5a Jornada de Recerca a l’Institut Català de la Salut

Barcelona, 8 de Novembre de 2012

La microbiota intestinal com a òrgan: impacte en fisiologia i patologia

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VHIR

Prokaryotes and Eukaryotes

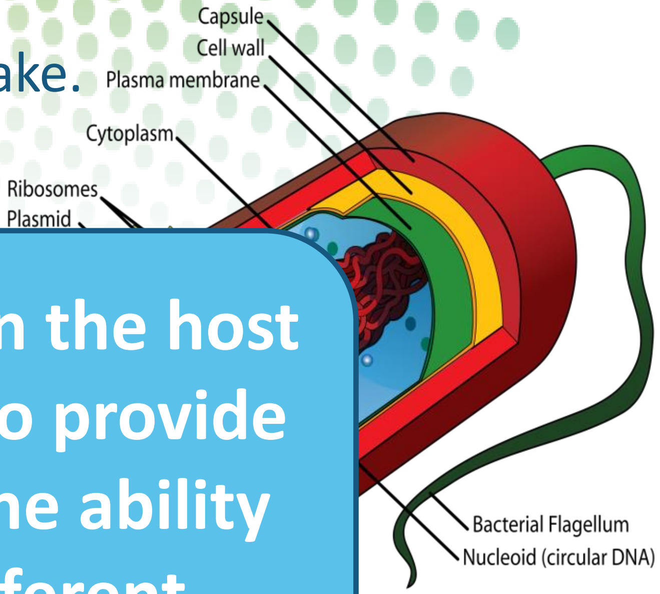
BACTERIA:

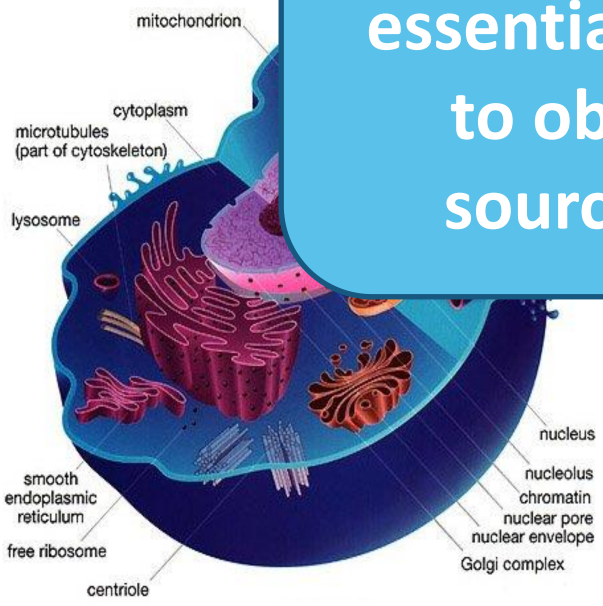
✓ Genetic material is not membrane bound: low number of genes, high incidence of foreign gene uptake.

✓ Multispecies communities required for survival.

