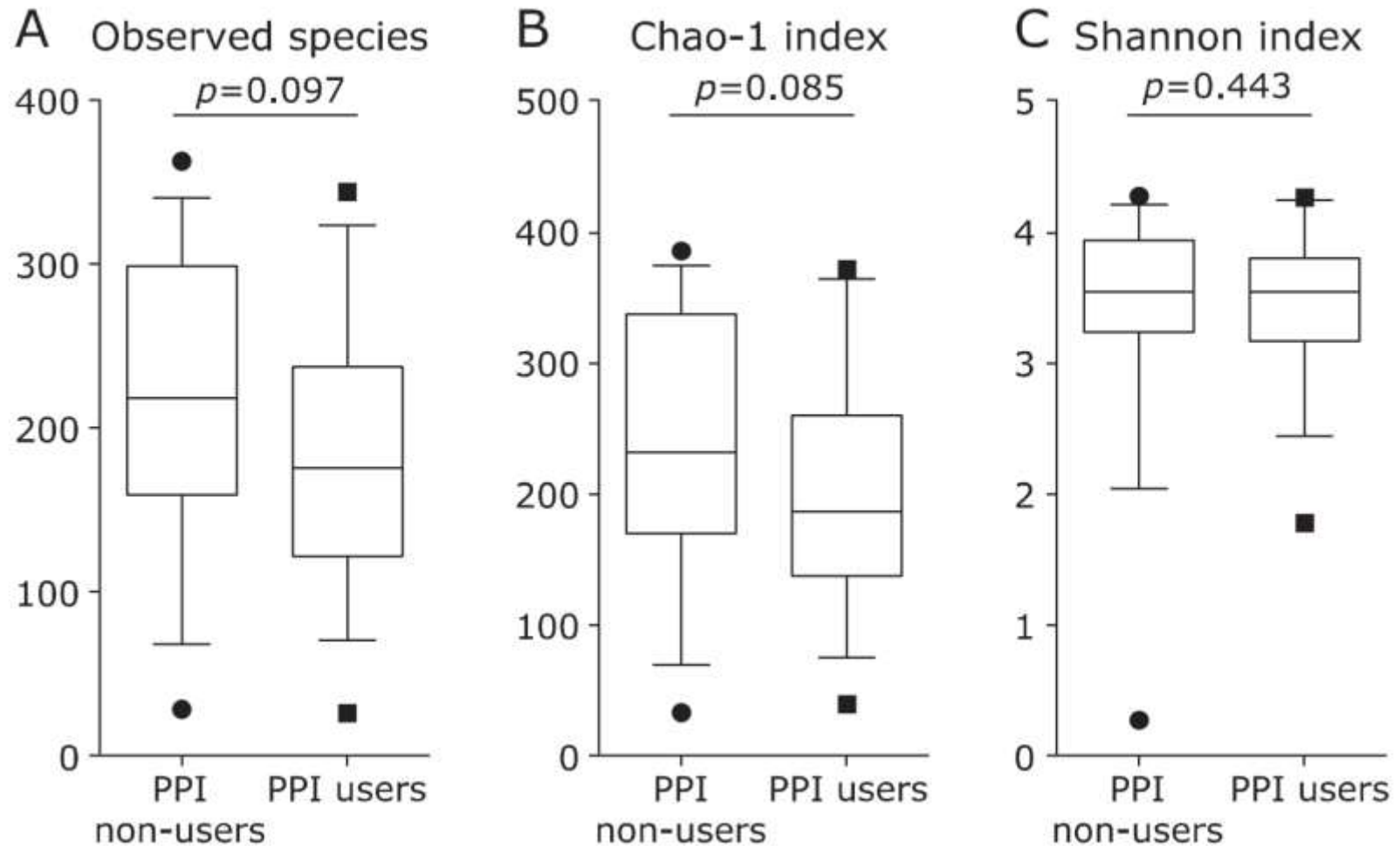
Proton pump inhibitors

Adverse drug reactions

- Τα τελευταία χρόνια, μελέτες έχουν δείξει ότι η εντερική χλωρίδα ασθενών που κάνουν χρήση PPIs εμφανίζει σημαντική μείωση της βιοποικιλότητας της, με σαφή τροποποίηση ορισμένων τάξεων μικροοργανισμών.
- Η αλλαγή αυτή φαίνεται να σχετίζεται με αυξημένο κίνδυνο διαταραχών του πεπτικού, όπως λοιμώξεις με κλωστηρίδιο Difficile.

**(Freedberg et al. Clin Lab Med 2014;
Seto et al. Microbiome 2014;
Clooney et al. Aliment Pharmacol Ther 2016;
Jackson et al. Gut 2016)**

α -Diversity Indices



Δίαιτα

- Η τροφή είναι ο σύνδεσμος της εντερικής χλωρίδας με το περιβάλλον
- Η διατροφή είναι κυρίαρχος παράγοντας, που επηρεάζει τη σύνθεση και τη δραστηριότητα της εντερικής χλωρίδας.
- Τόσο η βιοποικιλότητα όσο και οι μεταβολικές αποδόσεις της εντερικής χλωρίδας μεταβάλλονται μέσα σε λίγες ημέρες μετά από αλλαγές της δίαιτας, ένα αποτέλεσμα που πιστεύεται ότι συμβαίνει μέσω πολλαπλών αλληλένδετων οδών.

(David et al Nature 2014; Read and Holmes, Front Immunol. 2017)

Diet rapidly and reproducibly alters the human gut microbiome

Lawrence A. David^{1,2,#}, Corinne F. Maurice¹, Rachel N. Carmody¹, David B. Gootenberg¹, Julie E. Button¹, Benjamin E. Wolfe¹, Alisha V. Ling³, A. Sloan Devlin⁴, Yug Varma⁴, Michael A. Fischbach⁴, Sudha B. Biddinger³, Rachel J. Dutton¹, and Peter J. Turnbaugh^{1,*}

¹FAS Center for Systems Biology, Harvard University, Cambridge, MA, 02138, USA.

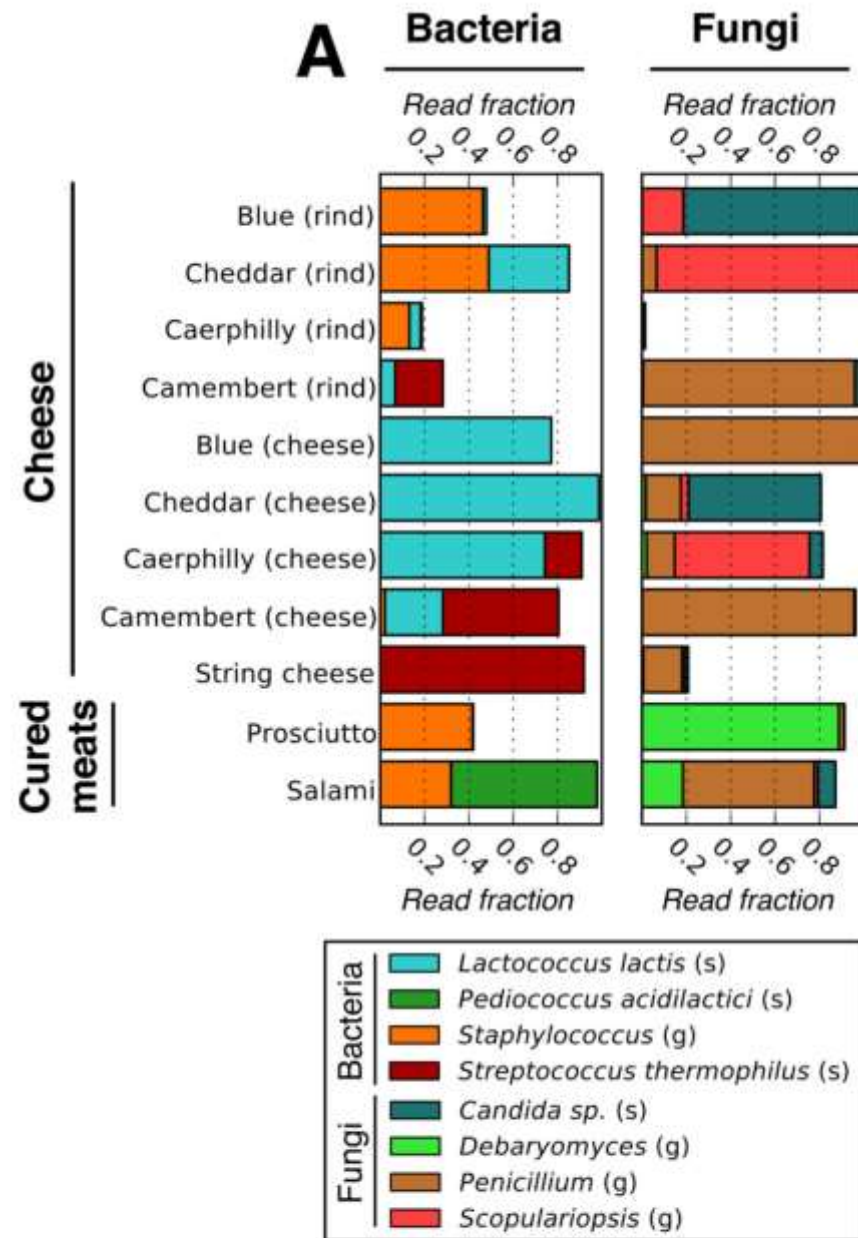
²Society of Fellows, Harvard University, Cambridge, MA, 02138, USA.

³Division of Endocrinology, Children's Hospital Boston, Harvard Medical School, Boston, MA, 02115, USA.

⁴Department of Bioengineering & Therapeutic Sciences and the California Institute for Quantitative Biosciences, University of California, San Francisco, San Francisco, CA, 94158, USA.

Abstract

Long-term diet influences the structure and activity of the trillions of microorganisms residing in the human gut^{1–5}, but it remains unclear how rapidly and reproducibly the human gut microbiome responds to short-term macronutrient change. **Here, we show that the short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and overwhelms inter-individual differences in microbial gene expression.** The animal-based diet increased the abundance of bile-tolerant microorganisms (*Alistipes*, *Bilophila*, and *Bacteroides*) and decreased the levels of Firmicutes that metabolize dietary plant polysaccharides (*Roseburia*, *Eubacterium rectale*, and *Ruminococcus bromii*). Microbial activity mirrored differences between



Colonization of the human gut by bovine bacteria present in Parmesan cheese

Christian Milani¹, Sabrina Duranti¹, Stefania Napoli², Giulia Alessandri³, Leonardo Mancabelli²,

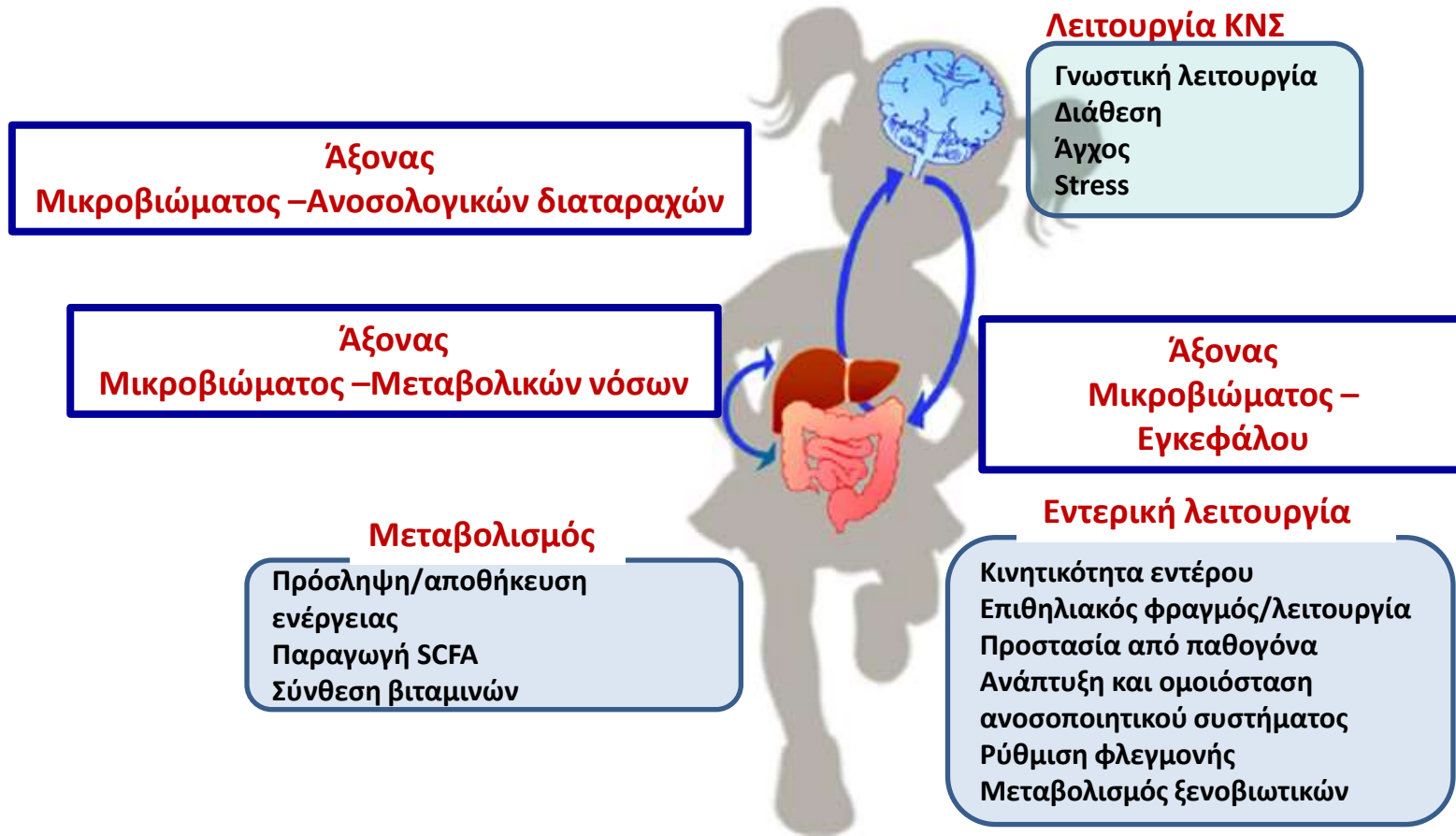
study showing that *Bifidobacterium mongoliense* strains from cheese can transiently colonize the human gut, a process that can be enhanced by cow milk consumption.

	Stool	Milk	Litter	Cheese	
<i>Atopostipes suicloacalis</i>	Red	Red	Purple	Purple	P2
<i>Corynebacterium stationis</i>	Red	Red	Blue	Blue	P1
<i>Corynebacterium variable</i>	Black	Red	Purple	Blue	P1
<i>Jeotgallicoccus psychrophilus</i>	Red	Red	Blue	Purple	P2
<i>Kocuria kristinae</i>	Red	Red	Black	Red	P3
<i>Lactobacillus delbrueckii</i>	Red	Black	Purple	Blue	P2
<i>Lactobacillus helveticus</i>	Black	Red	Blue	Blue	P2
<i>Oligella ureolytica</i>	Red	Red	Blue	Purple	P2
<i>Paraprevotella clara</i>	Red	Red	Black	Black	RE1
<i>Prevotella ruminicola</i>	Red	Red	Red	Red	RE1
<i>Pseudoclavibacter soli</i>	Black	Black	Blue	Blue	P1
<i>Pseudoflavonifractor capillosus</i>	Red	Black	Red	Black	P3
<i>Streptococcus thermophilus</i>	Black	Red	Blue	Blue	P2
<i>Treponema porcinum</i>	Red	Black	Red	Black	P3
<i>Bifidobacterium mongoliense</i>	Red	Black	Blue	Blue	P1



Εντερική χλωρίδα και νοσολογία

Μικροβίωμα – Ο ρόλος του στην υγεία?



(American Society for Nutrition Adv. Nutr. 2016;7:323-330)

Disease associations with the intestinal microbial community (examples).

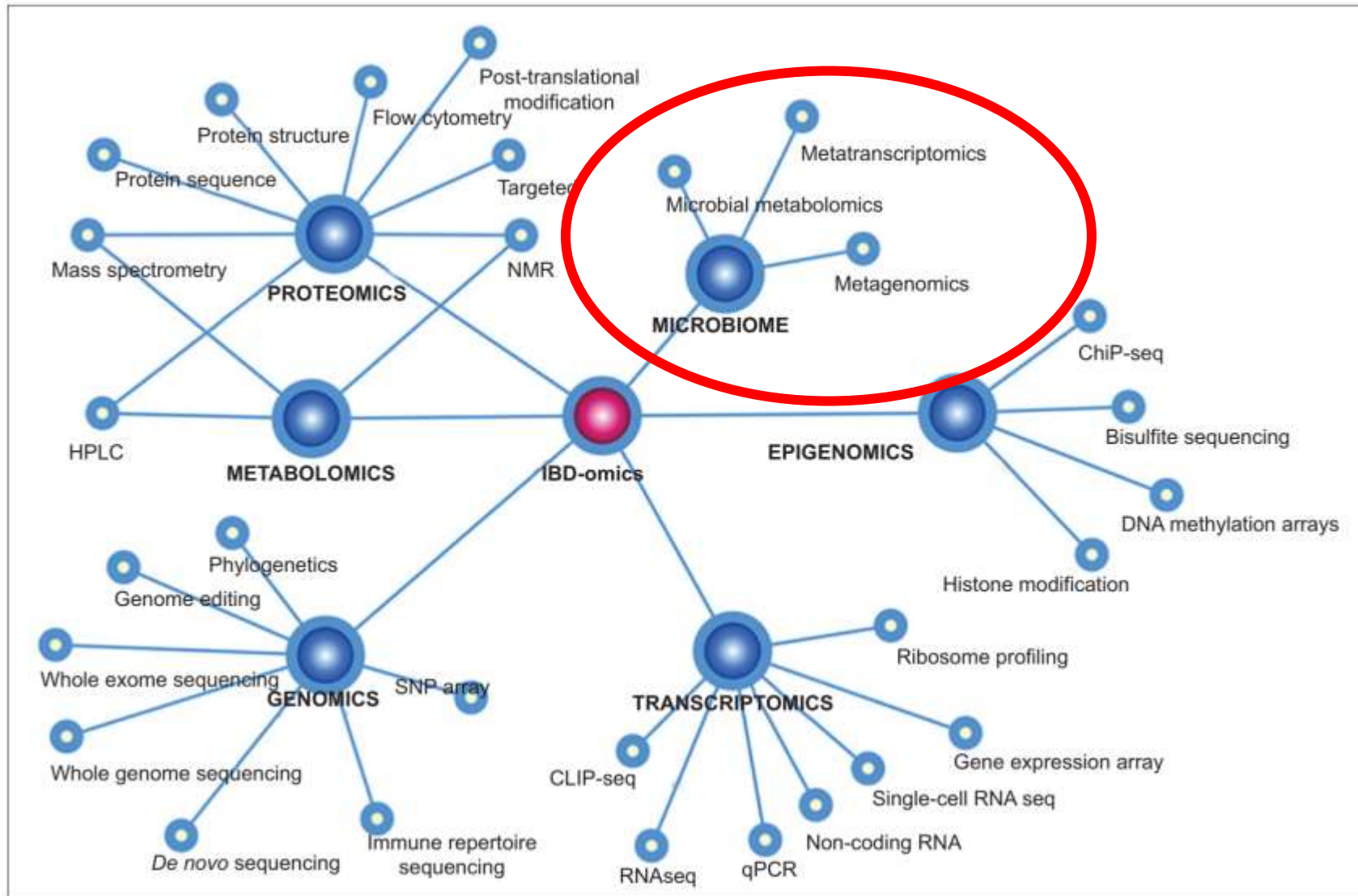
	References
Allergies/Allergy protection	[23,59,64–66]
Atherosclerosis/Thrombosis/Cardiovascular Diseases	[46–48,67–70]
Cancer	[40,71,72]
Diabetes mellitus	[42,73]
Immune-Mediated Inflammatory Diseases	
–Inflammatory bowel diseases	[26,27,30,31,74,75]
–Multiple sclerosis	[76,77]
–Rheumatoid arthritis	[78]
–Psoriasis	[79]
Kwashiorkor	[43,44]
Liver Diseases	[80,81]
Metabolic Syndrome/Obesity	[41,82–85]
Neurodevelopmental, Psychiatric and Neurodegenerative diseases	
–Autism	[49,86]
–Depression	[49,87]
–Alzheimer’s Disease, Parkinson’s Disease	[49,53,54,88,89]