✓ Complete c
from the env
compounds v

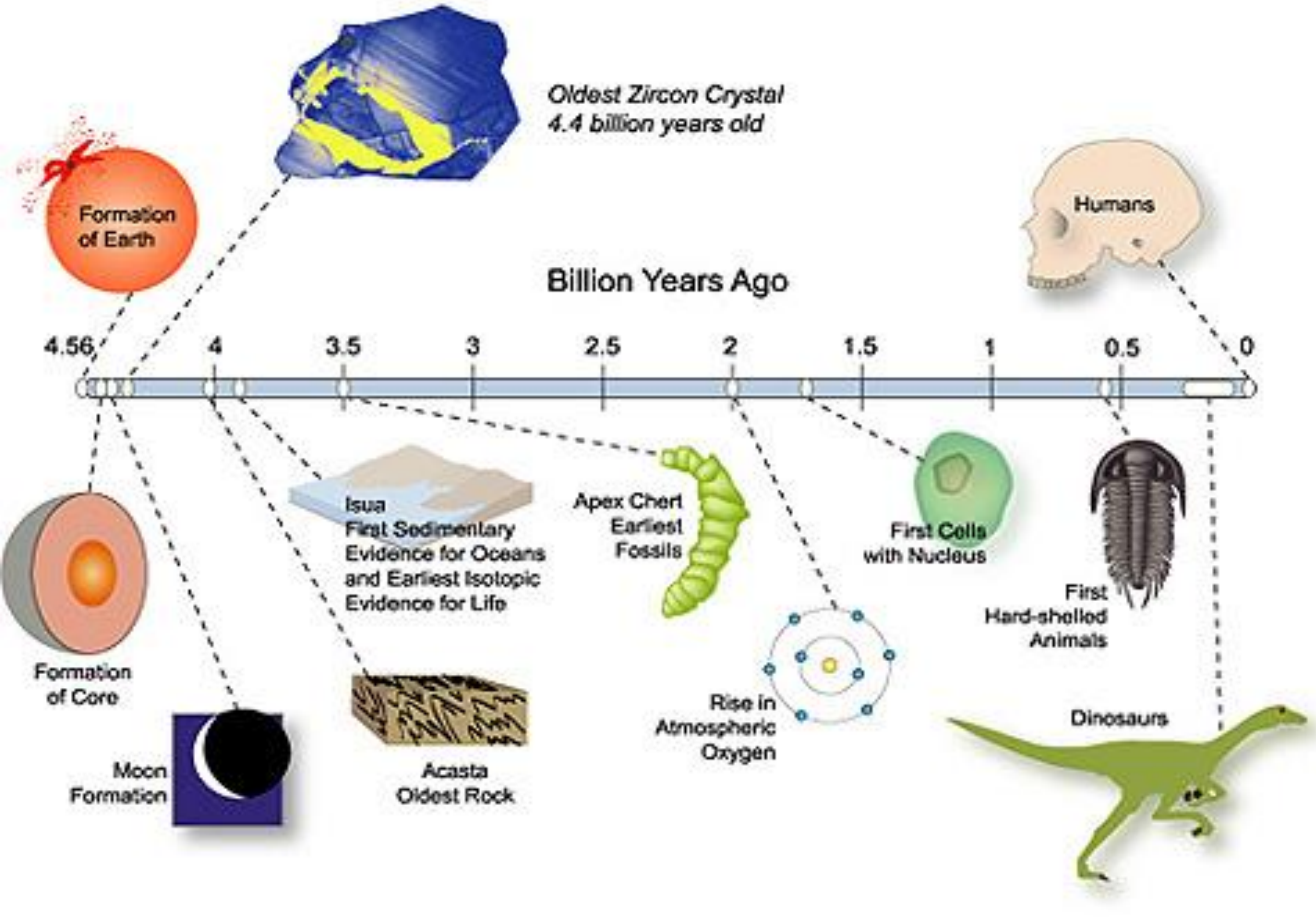
Gut Microbial communities in the host have evolutionary adapted to provide essential compounds and the ability to obtain energy from different sources (Moran, Curr Biol 2006)





es in a
f foreign gene
uptake

✓ Ancestral loss of genes for biosynthesis of essential compounds (amino-acids and vitamins) and for harvesting energy from complex sources.



Andree Valley

PRIMARY FUNCTIONS OF THE GUT MICROBIOTA

- **Metabolic functions:** fermentation of nondigestible dietary residue and endogenous mucus: salvage of energy as SCFA, production of vitamin K, absorption of ions.