Systems biology in inflammatory bowel diseases: on the way to precision medicine

Nikolas Dovrolis, Eirini Filidou, George Kolios

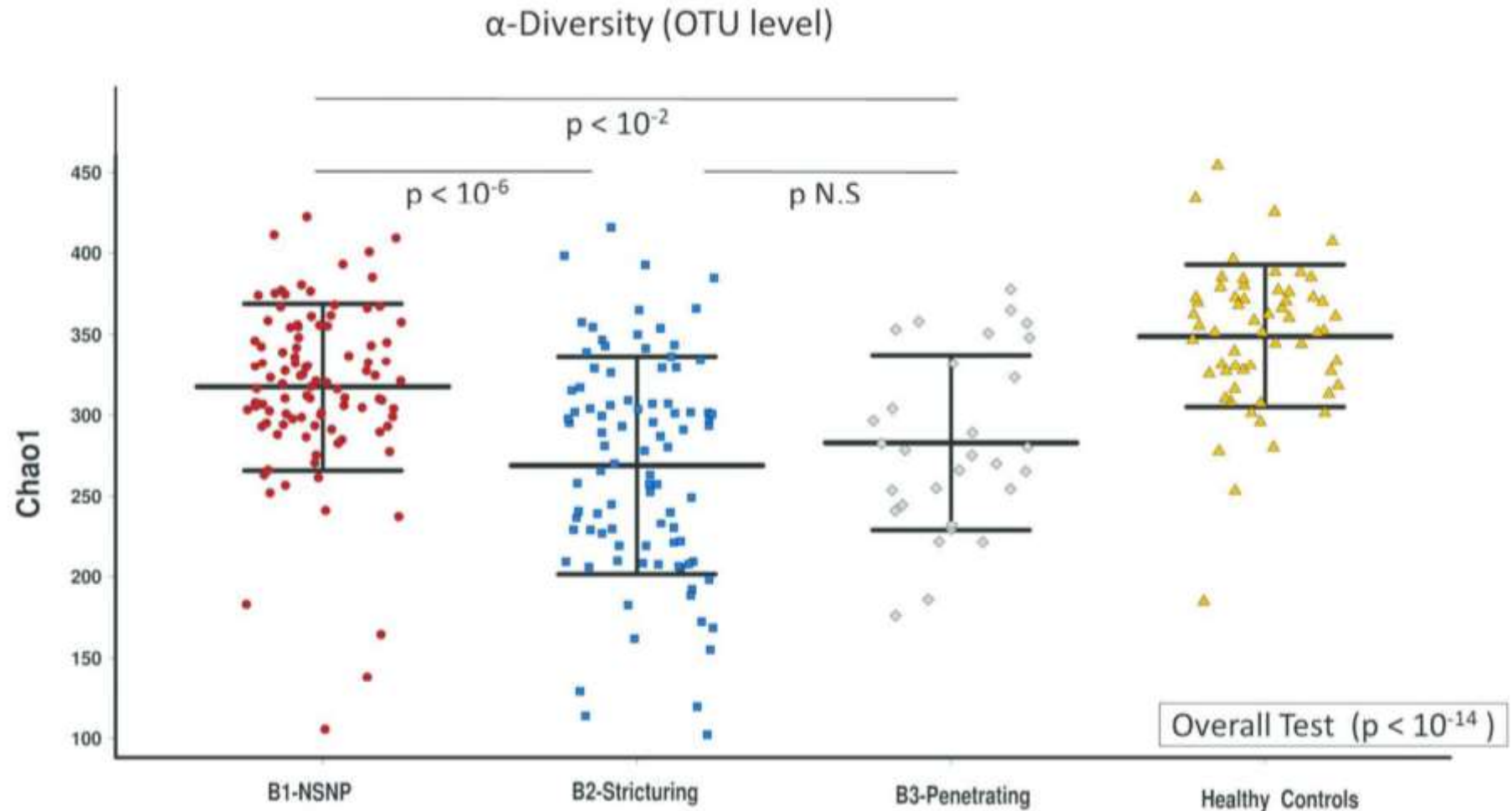
Democritus University of Thrace, Alexandroupolis, Greece

(Annals of Gastroenterology 2019)



Gut Microbial Signatures Underline Complicated Crohn's Disease but Vary Between Cohorts; An In Silico Approach

Nikolas Dovrolis, MSc,^{*,§} Ioannis Drygiannakis, MD, PhD,[†] Eirini Filidou, PhD,^{*}
Leonidas Kandilogiannakis, MSc,^{*} Konstantinos Arvanitidis, PhD,^{*} Ioannis Tentes, PhD,[‡]
George Kolios, MD, PhD,^{*} and Vassilis Valatas, MD, PhD[†]



(Dovrolis et al. Inflamm Bowel Dis. 2019)

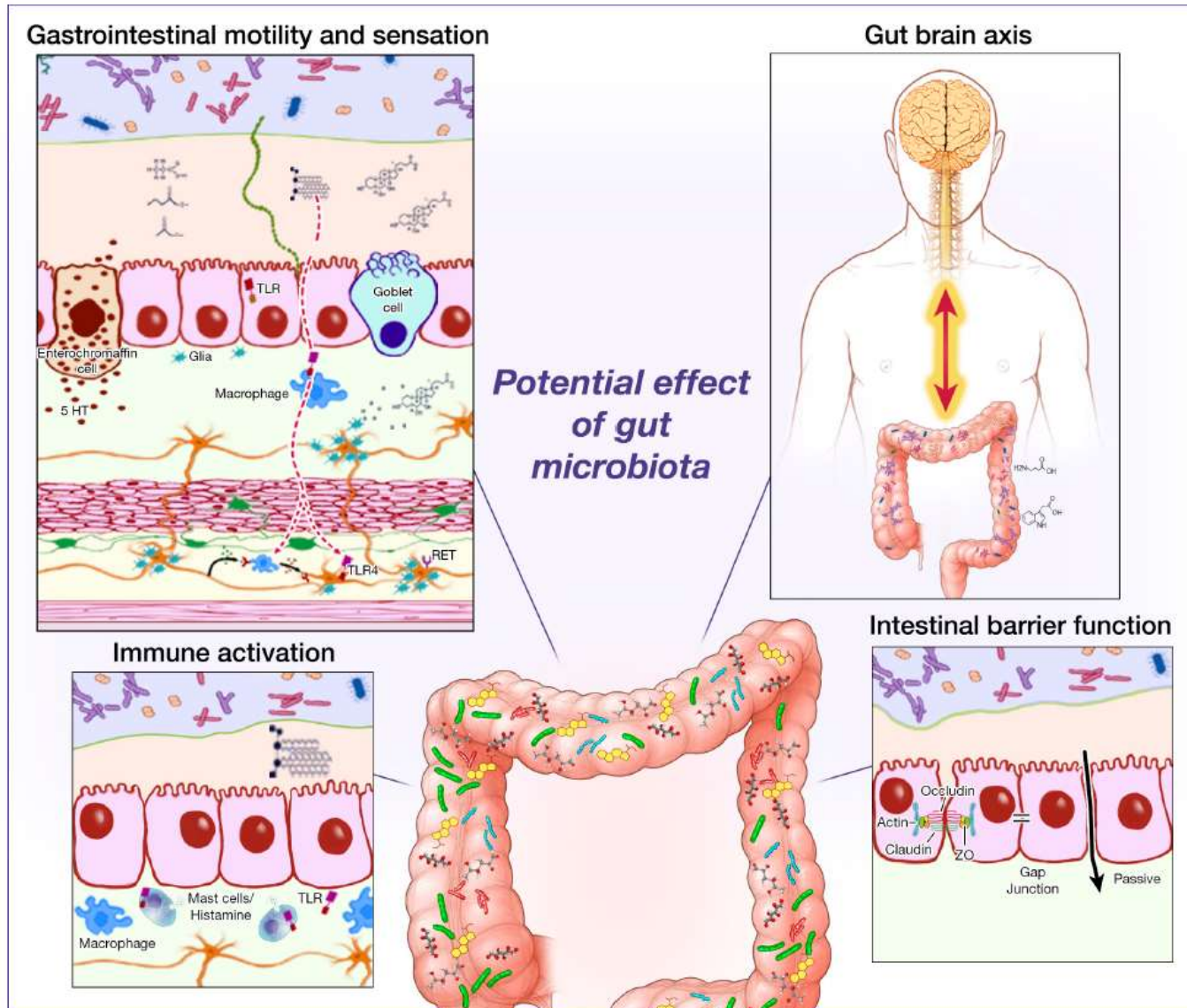
Broken Biome or Broken Host?

- **Is this an abnormal immune response to a normal microbiota or is this an appropriate response to an abnormal microbiota?**
- It is now clear from various animal models that both situations may arise and may overlap.
- Genetically determined anomalies of the innate immune system can lead to a modification of the microbiota, which becomes colitogenic upon transfer to an otherwise normal recipient.
- In addition, because the microbiota shapes the maturation of the immune system in early life, any disruption of the microbiota such as that caused by antibiotic exposure may lead to suboptimal immunity and/or risk of IBD in later life

(Sheehan & Shanahan. Gastroenterol Clin N Am - (2016))

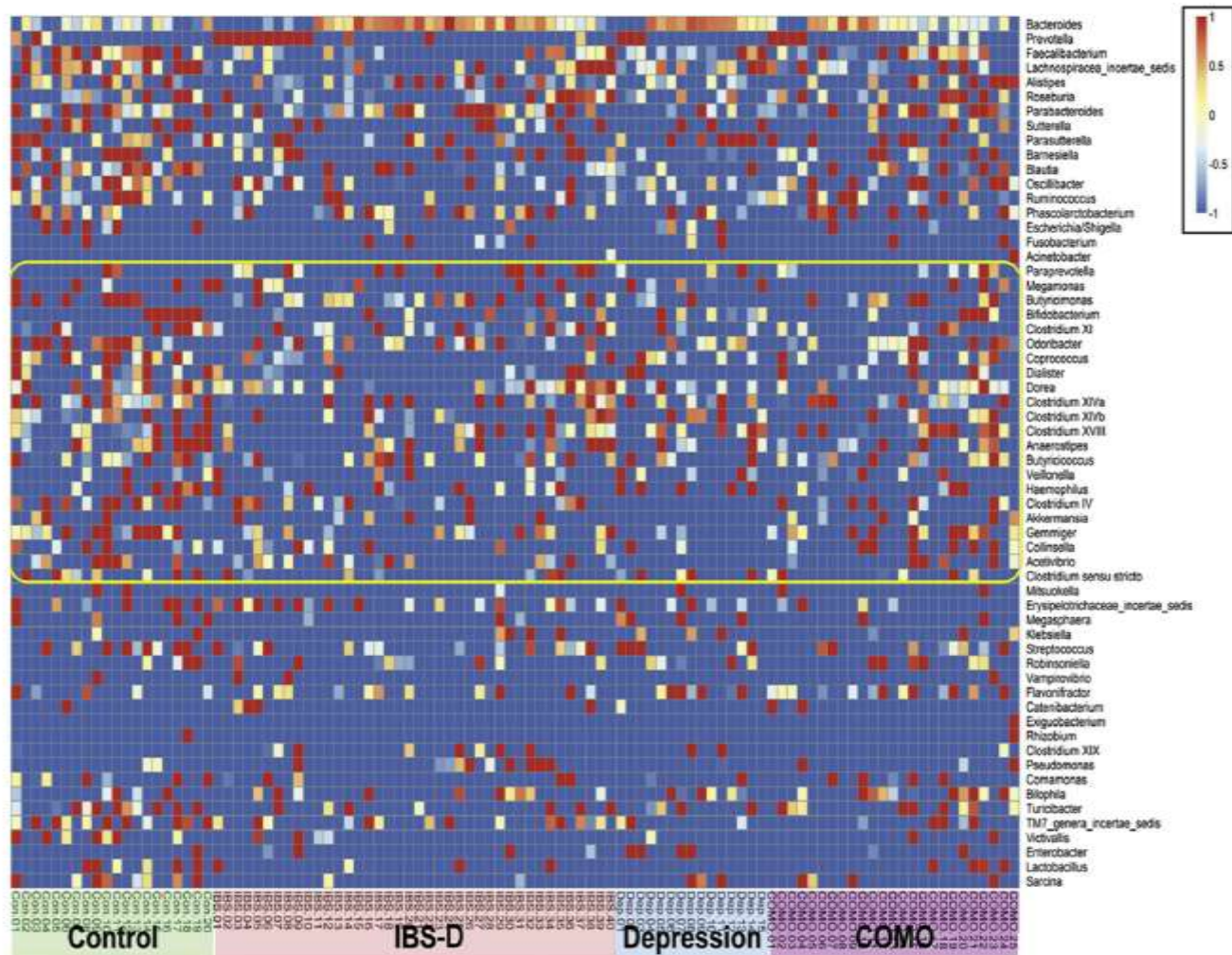
Irritable bowel syndrome: a gut microbiota-related disorder?

Yogesh Bhattarai,^{1,2} David A. Muniz Pedrego,^{1,2} and Purna C. Kashyap^{1,2}



Similar Fecal Microbiota Signatures in Patients With Diarrhea-Predominant Irritable Bowel Syndrome and Patients With Depression

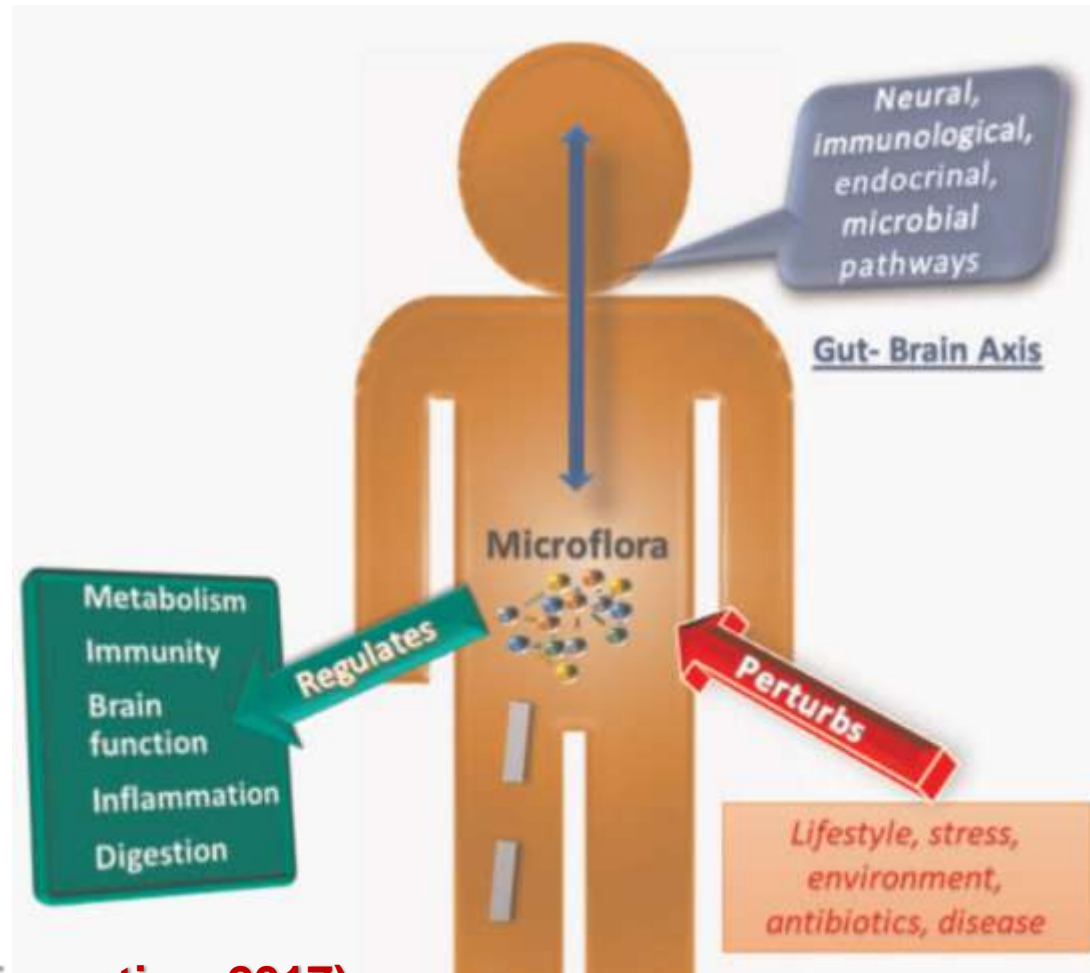
D



Computational profiling of the gut–brain axis: microflora dysbiosis insights to neurological disorders

Nikolas Dovrolis, George Kolios, George M. Spyrou and Ioanna Maroulakou

Corresponding author: Ioanna Maroulakou, Laboratory of Genetics, Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, 68100, Greece. Tel.: +30-25510-30666; E-mail: imaroula@mbg.duth.gr

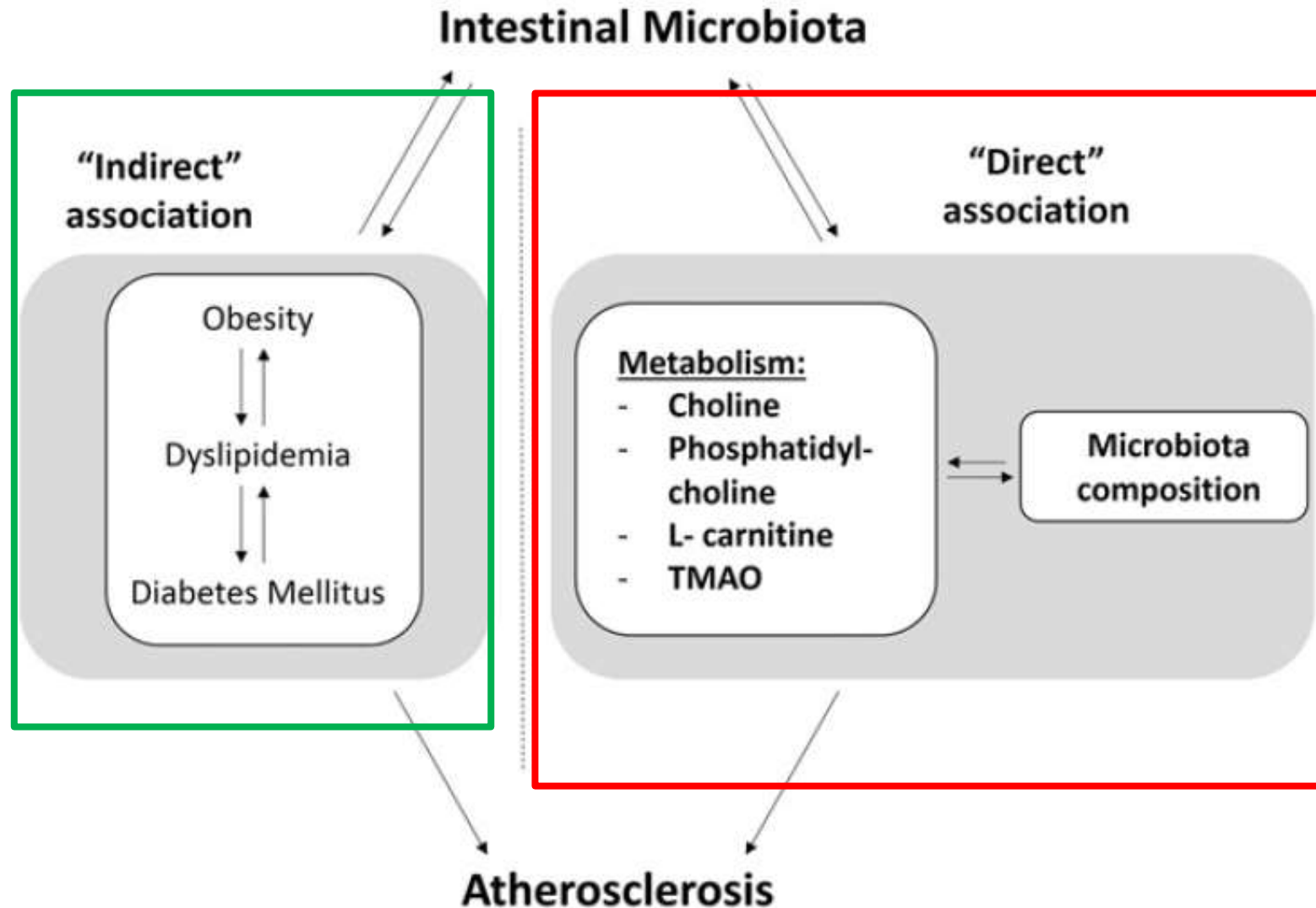


(Briefings in Bioinformatics, 2017)