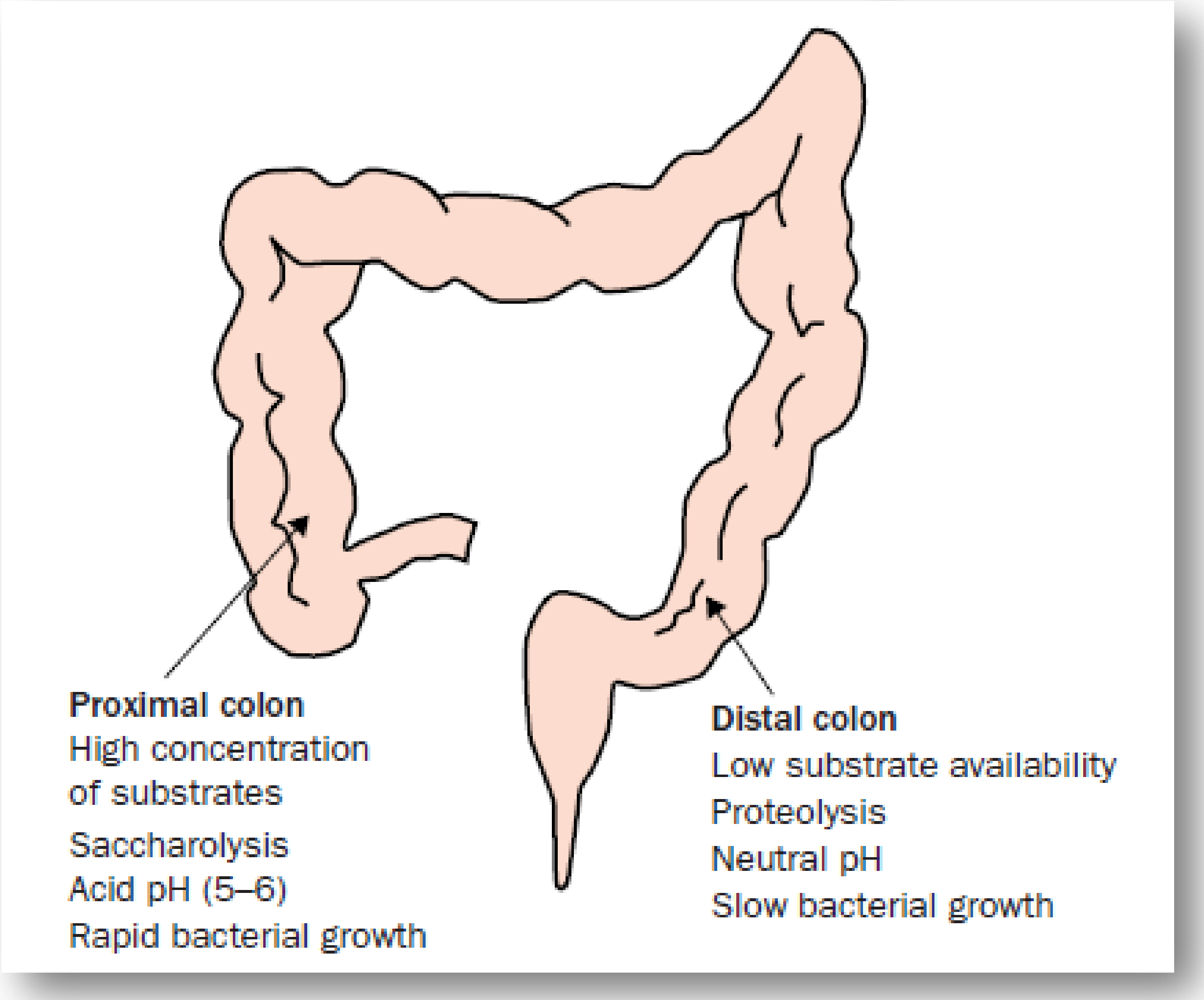
- **Defensive functions:** protection against pathogens (the barrier effect).

- **Trophic functions:** control of epithelial cell proliferation and differentiation; development and homeostasis of the immune system.

Vall
d'Hebron

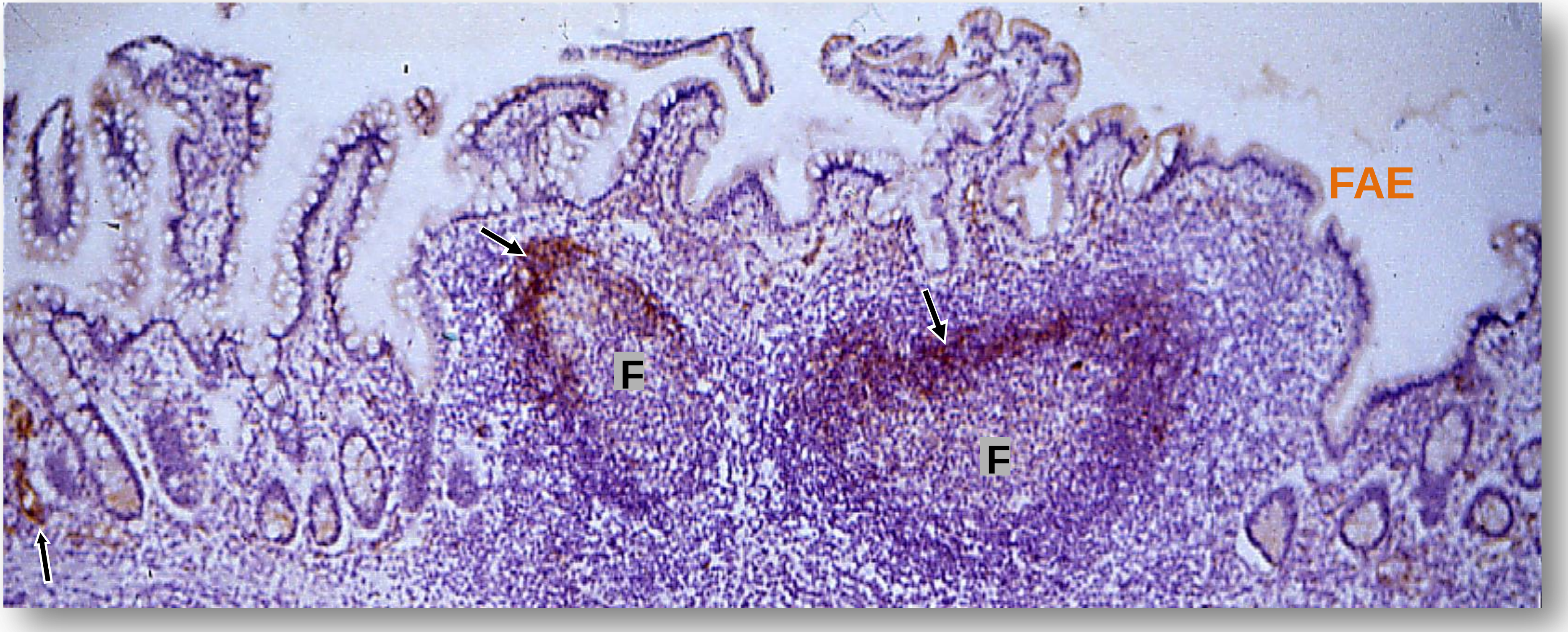
Guarner & Malagelada, Lancet 2003

The Gut Microbiota as a Metabolic Organ



Guarner & Malagelada, Lancet 2003

Induction of the Immune System

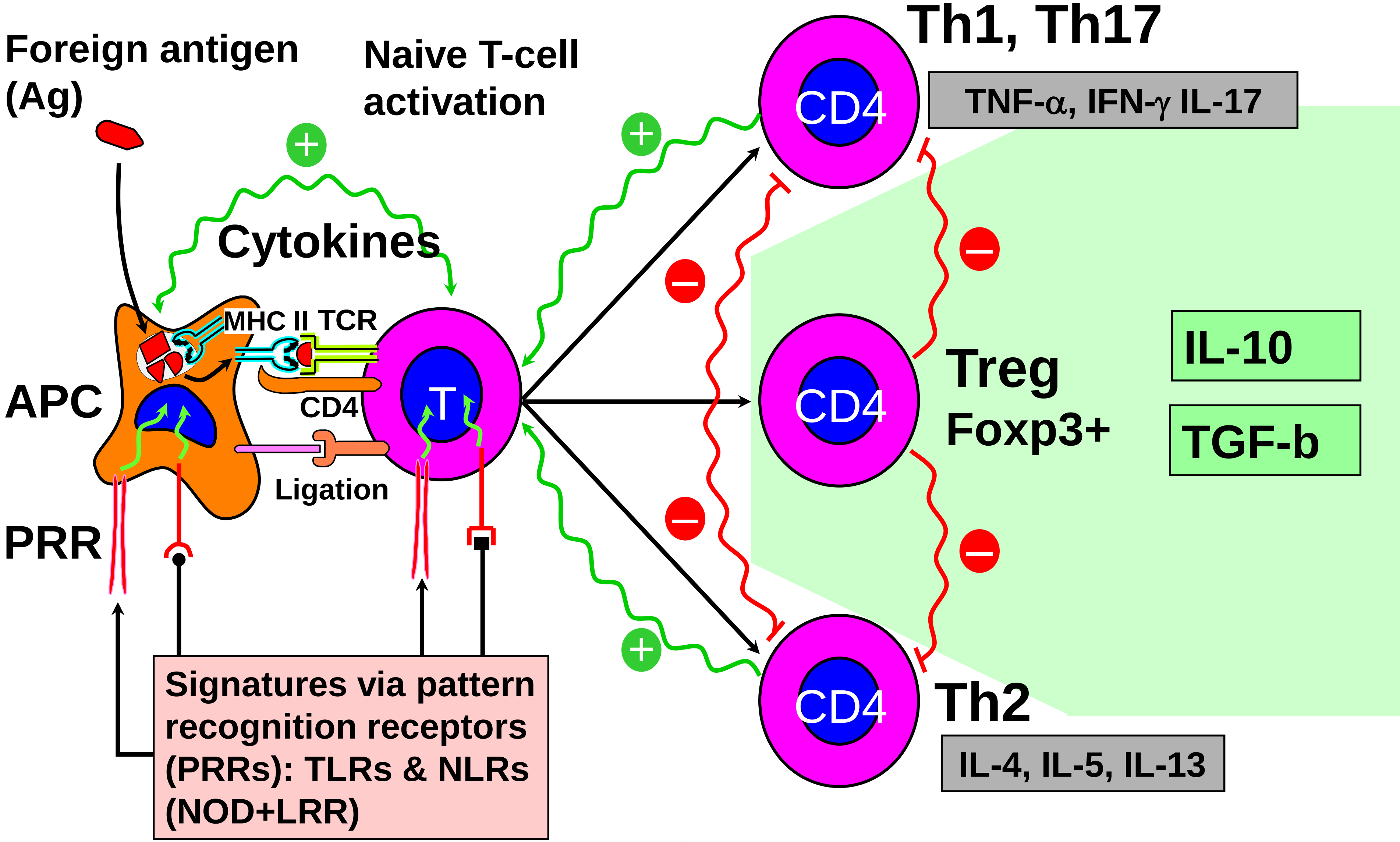


Gut-Associated Lymphoid Tissue structures are strategically situated in relation to the greatest concentration of microbiota

- **Peyer's patches:**
  distal ileum (nos. 100-250)
- **Isolated lymphoid follicles (ILFs):**
  large bowel (nos. ~ 30 000)