New Aspects on the Metabolic role of Intestinal Microbiota in the Development of Atherosclerosis

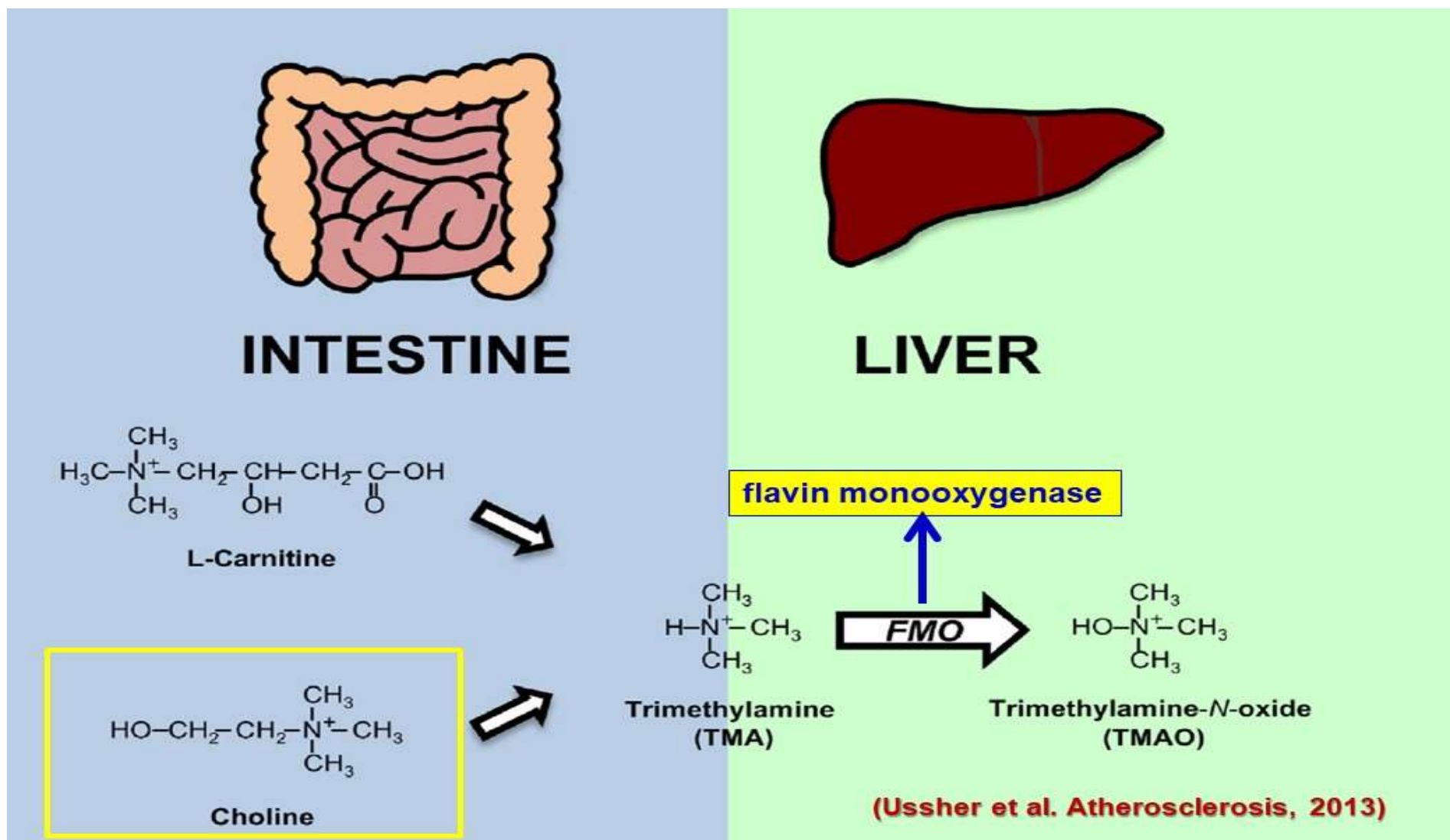


Ioannis Drosos, Anna Tavidou*, George Kolios

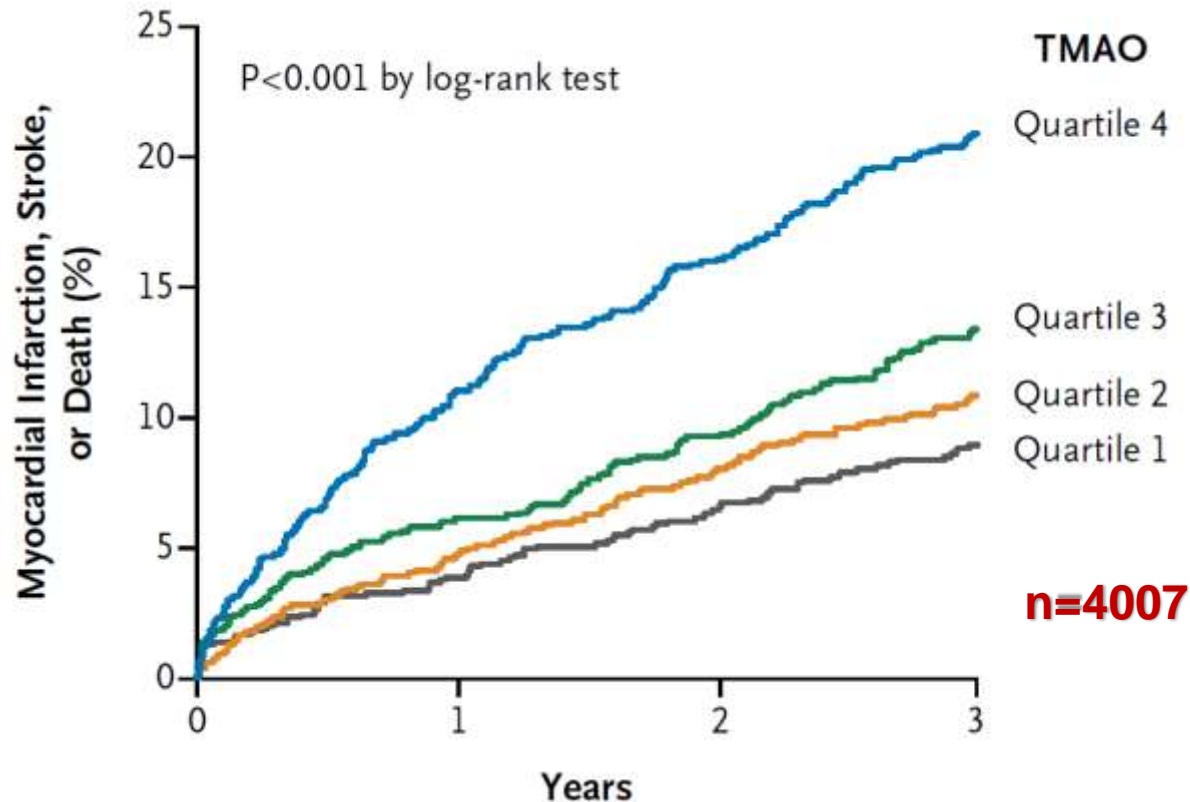


(Drosos et al. Metabolism 2015)

Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease



Kaplan–Meier Estimates of Major Adverse Cardiovascular Events, According to the Quartile of TMAO Level



The quartiles of TMAO levels are as follows:

- ✓ quartile 1, less than 2.43 μM ;
- ✓ quartile 2 2.43 to 3.66 μM ;
- ✓ quartile 3, 3.67 to 6.18 μM ; and
- ✓ quartile 4, more than 6.18 μM .

(Wilson Tang et al. NEJM 2013)

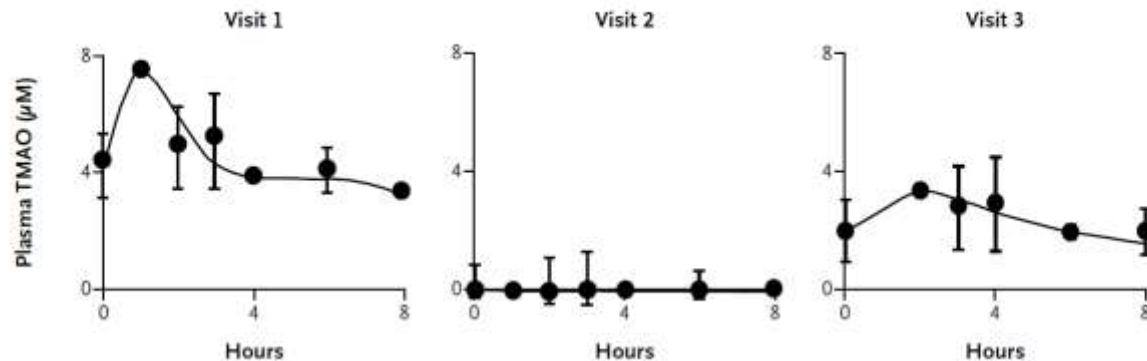
Table 2. Risk of a Major Adverse Cardiovascular Event at 3 Years, According to Quartile of TMAO Level.*

Risk of Event	TMAO Level							
	Quartile 1		Quartile 2		Quartile 3		Quartile 4	
	reference	hazard ratio (95% CI)	P value	hazard ratio (95% CI)	P value	hazard ratio (95% CI)	P value	
Unadjusted hazard ratio	1.00	1.24 (0.93–1.66)	0.15	1.53 (1.16–2.02)	0.003	2.54 (1.96–3.28)	<0.001	

Effects of a Phosphatidylcholine Challenge and Administration of Antibiotics on Mean Levels of Trimethylamine-*N*-Oxide (TMAO) and Its d9 Isotopologue (d9-TMAO).

Visit 1 → Antibiotics (gut flora suppression) → Visit 2 → Reacquisition of gut flora → Visit 3

C Plasma TMAO



D Plasma d9-TMAO



Dietary phosphatidylcholine challenge:
✓ deuterium-labeled phosphatidylcholine (250 mg d9-phosphatidylcholine) and
✓ two hard-boiled eggs (appr. 250 mg of total choline each).

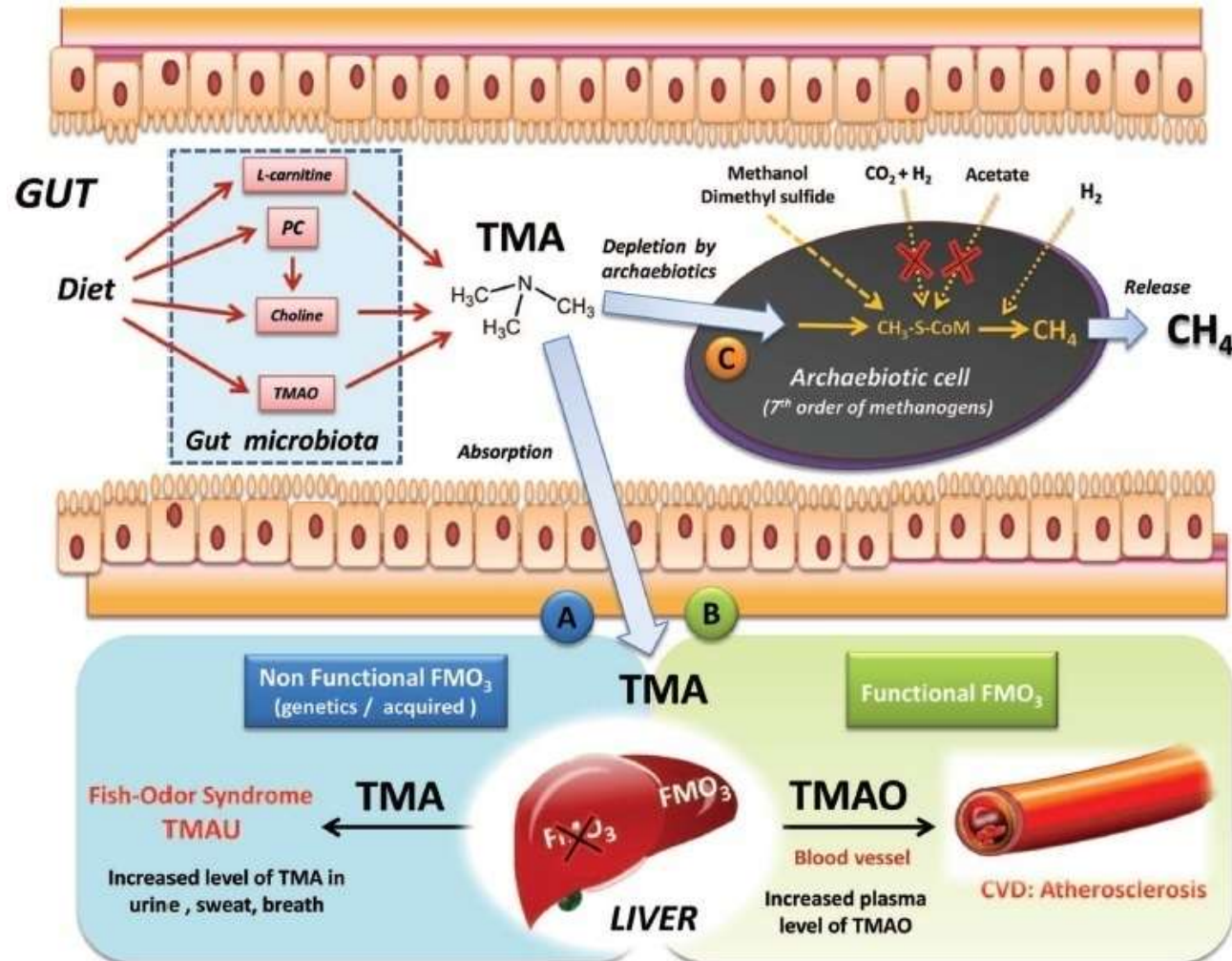
Broad-spectrum antibiotics for 1 week:
✓ metronidazole (500 mg twice daily)
✓ ciprofloxacin (500 mg once daily)

CONCLUSIONS

The production of TMAO from dietary phosphatidylcholine is dependent on metabolism by the intestinal microbiota. Increased TMAO levels are associated with an increased risk of incident major adverse cardiovascular events.

Archaeobiotics

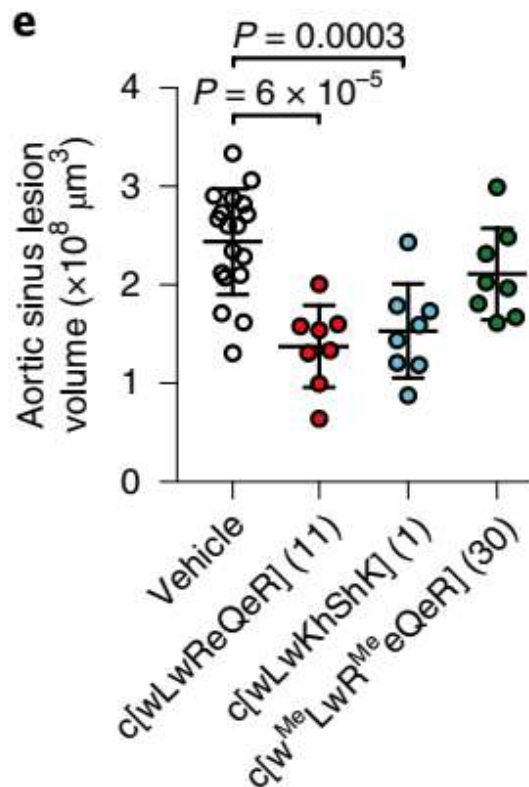
Proposed therapeutic use of **archaea** to prevent trimethylaminuria and cardiovascular disease



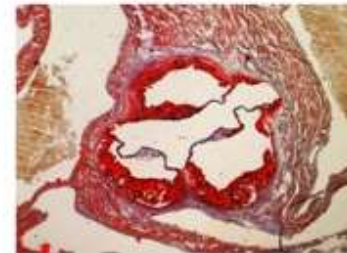
(Brugere et al. Gut Microbes 2014)

Directed remodeling of the mouse gut microbiome inhibits the development of atherosclerosis

of the mouse gut microbiome to discover molecules that can selectively modify bacterial growth. This approach was used to identify cyclic D,L- α -peptides that remodeled the Western diet (WD) gut microbiome toward the low-fat-diet microbiome state. Daily oral administration of the peptides in WD-fed *LDLR*^{-/-} mice reduced plasma total cholesterol levels and atherosclerotic plaques. Depletion of the microbiome with antibiotics abrogated these effects. Peptide treatment reprogrammed the microbiome transcriptome, suppressed the production of pro-inflammatory cytokines (including interleukin-6, tumor necrosis factor- α and interleukin-1 β), rebalanced levels of short-chain fatty acids and bile acids, improved gut barrier integrity and increased intestinal T regulatory cells. Directed chemical manipulation provides an additional tool for deciphering the chemical biology of



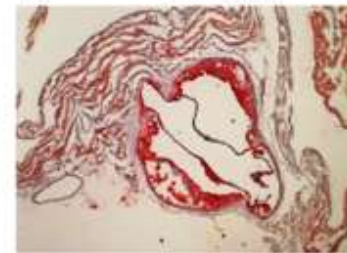
WD +
vehicle



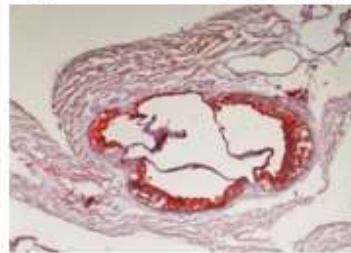
WD +
c[wLwReQeR] (11)



WD +
c[wLwKhShK] (1)