Brandtzaeg, Immunological Investigations 2010

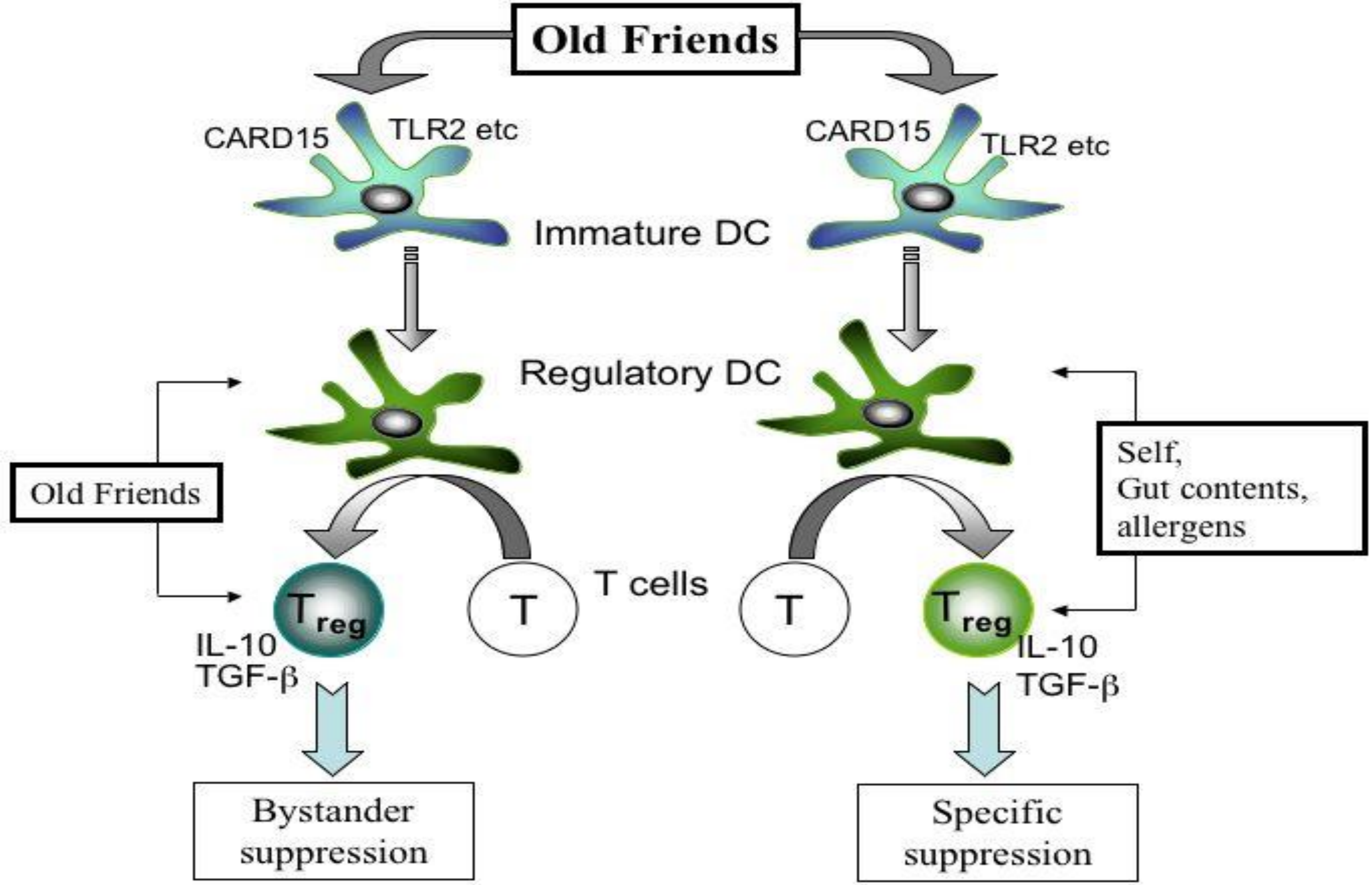
Decision making in the adaptive (acquired) immune system is instructed by the microbial impact on APCs and T cells



Signatures via pattern recognition receptors (PRRs): TLRs & NLRs (NOD+LRR)