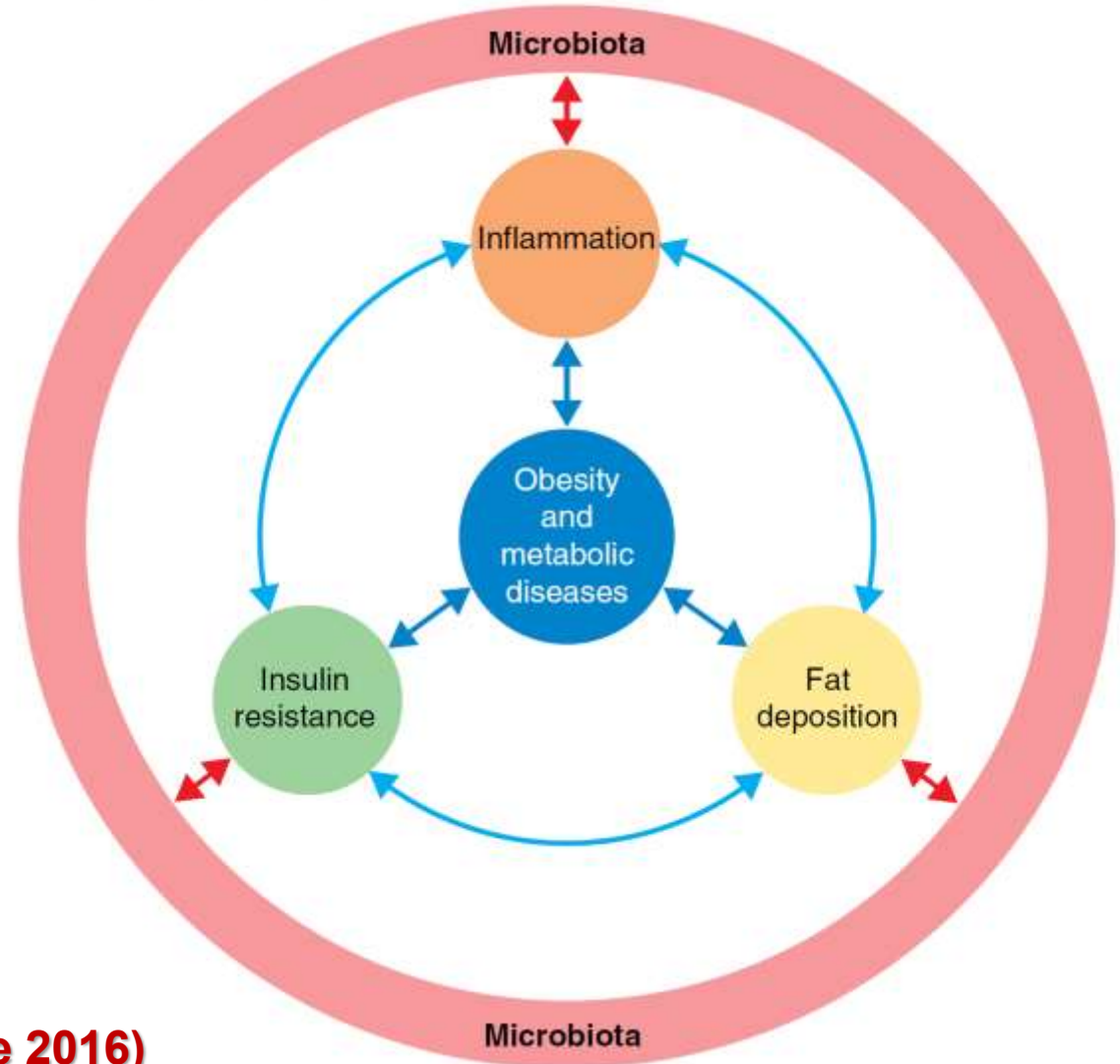
WD +
c[w^{Me}LwR^{Me}eQeR] (30)



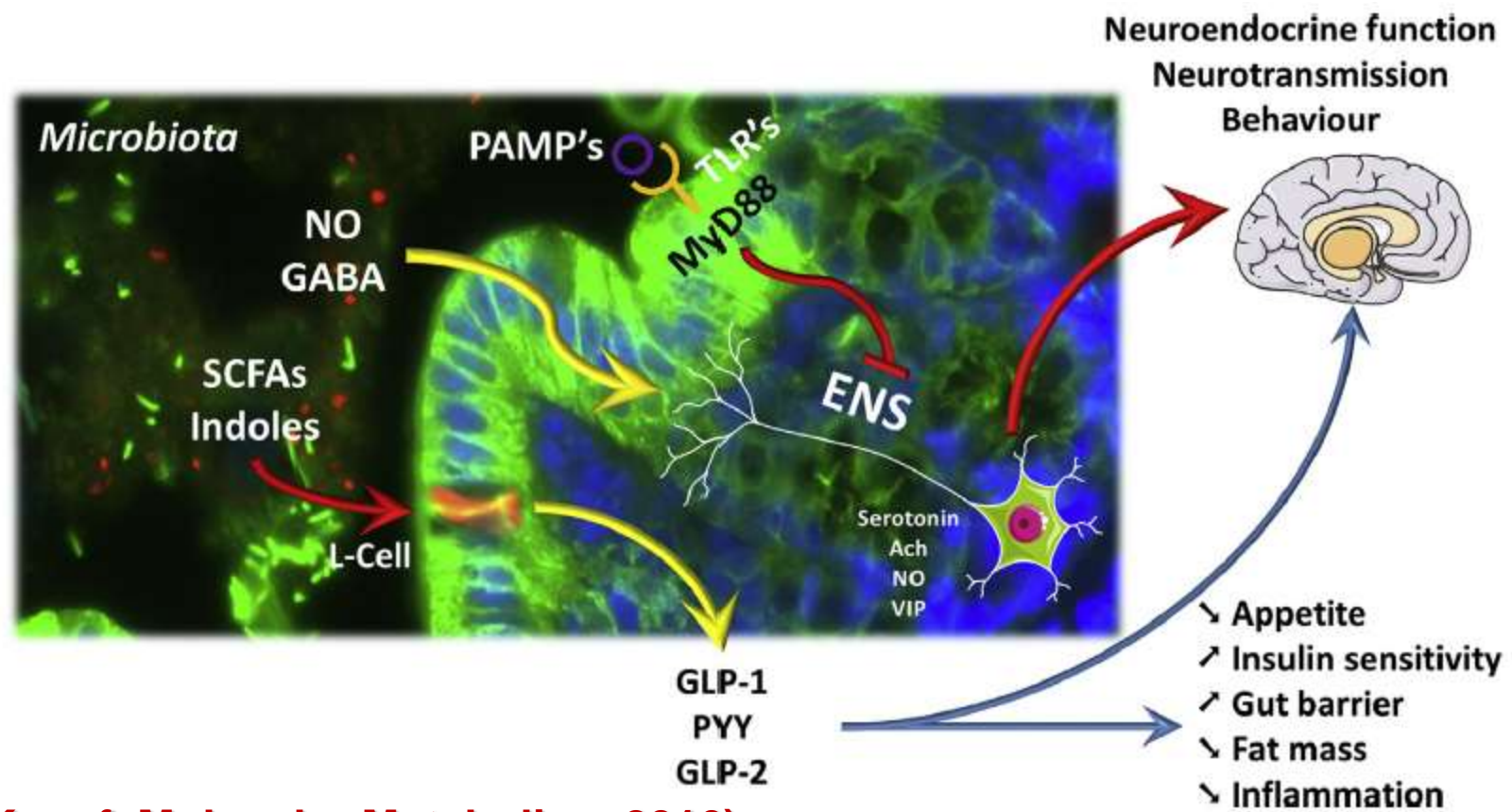
(Chen et al. Nature Biotech. 2020)

Gut Microbiota and metabolic disease

- The gut microbiota can contribute to:
 - ✓ host insulin resistance,
 - ✓ low grade inflammation, and
 - ✓ fat deposition
- through a range of molecular interactions with the host and therefore can indirectly participate in the onset of obesity and metabolic diseases



(Boulangé et al. Genome Medicine 2016)



(Cani & Knauf. Molecular Metabolism 2016)

- **SCFAs and Indoles** are recognized by specific Gprotein coupled receptors expressed at the surface of enteroendocrine cells such as L-cells, producing GLP-1, GLP-2, and PYY.
- Secondary messengers, including NO, serotonin, acetylcholine (Ach) or vasoactive intestinal polypeptide (VIP) release, are involved in the brain axis.
- **Pathogen-associated molecular patterns (PAMPs)** are recognized by Toll-Like receptors (TLR's) regulate numerous metabolic functions such as for instance leptin sensitivity, gut hormones signaling to the brain, hence controlling whole-body energy homeostasis.

Acetate mediates a microbiome–brain– β -cell axis to promote metabolic syndrome

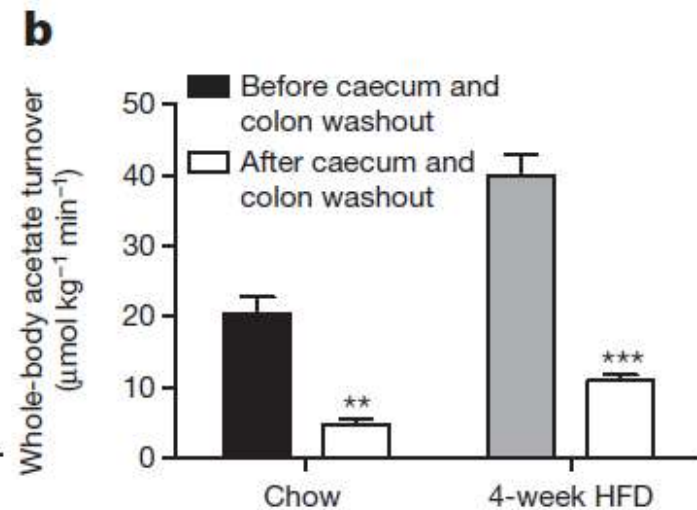
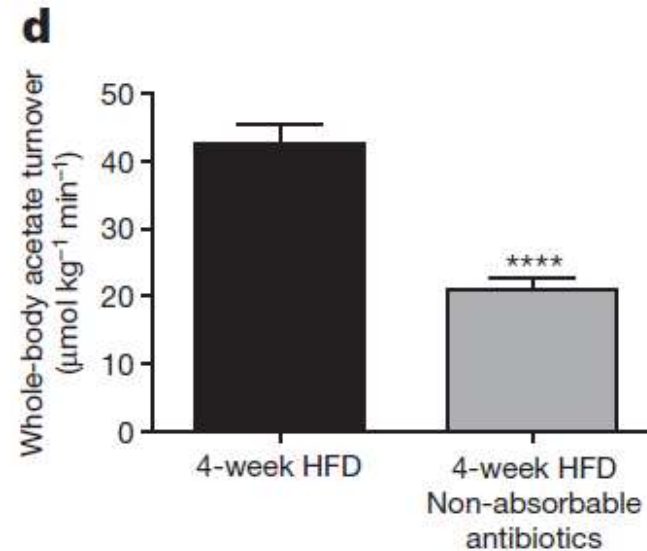
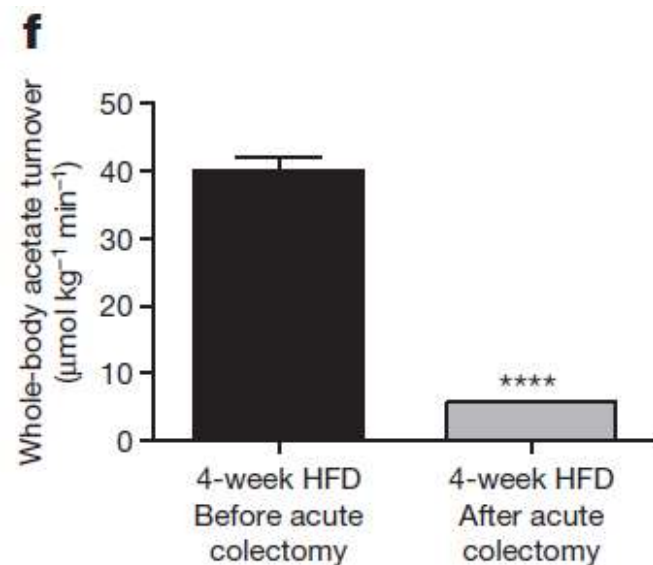
Rachel J. Perry¹, Liang Peng¹, Natasha A. Barry^{2,3}, Gary W. Cline¹, Dongyan Zhang⁴, Rebecca L. Cardone¹, Kitt Falk Petersen^{1,5}, Richard G. Kibbey^{1,6}, Andrew L. Goodman^{2,3} & Gerald I. Shulman^{1,4,5,6}

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA

centrations may not fully represent the SCFA load presented to the body, we developed a method to measure whole-body turnover rates of acetate, propionate, and butyrate by gas chromatography–mass spectrometry (GC–MS; as described in the Supplementary Methods) and found that, in contrast to propionate and butyrate, whole-body acetate turnover as well as plasma and faecal acetate concentrations were markedly increased in insulin-resistant rats after 3 days or 4 weeks on a high-fat diet (HFD) (Fig. 1a, b and Extended Data Fig. 2a–j).

Next we sought to determine the source of the increased acetate turnover in HFD-fed rats. We measured tissue acetate concentrations and

ectopic lipid deposition in liver and skeletal muscle, and liver and muscle insulin resistance (Extended Data Fig. 1). The increased acetate production that occurs when the gut microbiota are exposed to calorically dense nutrients may mediate an important positive feedback loop between the gut microbiota and the CNS that promotes hyperphagia (due to increased ghrelin secretion) and increased energy storage as fat (due to increased GSIS) in foraging animals when they stumble across calorically dense foodstuffs in the wilderness. However, in the setting of chronic exposure to calorically



(Nature. 2016, Jun 8)

Microbial signals to the brain control weight

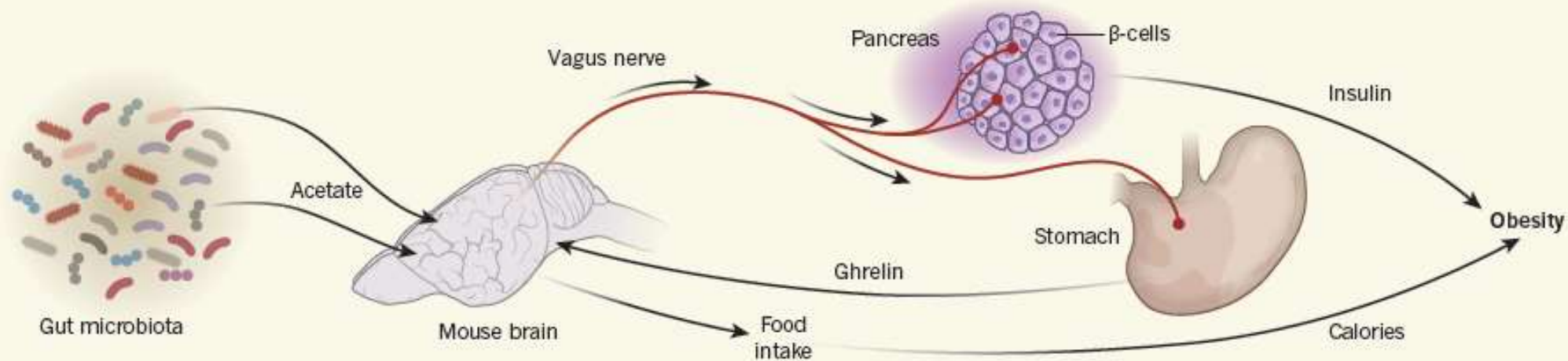


Figure 1 | A mechanism for microbiota-mediated weight gain. Perry *et al.*² report that, in rodents, production of acetate molecules from dietary nutrients by the bacteria that colonize the gut (the microbiota) increases the brain's stimulation of the parasympathetic nervous system, which includes the vagus nerve. Signals from the vagus nerve trigger

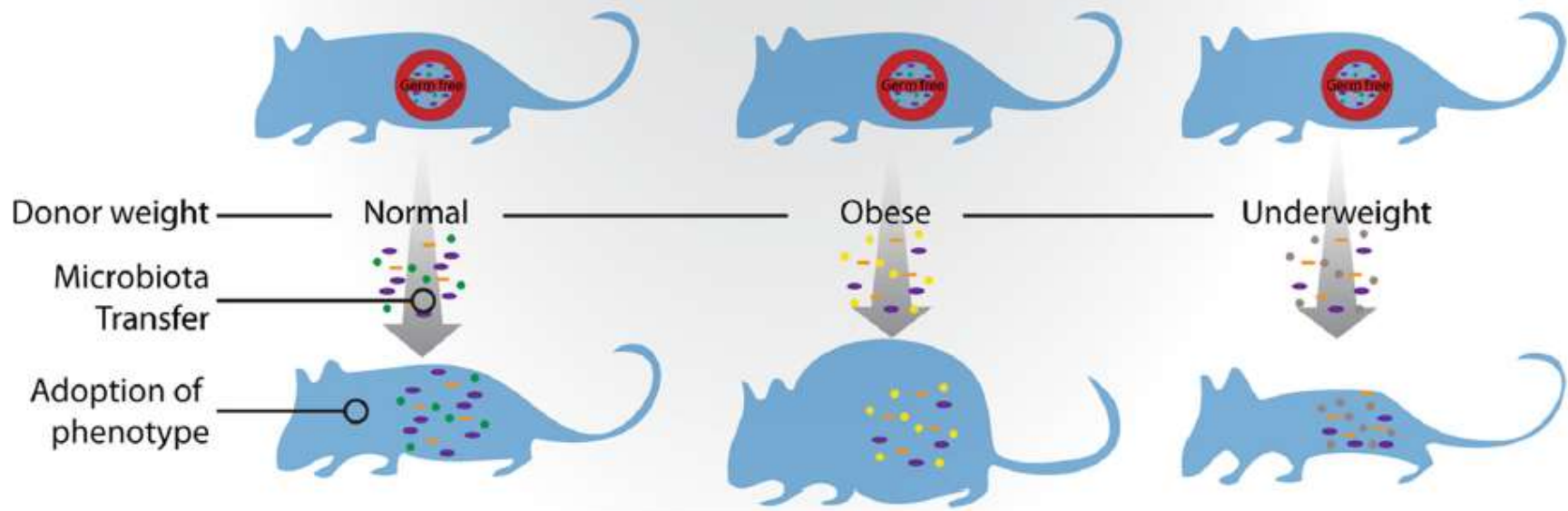
secretion of the 'hunger hormone' ghrelin from the stomach, leading to increased food intake. The vagus nerve also potentiates glucose-stimulated insulin secretion from β -cells in the pancreas, promoting calorie storage and fat gain. In this way, the gut microbiota influences obesity.

(Trajkovs K I & Wollheim. Nature 2016)

Μικροβιακή χλωρίδα και παχυσαρκία

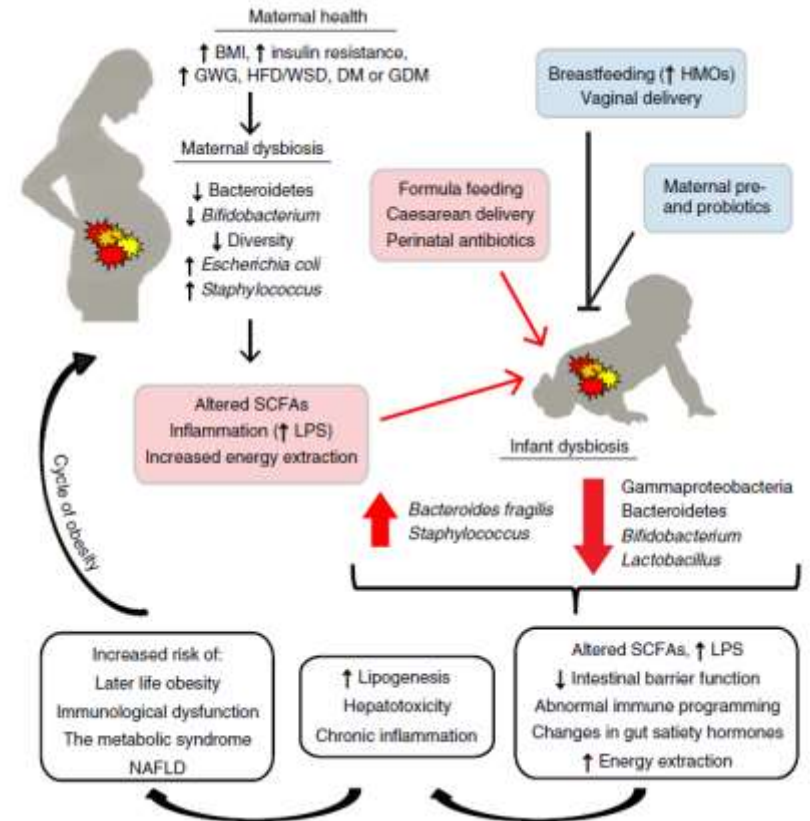
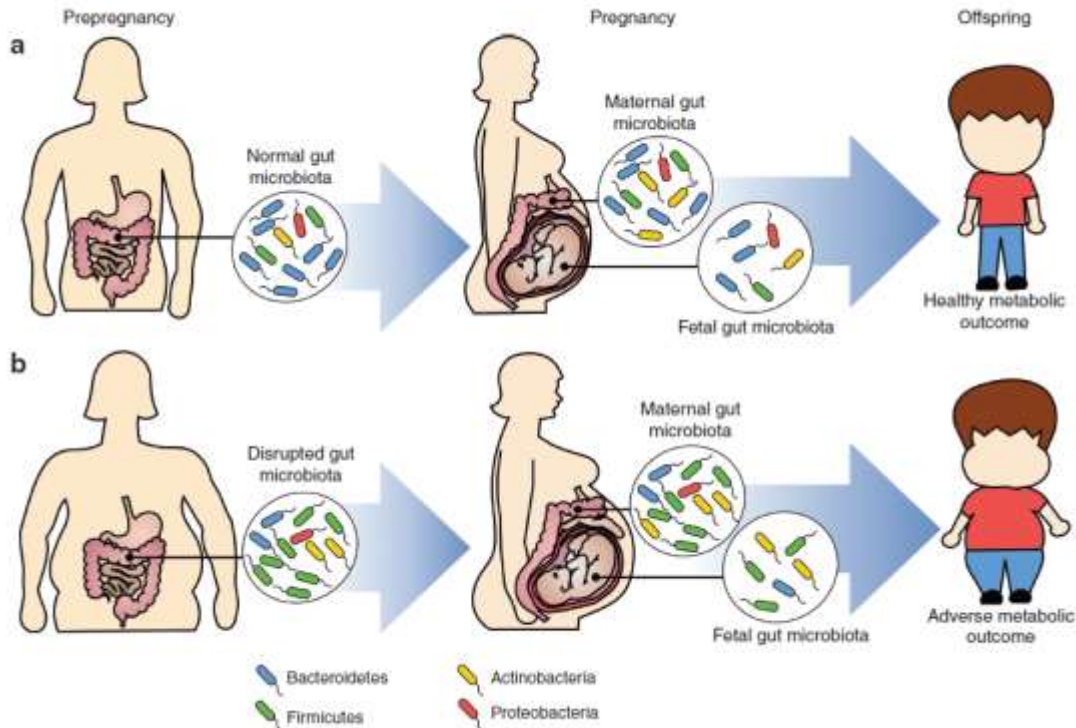
B

Germ-free animals adopt phenotype of microbiota donor



(Clarke et al. Molecular Endocrinology 2014)

Παχυσαρκία μητέρας και μικροβίωμα εμβρύου



(Gohir et al. Pediatr Res. 2015)

(Soderborg et al. Diabetologia 2016)

Germ and joints: the contribution of the human microbiome to rheumatoid arthritis

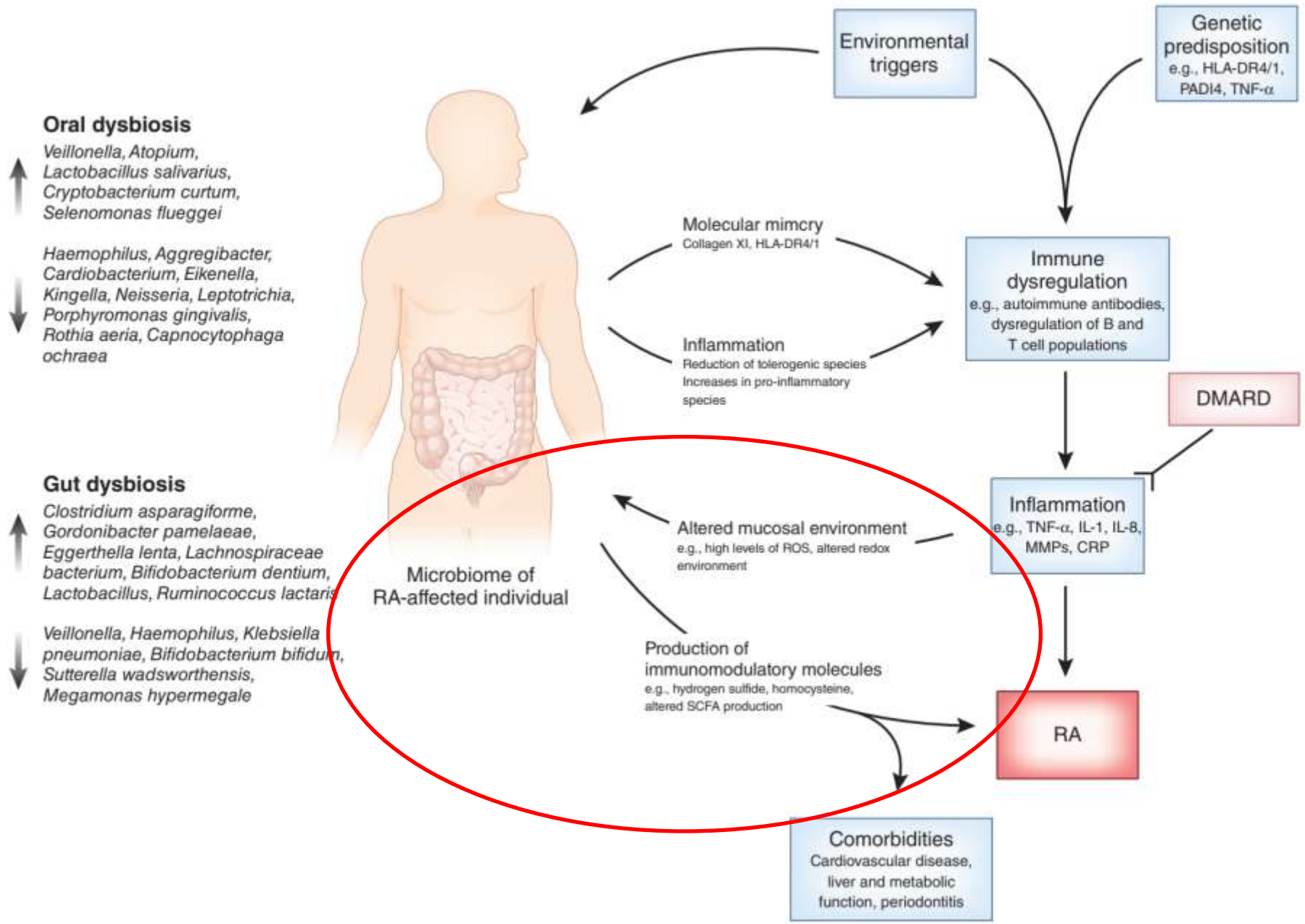
Geraint B Rogers

Rheumatoid arthritis (RA) is a debilitating autoimmune disorder, the etiology of which is poorly understood. A new study reveals **dysbiosis in gut and oral microbiomes** of affected individuals, potentially providing a basis for patient stratification and clues to **pathophysiological mechanisms of RA onset and progression**.

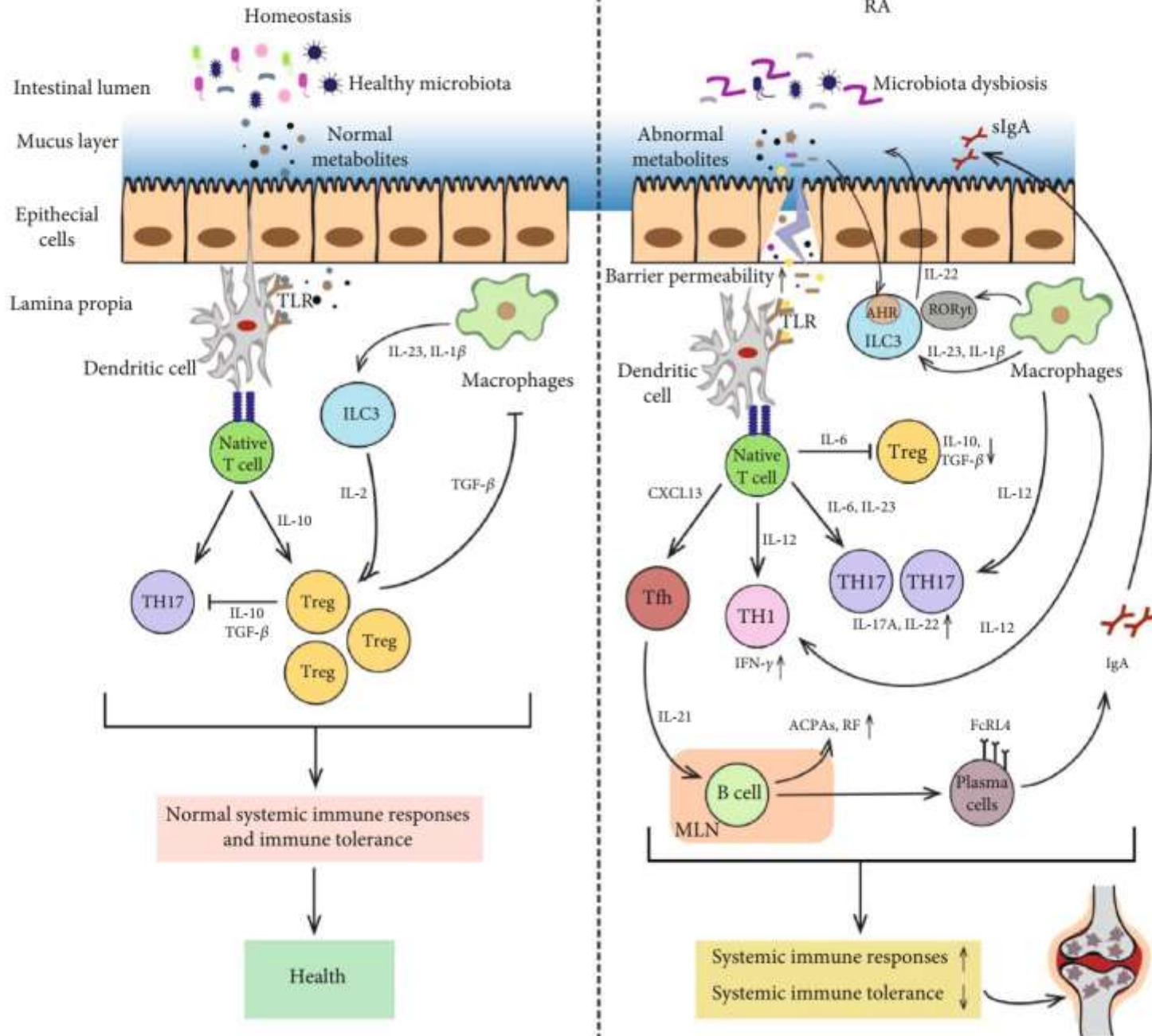
The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment

Xuan Zhang^{1,13}, Dongya Zhang^{2,3,13}, Huijue Jia^{2,3,13}, Qiang Feng^{2,4,5,13}, Donghui Wang^{2,5,13}, Di Liang^{1,13},

We carried out **metagenomic shotgun sequencing and a metagenome-wide association study (MGWAS) of fecal, dental and salivary samples from a cohort of individuals with rheumatoid arthritis (RA) and healthy controls**. Concordance was observed between the gut and oral microbiomes, suggesting overlap in the abundance and function of species at different body sites. Dysbiosis was detected in the gut and oral microbiomes of RA patients, but it was partially resolved after RA treatment. **Alterations in the gut, dental or saliva microbiome distinguished individuals with RA from healthy controls, were correlated with clinical measures and could be used to stratify individuals on the basis of their response to therapy**. In particular, *Haemophilus* spp. were depleted in individuals with RA at all three sites and negatively correlated with levels of serum autoantibodies, whereas *Lactobacillus salivarius* was over-represented in individuals with RA at all three sites and was present in increased amounts in cases of very active RA. Functionally, the redox environment, transport and metabolism of iron, sulfur, zinc and arginine were altered in the microbiota of individuals with RA. Molecular mimicry of human antigens related to RA was also detectable. **Our results establish specific alterations in the gut and oral microbiomes in individuals with RA and suggest potential ways of using microbiome composition for prognosis and diagnosis**.



(Geraint B Rogers Nat. Med. 21, 839–841 2015)



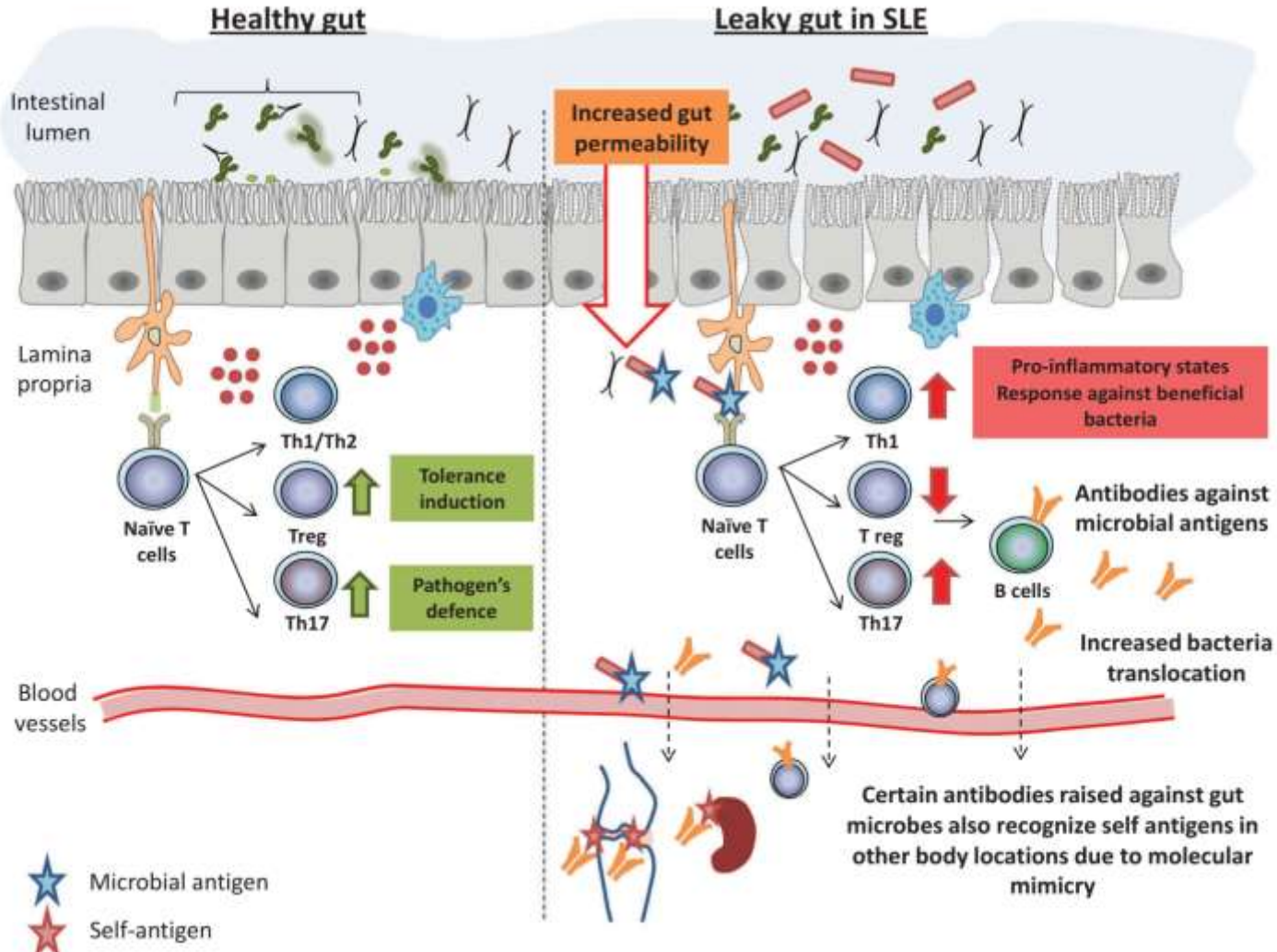
(Xu et al. Med.Inflamm.2020)

SPECIAL REPORT



The role of gut microbiota in lupus: what we know in 2018?

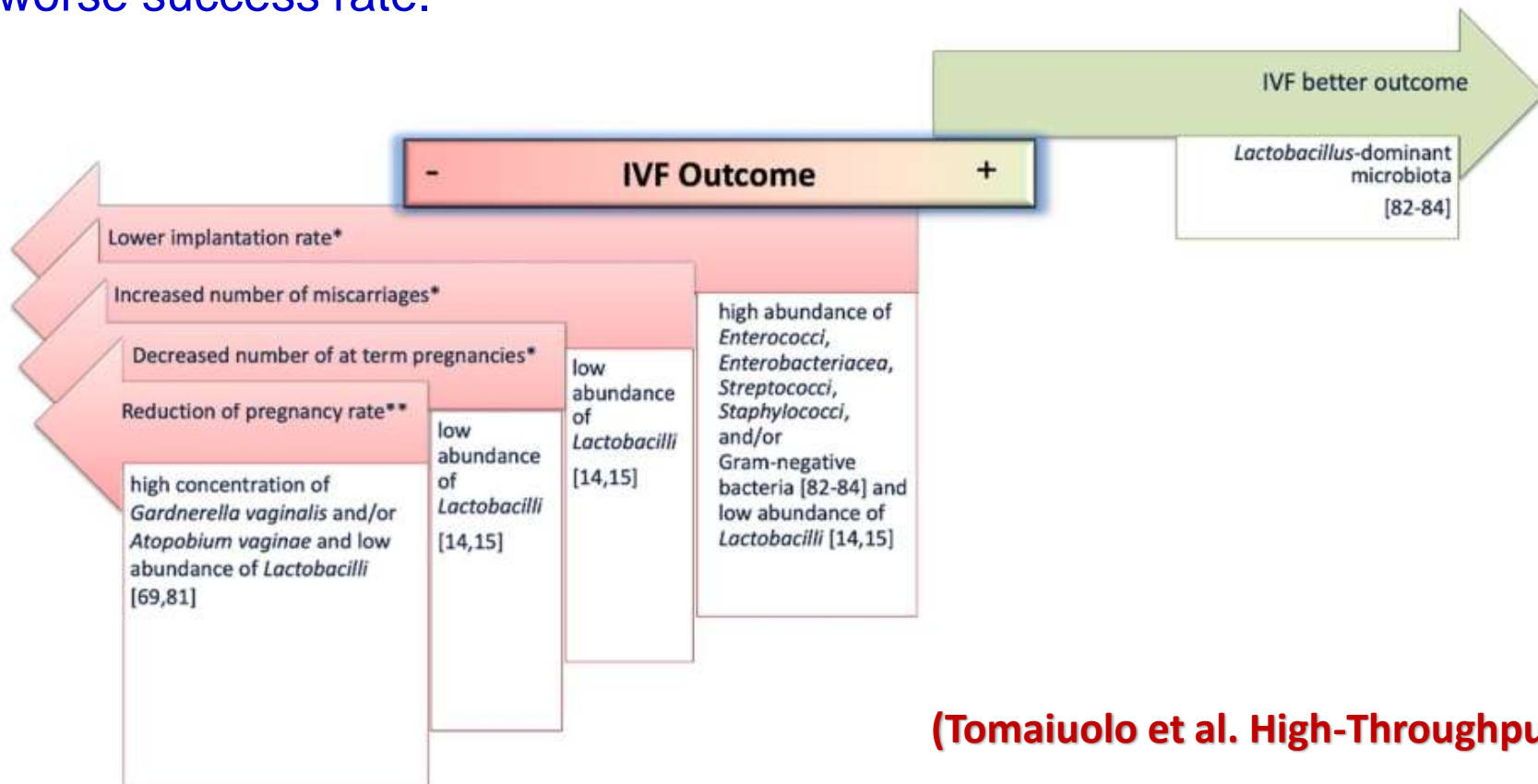
Lorena Ruiz^a, Patricia López^{b,c}, Ana Suárez^{b,c}, Borja Sánchez^a and Abelardo Margolles^a



Microbiota and Human Reproduction: The Case of Female Infertility

Rossella Tomaiuolo ^{1,2,3}, Iolanda Veneruso ^{2,3}, Federica Cariatì ^{1,3} and Valeria D'Argenio ^{3,4,*} 

Female reproductive system microbiome alterations have been associated with different IVF outcomes. While *Lactobacilli* spp. have been reported to exert beneficial effects, specific endometrial * or vaginal ** dysbiosis has been related to a worse success rate.