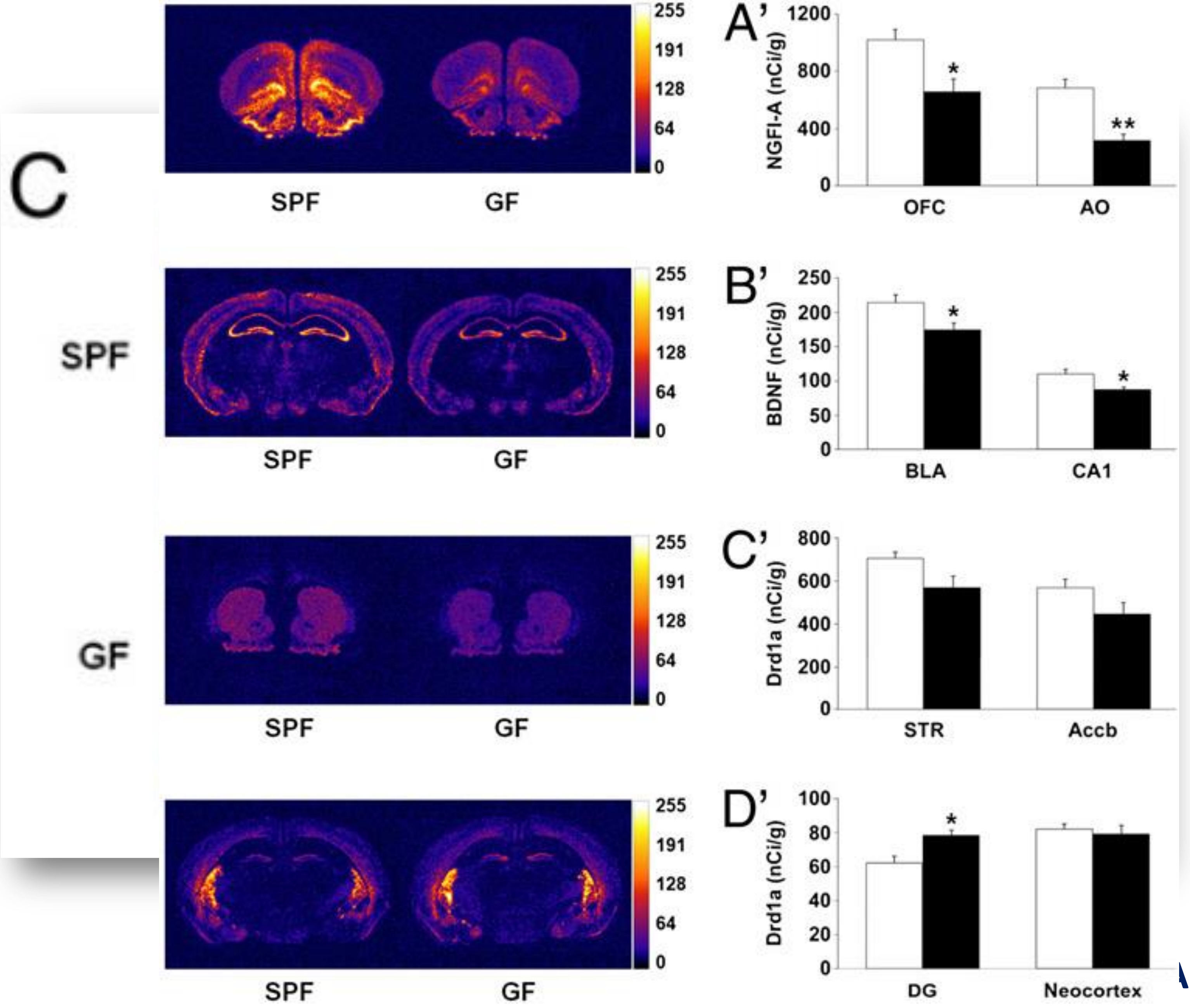
By Per Brandtzaeg in Guarner et al, Nature Clin Practice 2006