(Tomaiuolo et al. High-Throughput 2020)

The Disordered Vaginal Microbiota Is a Potential Indicator for a Higher Failure of *in vitro* Fertilization

Yao Kong^{1,2†}, Zhaoxia Liu^{1,2*†}, Qingyao Shang³, Yuan Gao³, Xia Li², Cihua Zheng⁴, Xiaorong Deng⁴ and Tingtao Chen^{1,3*}

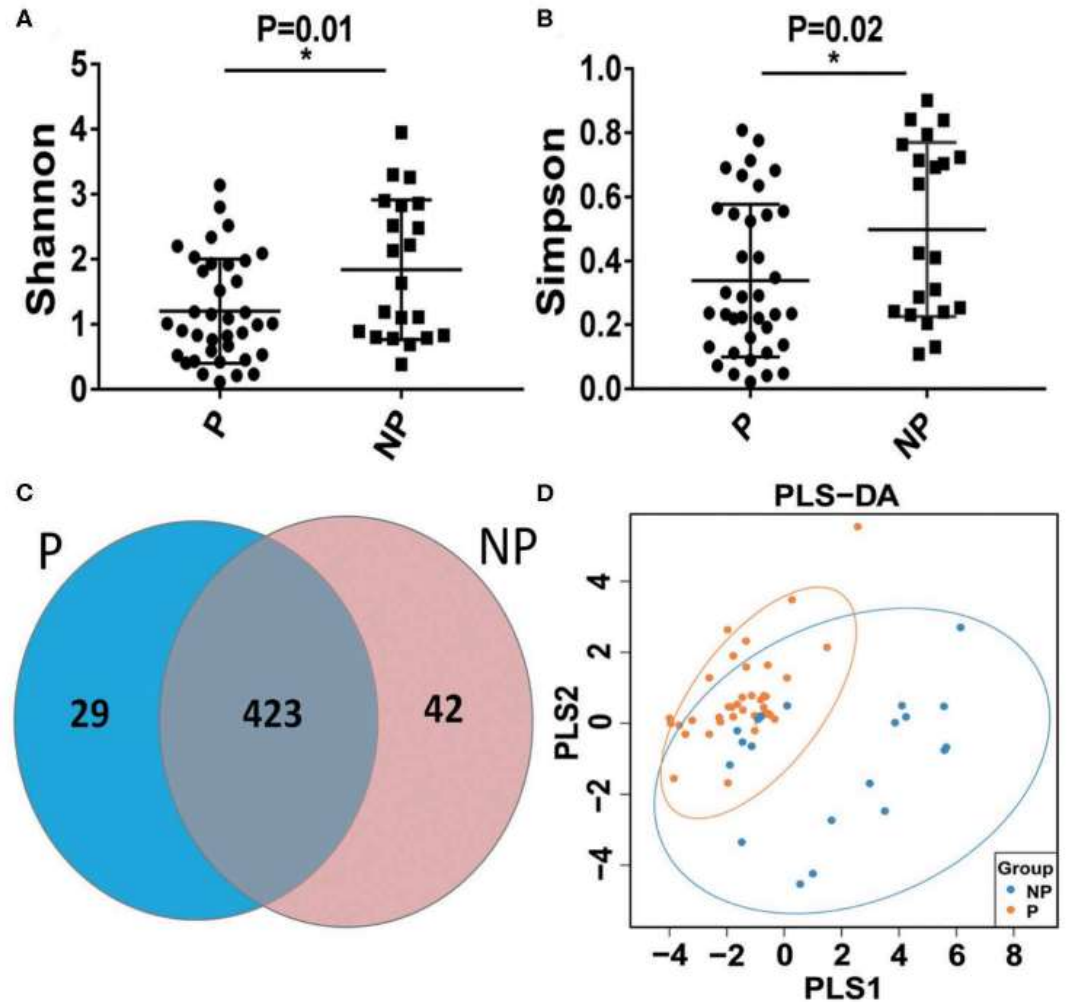
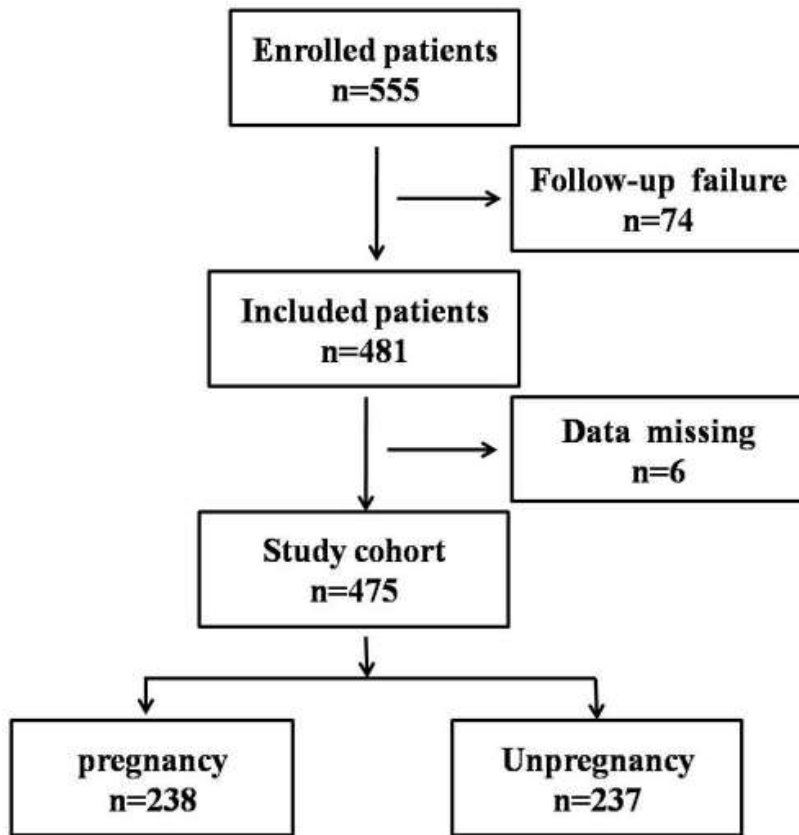
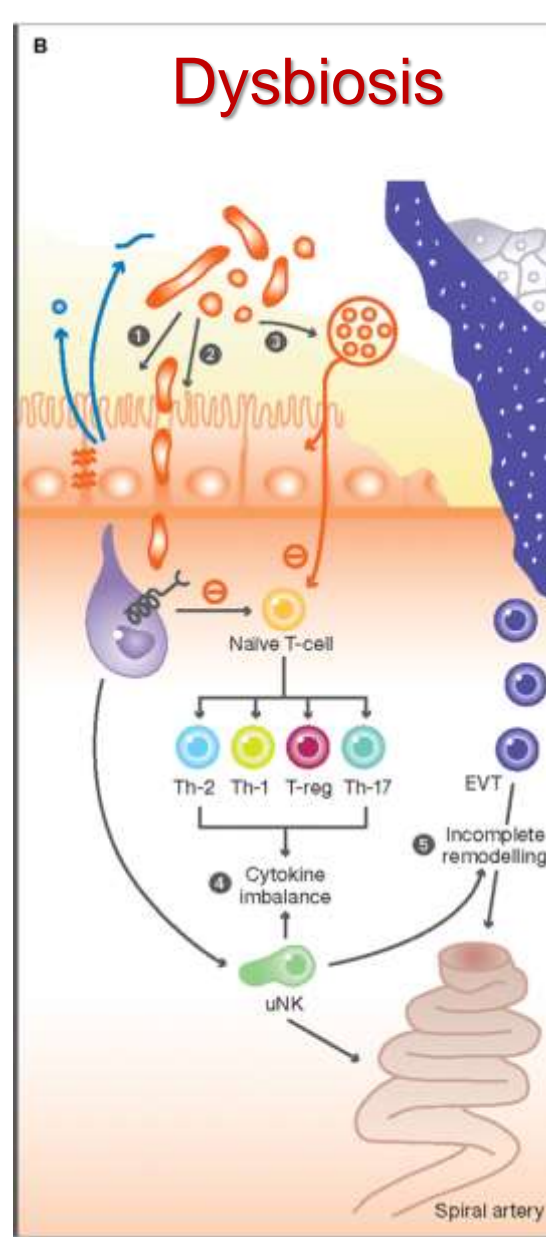
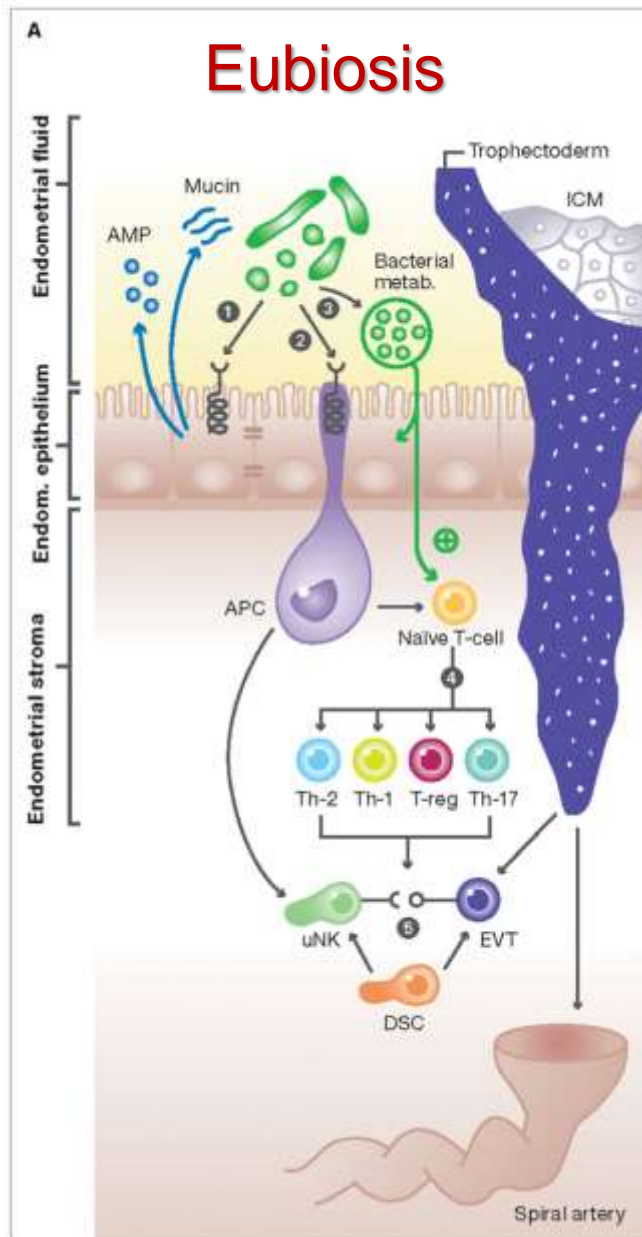


FIGURE 1 | Flowchart showing the process of patient enrollment. P, pregnancy success after embryo transfer; NP, pregnancy failure after embryo transfer.



(Al-Nasiry et al. Frontiers in Immunology. 2020)

Gut microbiota in colorectal cancer: mechanisms of action and clinical applications (Nature reviews | Gastroenterology & Hepatology 2019)

Sunny H. Wong^{1,2} and Jun Yu^{1,2*}

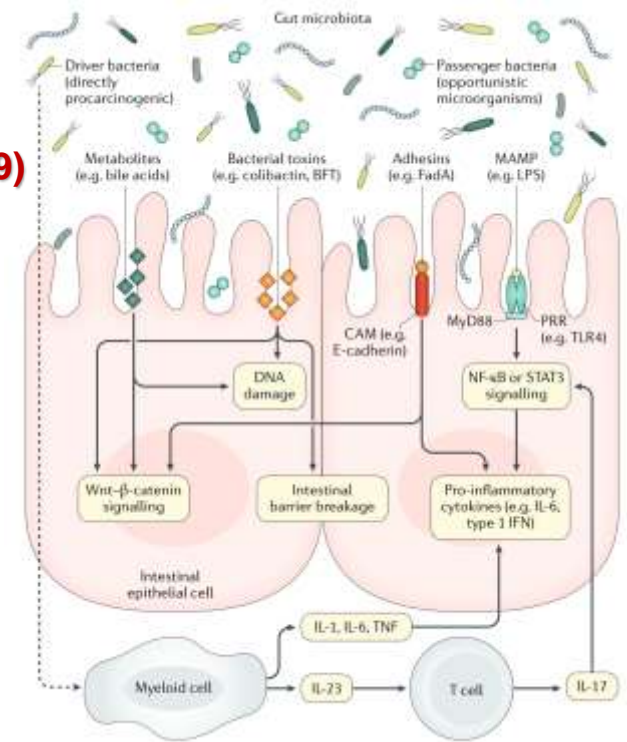
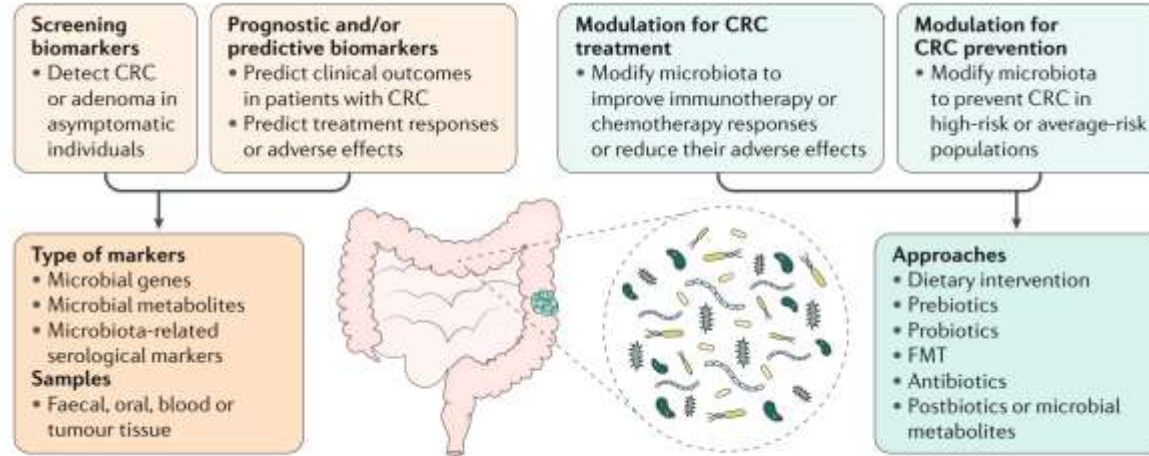
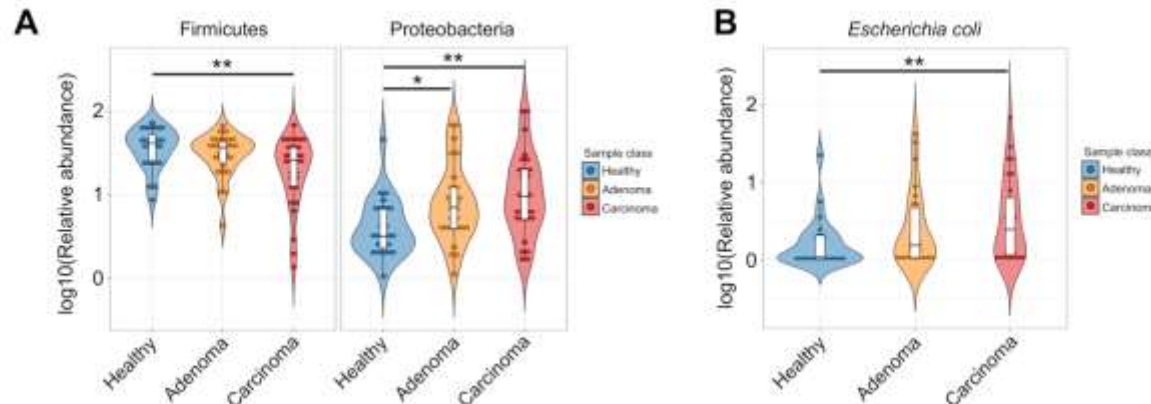


Fig. 2 | Potential clinical applications related to gut microbiota in colorectal cancer. Several potential clinical

Altered Fecal Small RNA Profiles in Colorectal Cancer Reflect Gut Microbiome Composition in Stool Samples

Sonia Tarallo,^a Giulio Ferrero,^b Gaetano Gallo,^{c,d} Antonio Francavilla,^a Giuseppe Clerico,^d Alberto Realis Luc,^d



(Tarallo et al. mSystems. 2019)

Cancer and the Microbiome—Influence of the Commensal Microbiota on Cancer, Immune Responses, and Immunotherapy



Vyara Matson

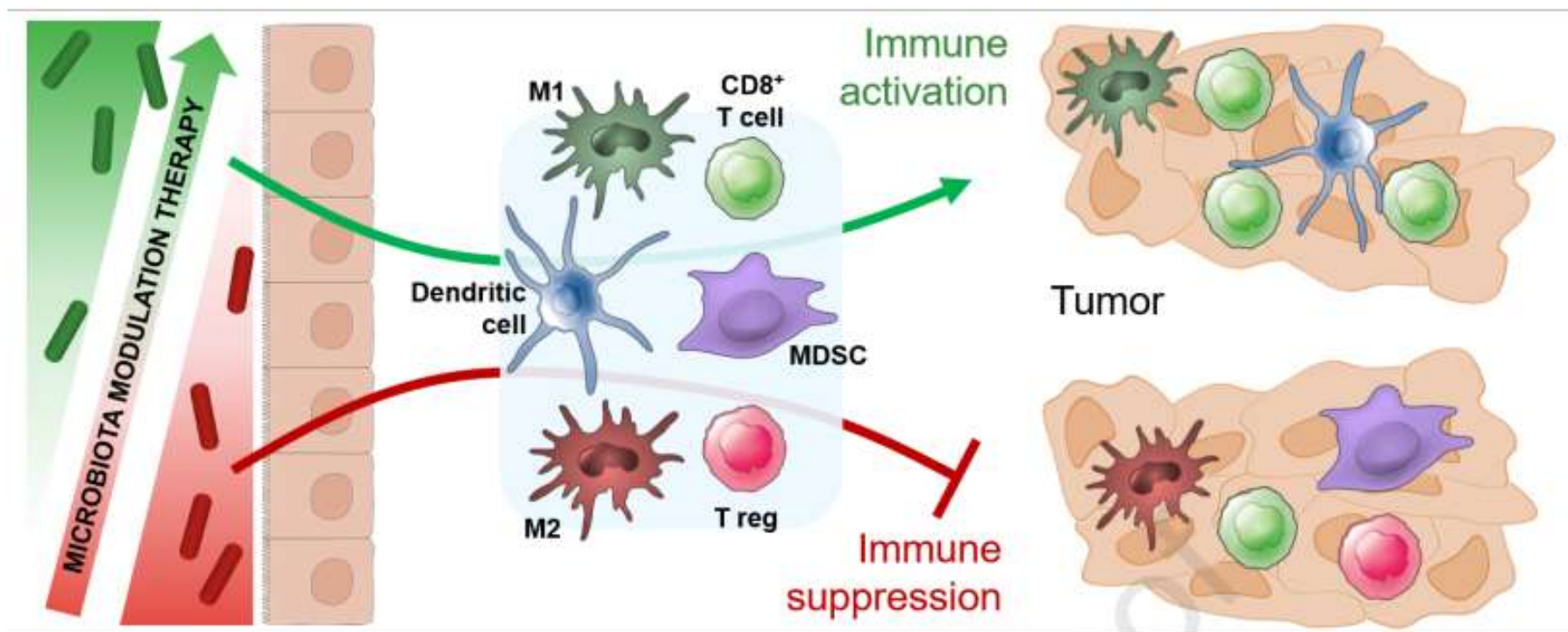


Carolina Soto Chervin



Thomas F. Gajewski

The University of Chicago, Chicago, Illinois



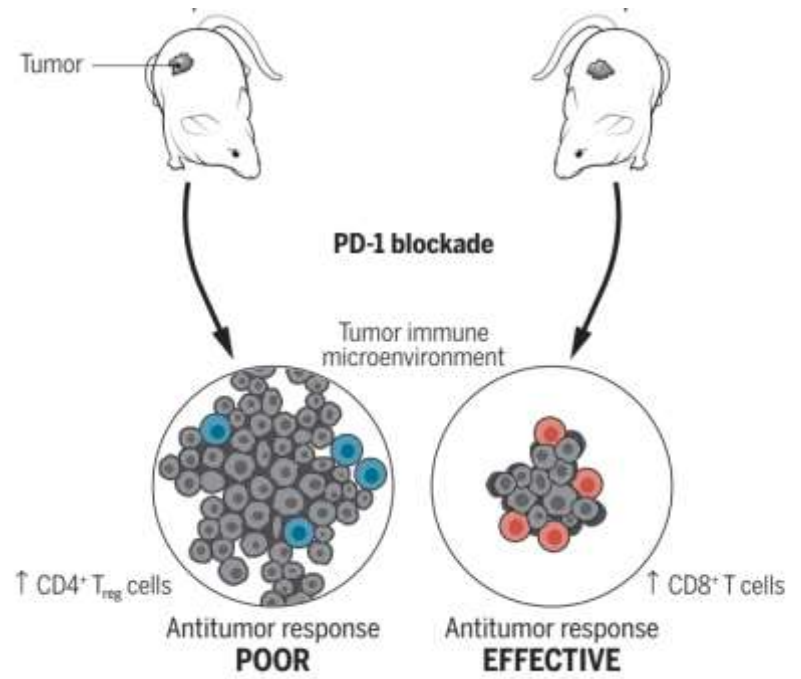
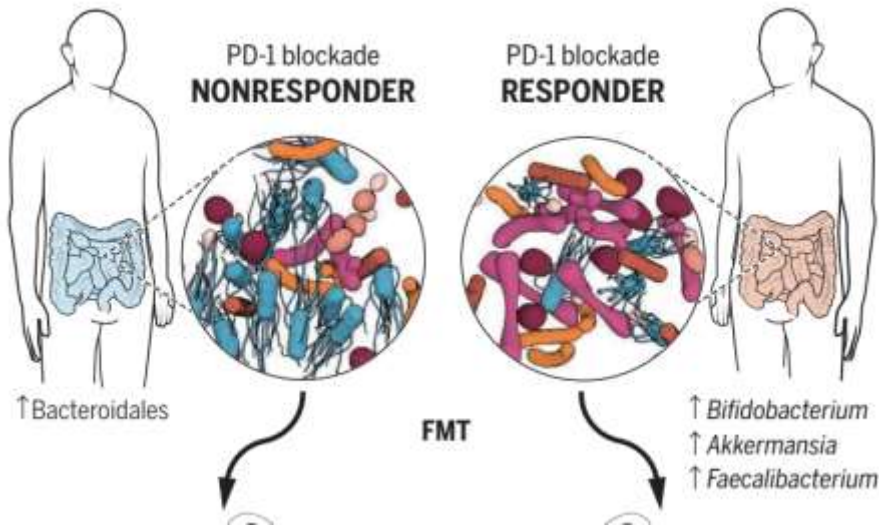
(Gastroenterology 2021)

Precision medicine using microbiota

Intestinal microbiota influence cancer patient responses to immunotherapy

therapeutic drugs. For example, bacteria modulate the antitumor efficacy in pre-clinical models of various chemotherapies (2-4) and immunotherapeutic agents (5, 6). Conceptually, these findings suggest that

immune system are essential for optimal drug efficacy. However, there is limited information regarding the functional impact of the composition of the human microbiome and therapeutic outcomes in cancer patients. On pages 91, 97, and 104 of this



(Christian Jobin Science 2018)

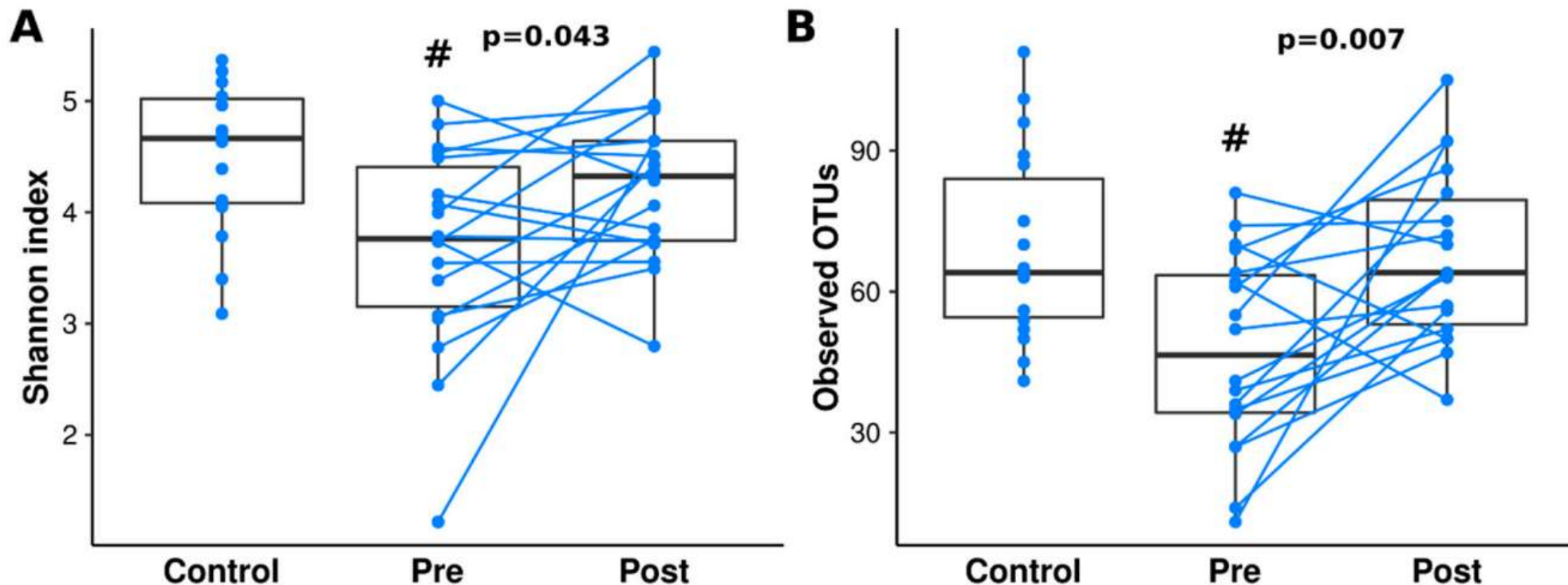
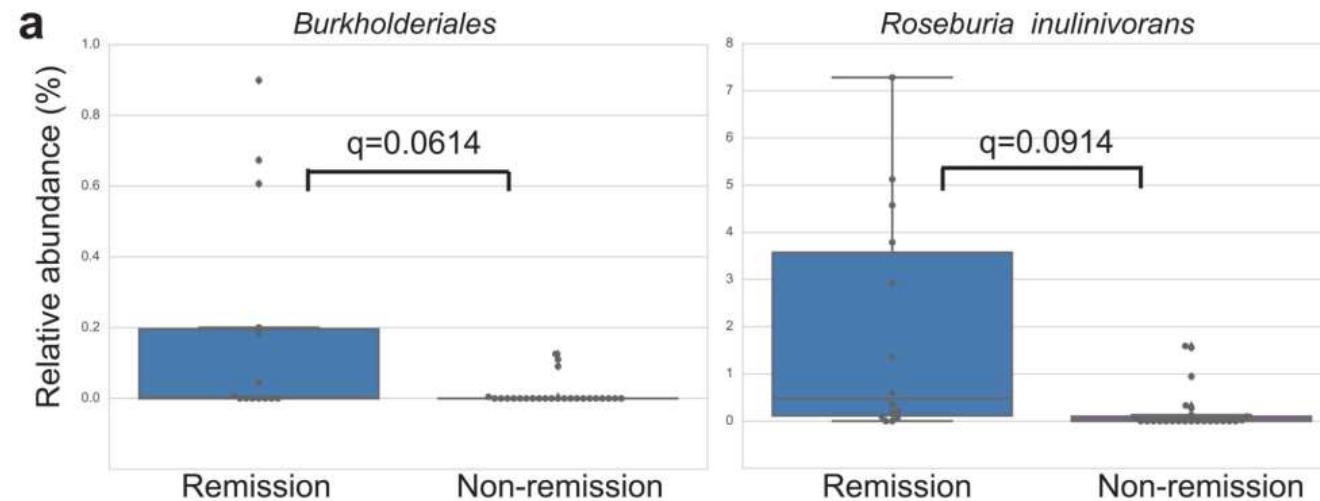
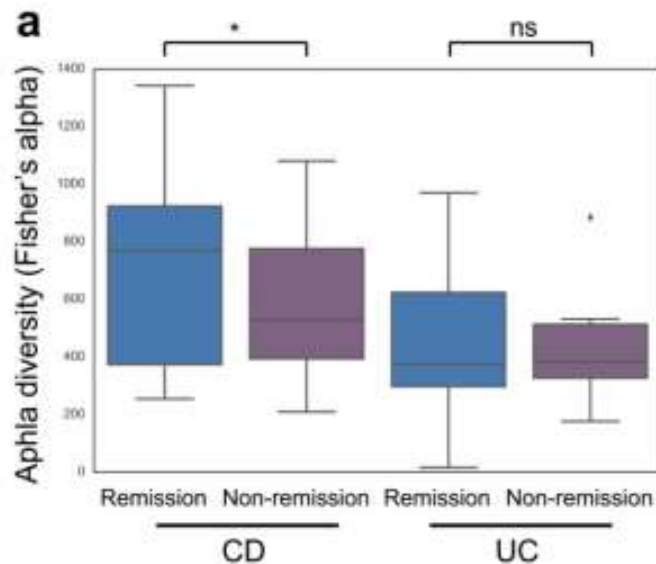


Figure 1. Differences in alpha diversity analysis of control and patients with CD (Crohn's disease) pre- and post-IFX therapy. Alpha diversity was measured by the Shannon index (A), observed OTUs (B), Faith's phylogenetic diversity (C), and Pielou's measure of species evenness (D). Kruskal-Wallis differences in calculated alpha diversity metrics between controls and patients with CD after induction therapy (Figure 1). This confirms that short-term induction IFX treatment reduces dissimilarity between patients with CD and controls, but it does not restore a typical healthy microbiome. Our analysis also revealed that there are 32 species present in patients with CD both before and after therapy, which are (J. Clin. Med. 2020, 9, 687)

Gut microbiome function predicts response to anti-integrin biologic therapy in Inflammatory Bowel diseases

Ashwin N Ananthakrishnan^{*,1,2}, Chengwei Luo^{*,3}, Vijay Yajnik^{1,2}, Hamed Khalili^{1,2}, John J Garber¹, Betsy W Stevens², Thomas Cleland¹, and Ramnik J Xavier^{1,2,3,4}

and weeks 14, 30, and 54 after therapy initiation were assessed. Community α -diversity was significantly higher, and *Roseburia inulinivorans* and a *Burkholderiales* species were more abundant at baseline among CD patients achieving week 14 remission. Several significant

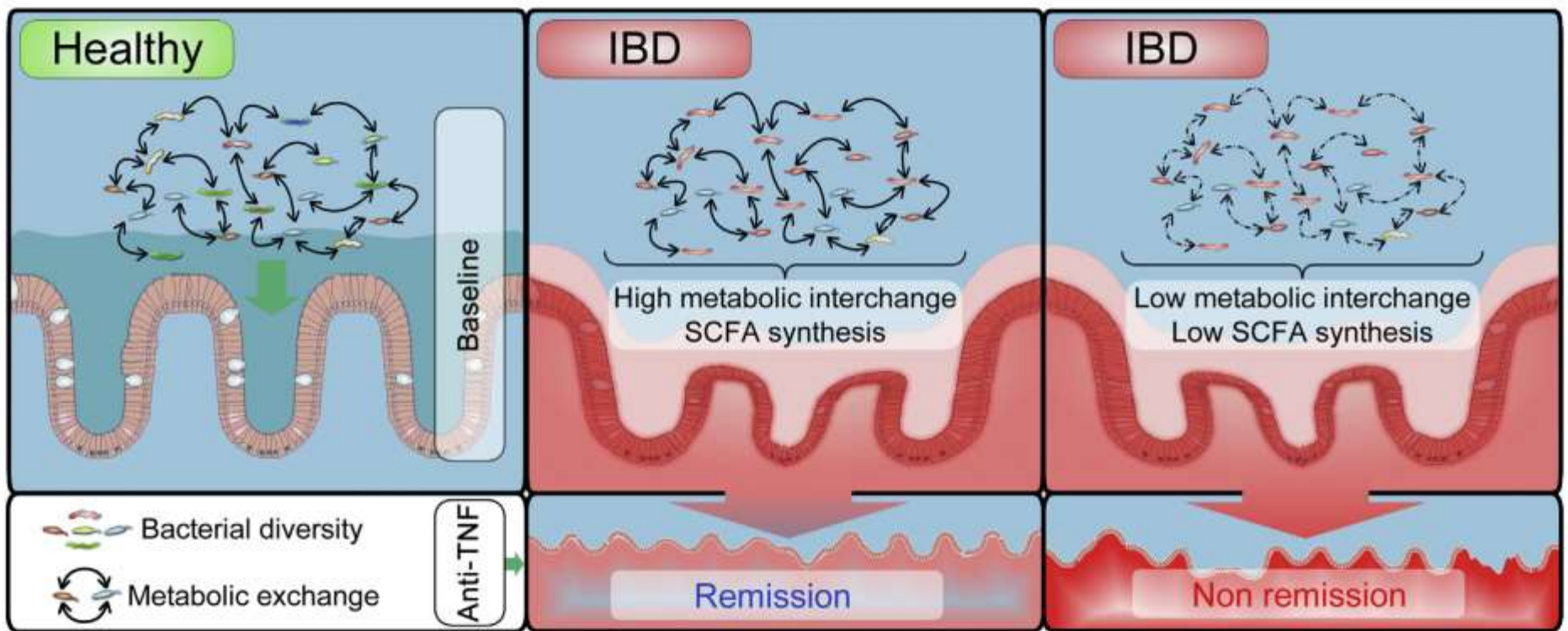


(Cell Host Microbe. 2017)



Metabolic Functions of Gut Microbes Associate With Efficacy of Tumor Necrosis Factor Antagonists in Patients With Inflammatory Bowel Diseases

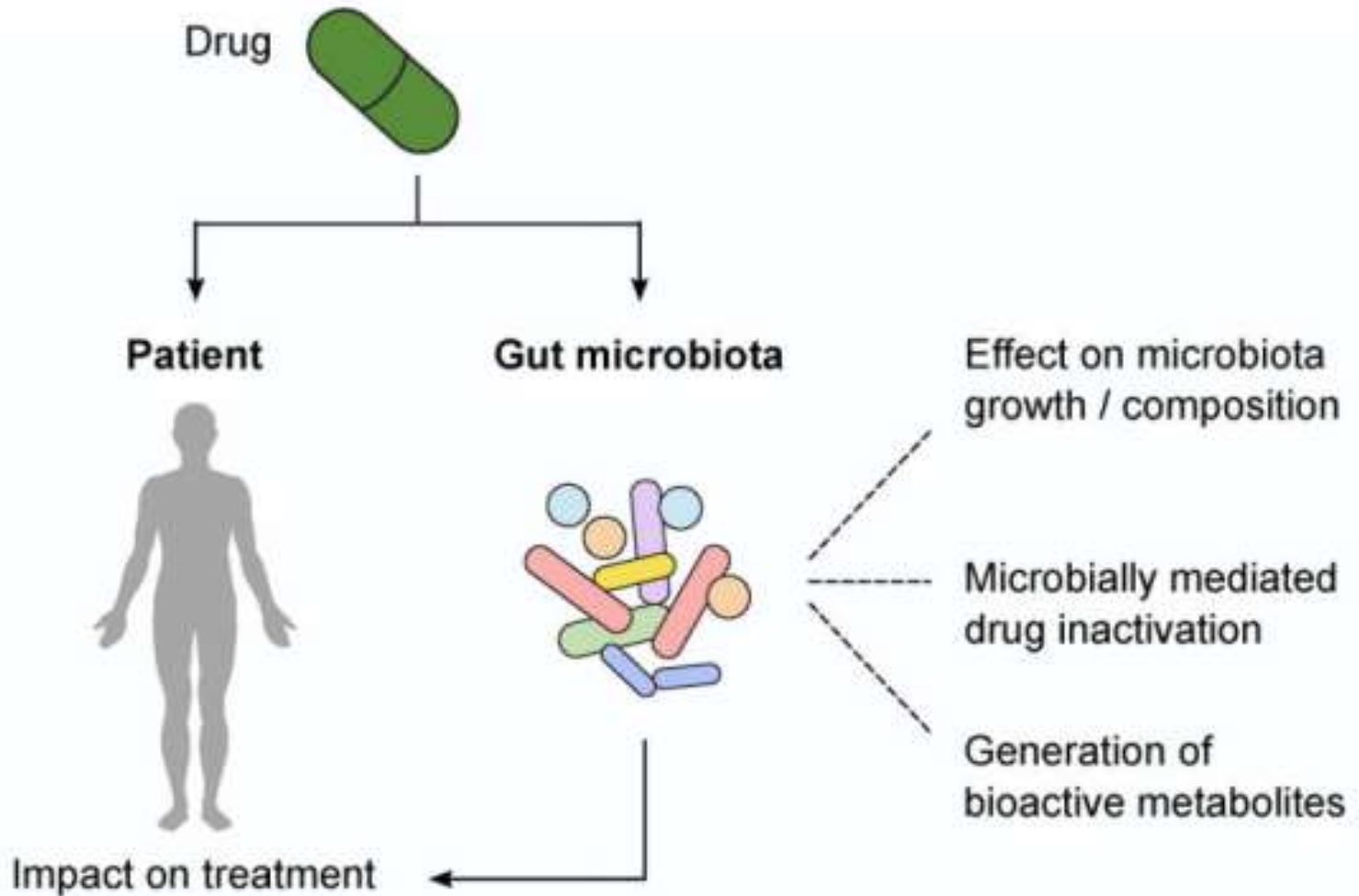
Konrad Aden,^{1,2,*} Ateequr Rehman,^{1,*} Silvio Waschina,^{3,*} Wei-Hung Pan,¹ Alesia Walker,⁴ Marianna Lucio,⁴ Alejandro Mena Nunez,¹ Richa Bharti,¹ Johannes Zimmerman,³ Johannes Bethge,² Berenice Schulte,² Dominik Schulte,² Andre Franke,¹ Susanna Nikolaus,² Johann Oltmann Schroeder,² Doris Vandeputte,⁵ Jeroen Raes,⁵ Silke Szymczak,⁶ Georg H. Waetzig,⁷ Rainald Zeuner,² Philippe Schmitt-Kopplin,⁴ Christoph Kaleta,^{3,§} Stefan Schreiber,^{1,2,§} and Philip Rosenstiel^{1,§}



Precision medicine goes microscopic: engineering the microbiome to improve drug outcomes

Kathy N. Lam^{1,+}, Margaret Alexander^{1,+}, Peter J. Turnbaugh^{1,2,3,*}

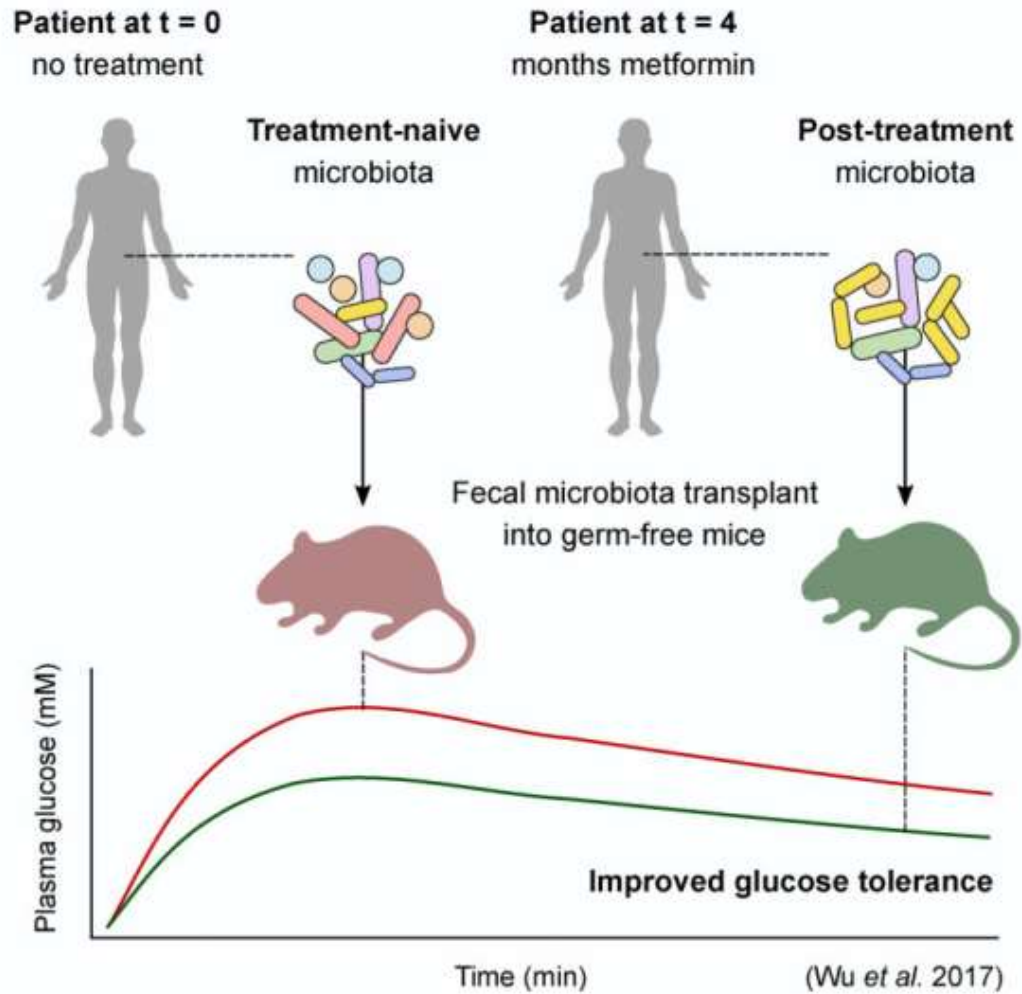
A



(Cell Host Microbe 2019)

Precision medicine goes microscopic: engineering the microbiome to improve drug outcomes

Kathy N. Lam^{1,+}, Margaret Alexander^{1,+}, Peter J. Turnbaugh^{1,2,3,*}





(Cell Host Microbe 2019)

Microflora-Microbiome

New players?

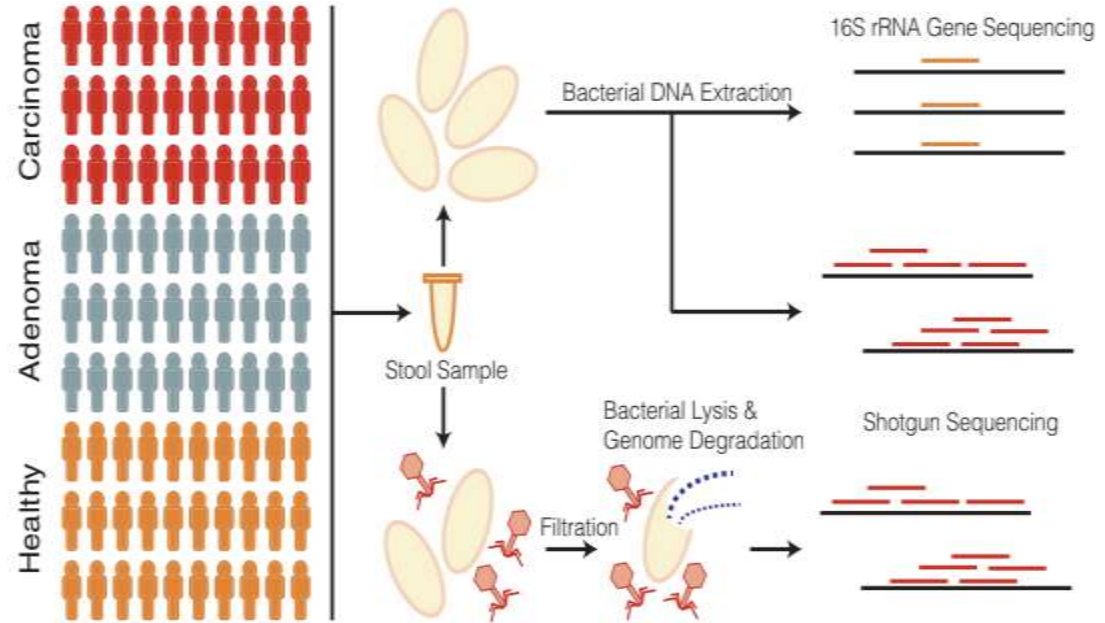
Diagnostic Potential and Interactive Dynamics of the Colorectal Cancer Virome

(MBio. 2018)

Geoffrey D. Hannigan,^a  Melissa B. Duhaime,^b Mack T. Ruffin IV,^c Charlie C. Koumpouras,^a  Patrick D. Schloss^a

^aDepartment of Microbiology and Immunology, University of Michigan, Ann Arbor, Michigan, USA

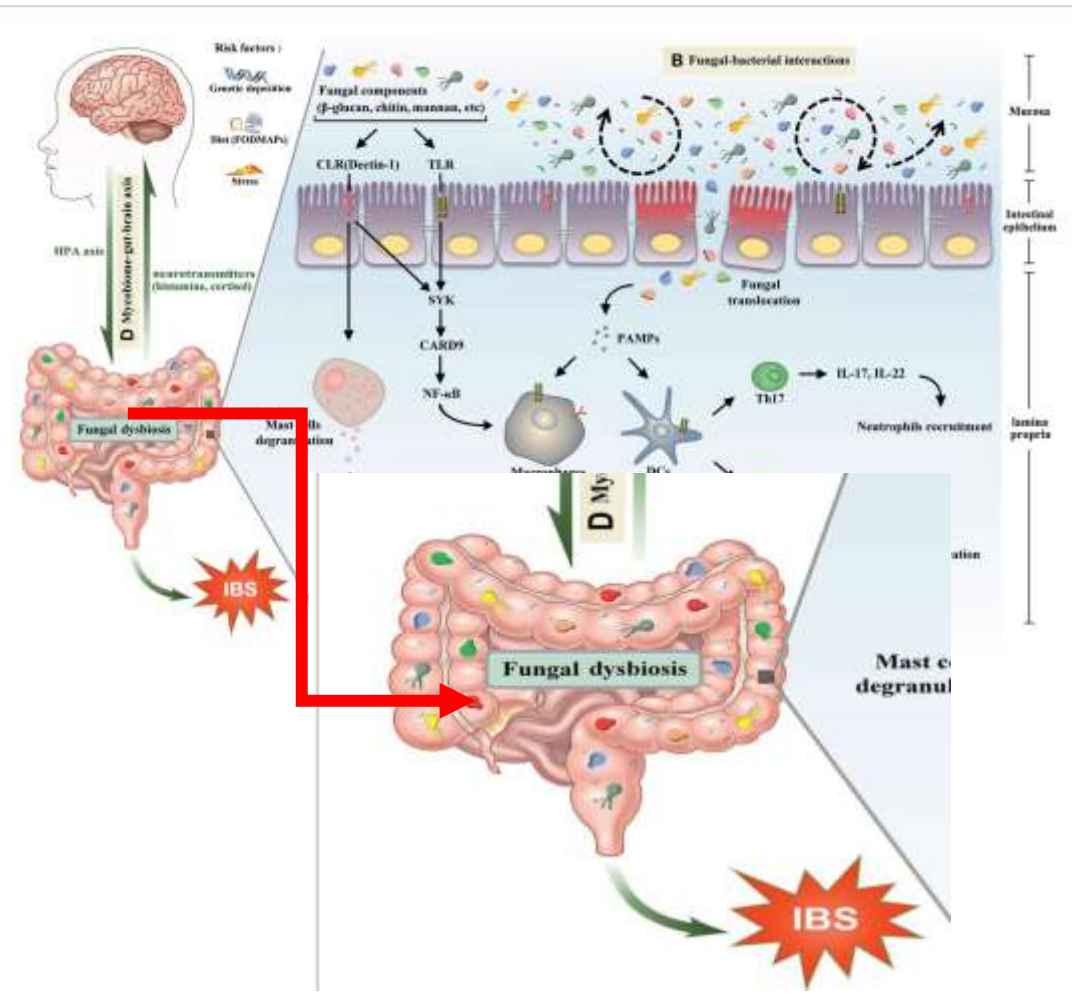
Our study cohort consisted of stool samples collected from 90 human subjects, 30 of whom had healthy colons, 30 of whom had adenomas, and 30 of whom had carcinomas. Half of each stool sample was used to sequence the bacterial communities using both 16S rRNA gene and **shotgun sequencing techniques**. The other half of each stool sample was purified for virus-like particles (VLPs) before genomic DNA extraction and shotgun metagenomic sequencing were performed.



network hubs. These results provide foundational evidence that bacteriophage communities are associated with colorectal cancer and potentially impact cancer progression by altering the bacterial host communities.

The Potential Role of Gut Mycobiome in Irritable Bowel Syndrome

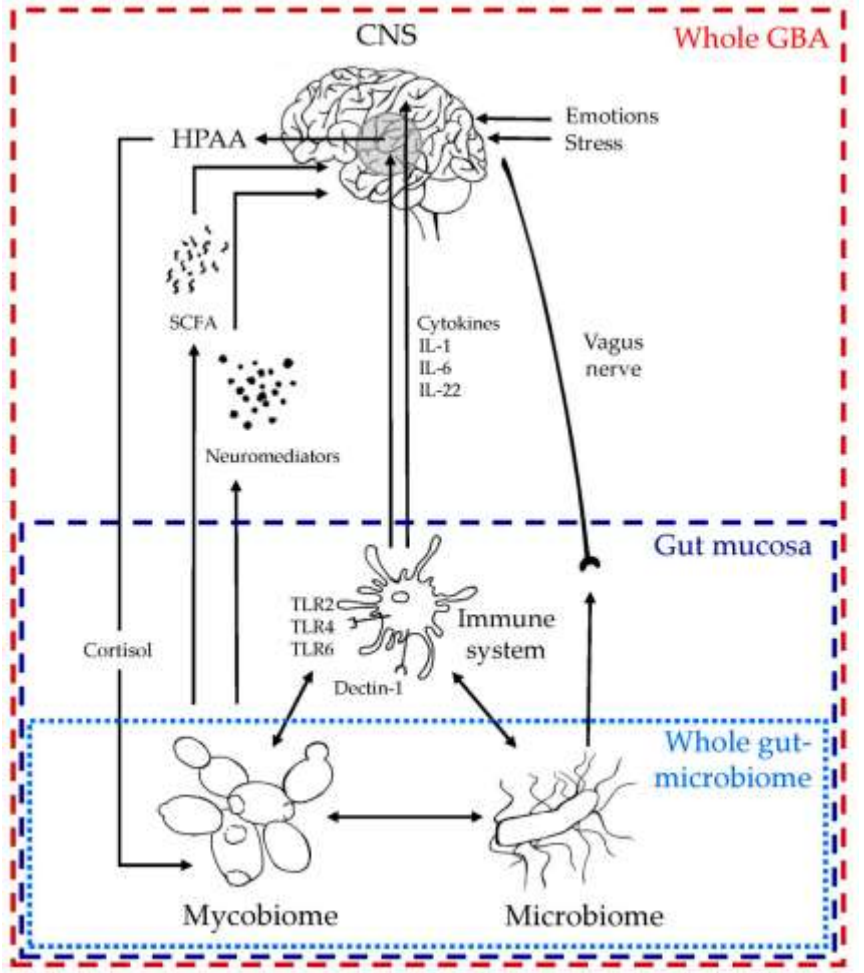
Yu Gu¹, Guoqiong Zhou¹, Xiali Qin, Shumin Huang, Bangmao Wang and Hailong Cao^{*}
 Department of Gastroenterology and Hepatology, Tianjin Medical University General Hospital, Tianjin, China



(Front in Microbiol 2019)

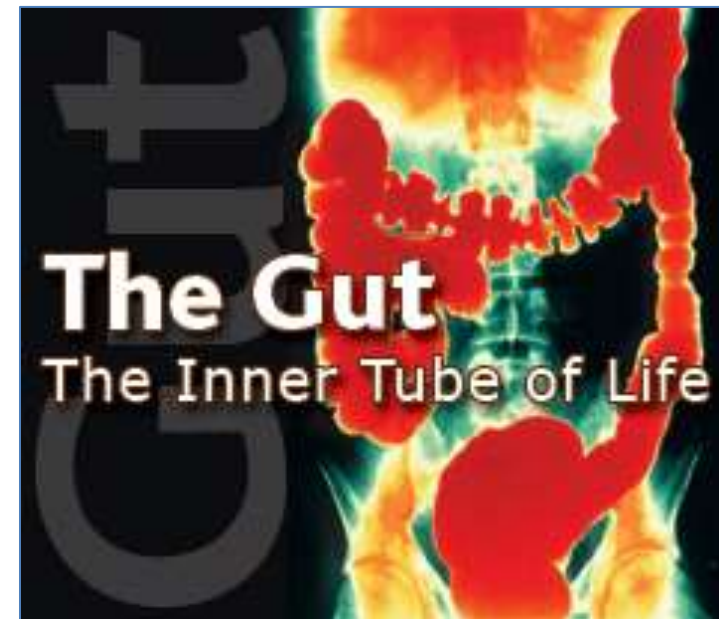
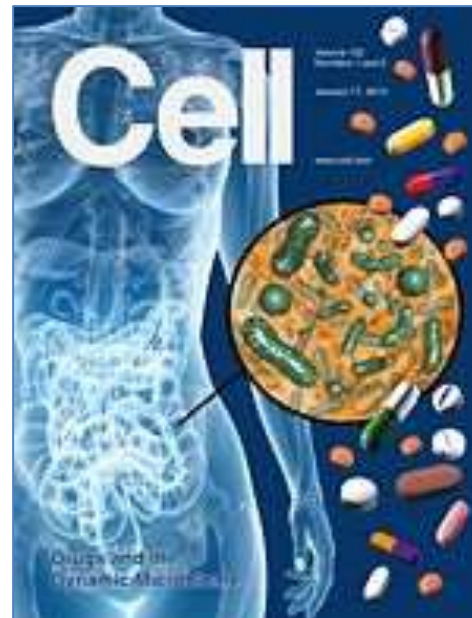
The Mycobiome: A Neglected Component in the Microbiota-Gut-Brain Axis

Raphaël Enaud^{1,2,3,*}, Louise-Eva Vandendorgh^{1,3,4}, Noémie Coron^{1,2,3}, Thomas Bazin^{1,2}




(Microorganisms 2018)

Το εντερικό μικροβίωμα στο κέντρο της σύγχρονης έρευνας



Το εντερικό μικροβίωμα στο επίκεντρο της σύγχρονης χρηματοδότησης



www.theatlantic.com/science/archive/2016/05/white-house-launches-the-national-microbiome-initiative/482598/

The Atlantic

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The White House Launches the National Microbiome Initiative

Half a billion dollars are being pledged to study the microbes in humans, crops, soils, oceans, and more.

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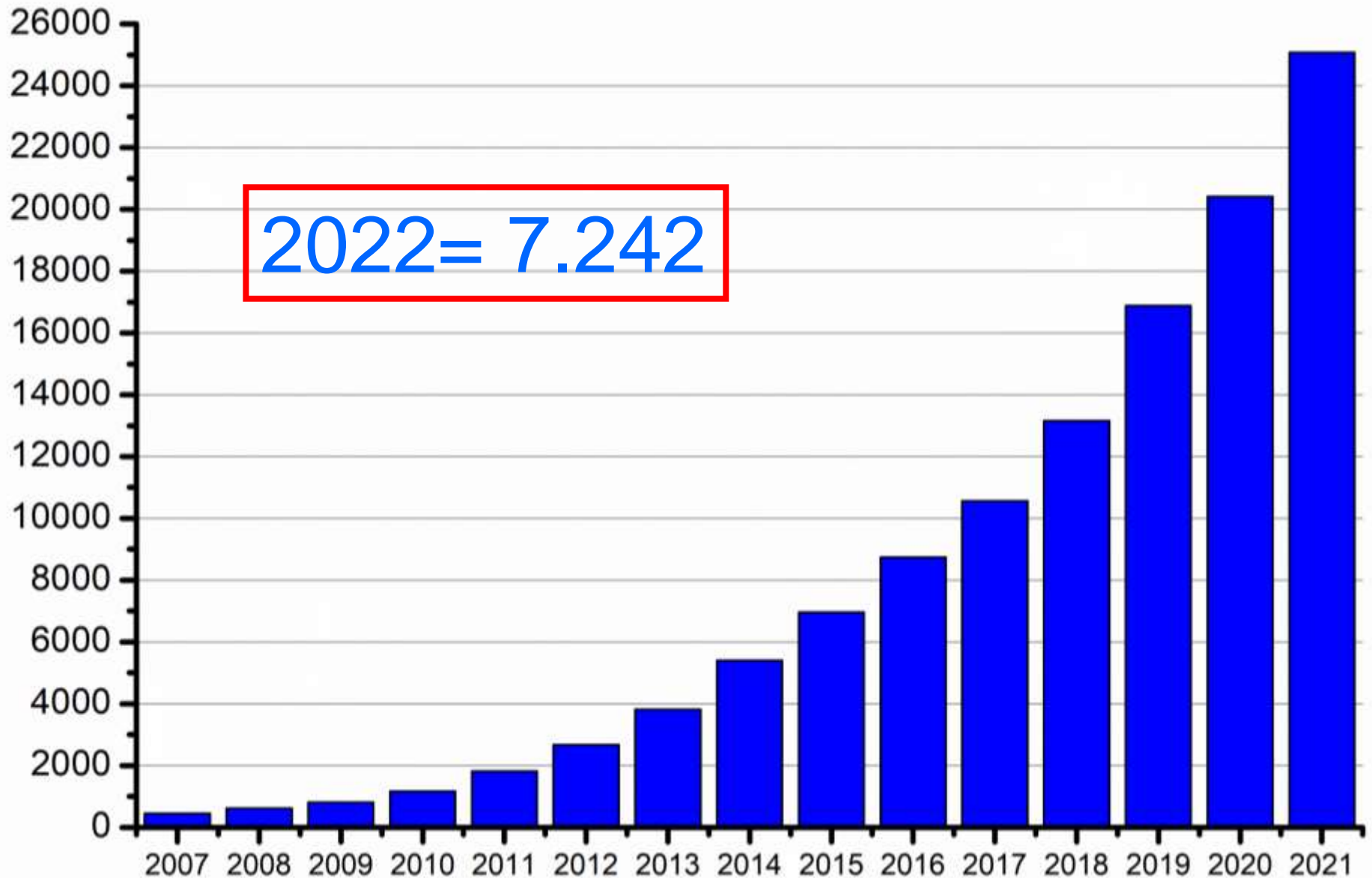
Το εντερικό μικροβίωμα στο στόχαστρο της σύγχρονης φαρμακοβιομηχανίας



The screenshot shows a web browser displaying an article from The Wall Street Journal. The article title is "Microbiome Companies Attract Big Investments" and the subtitle is "Companies explore microbiome science to achieve breakthroughs in medicine, agriculture". The article is categorized under "BUSINESS | JOURNAL REPORTS: LEADERSHIP". A video player is visible below the article title, showing a scene with laboratory equipment. To the right of the article, there is a "Recommended Videos" section with a video titled "Kaine vs. Pence: Key Takeaways from VP Debate".

- ❖ Global sales of Probiotics – 31.1 billion in 2015
- ❖ Fastest growing segment of the global dietary supplement & functional food industries

Papers in PubMed with Keyword Microbiome vs Year



Σαν συμπέρασμα...

- Ο άνθρωπος και η μικροβιακή του χλωρίδα είναι δύο συμβιωτικοί οργανισμοί,
- Που έχουν απόλυτη ανάγκη ο ένας τον άλλον
- Παίζει σημαντικό ρόλο στην παθοφυσιολογία και ανοσολογία του γαστρεντερικού σωλήνα.
- Διαταραχές της εντερικής χλωρίδας φαίνεται να εμπλέκονται στην παθογένεια πολλών διαταραχών, πέραν του πεπτικού.
- Η δυσβίωση της εντερικής χλωρίδας φαίνεται να συνδέεται άμεσα με τους παθογενετικούς μηχανισμούς ανοσολογικών διαταραχών
- Η περαιτέρω μελέτη της χλωρίδας και του μικροβιώματος πιθανόν να μας αποκαλύψει παθογενετικούς μηχανισμούς ή/και να αποτελέσει στόχο νέων θεραπευτικών στρατηγικών.