THE ‘OLD FRIENDS’ HYPOTHESIS



By Graham Rook in Guarner et al, Nature Clin Practice 2006

GUT MICROBIOTA, BRAIN DEVELOPMENT

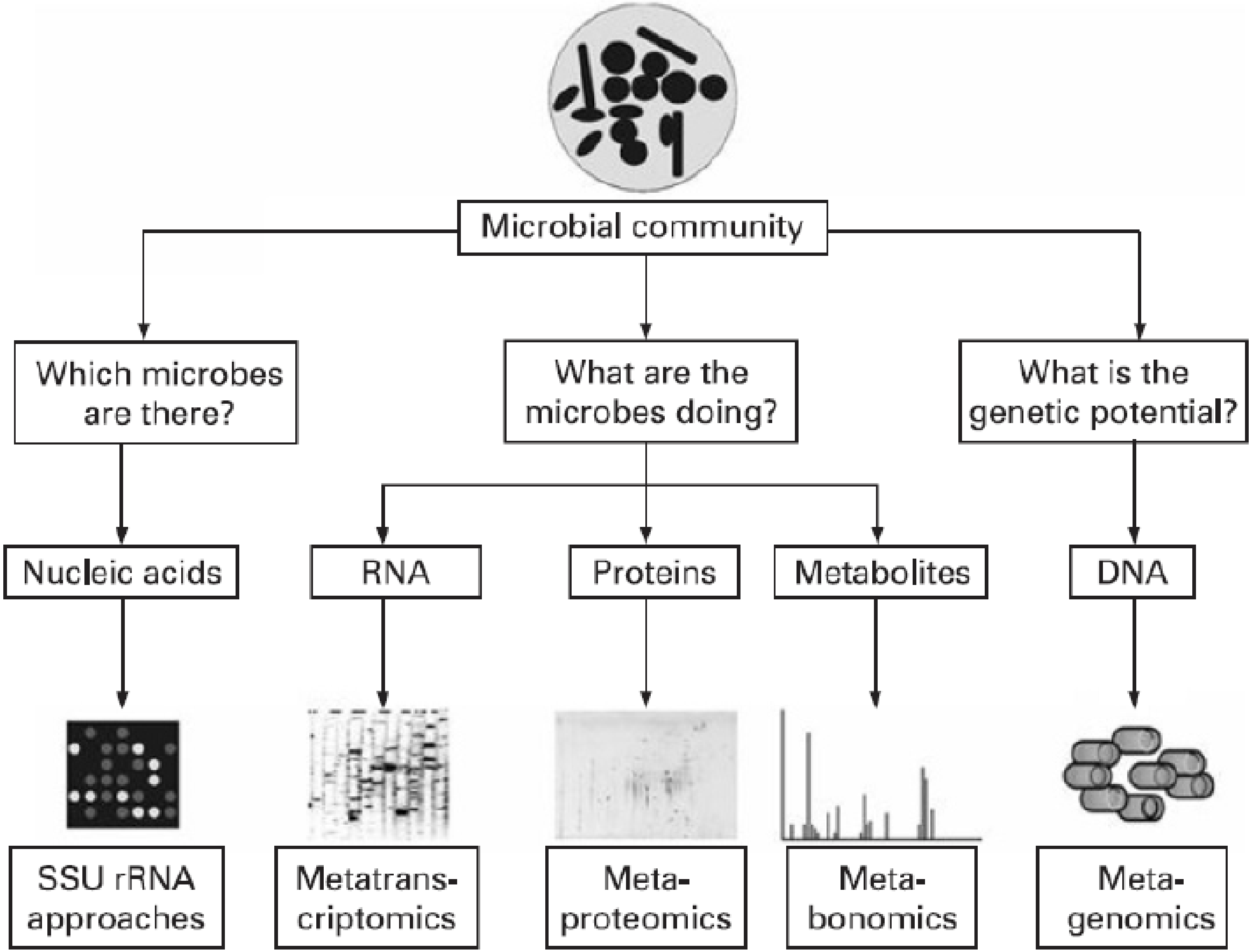


S 2011

MICROBIOTA DYSFUNCTION AND DISEASE

- infectious diarrhea, antibiotic-associated diarrhea
- septic complications: multisystem organ failure, diverticulitis, appendicitis
- functional disorders: constipation, bloating, irritable bowel syndrome
- obesity, type 2 diabetes, metabolic syndrome
- atopy, inflammatory bowel diseases, certain autoimmune disorders (?)
- colon cancer
- anxiety, autism (?)

Profiling Complex Microbial Communities



Microbial community

Which microbes are there?

What are the microbes doing?

What is the genetic potential?

Nucleic acids

RNA

Proteins

Metabolites

DNA

SSU rRNA approaches

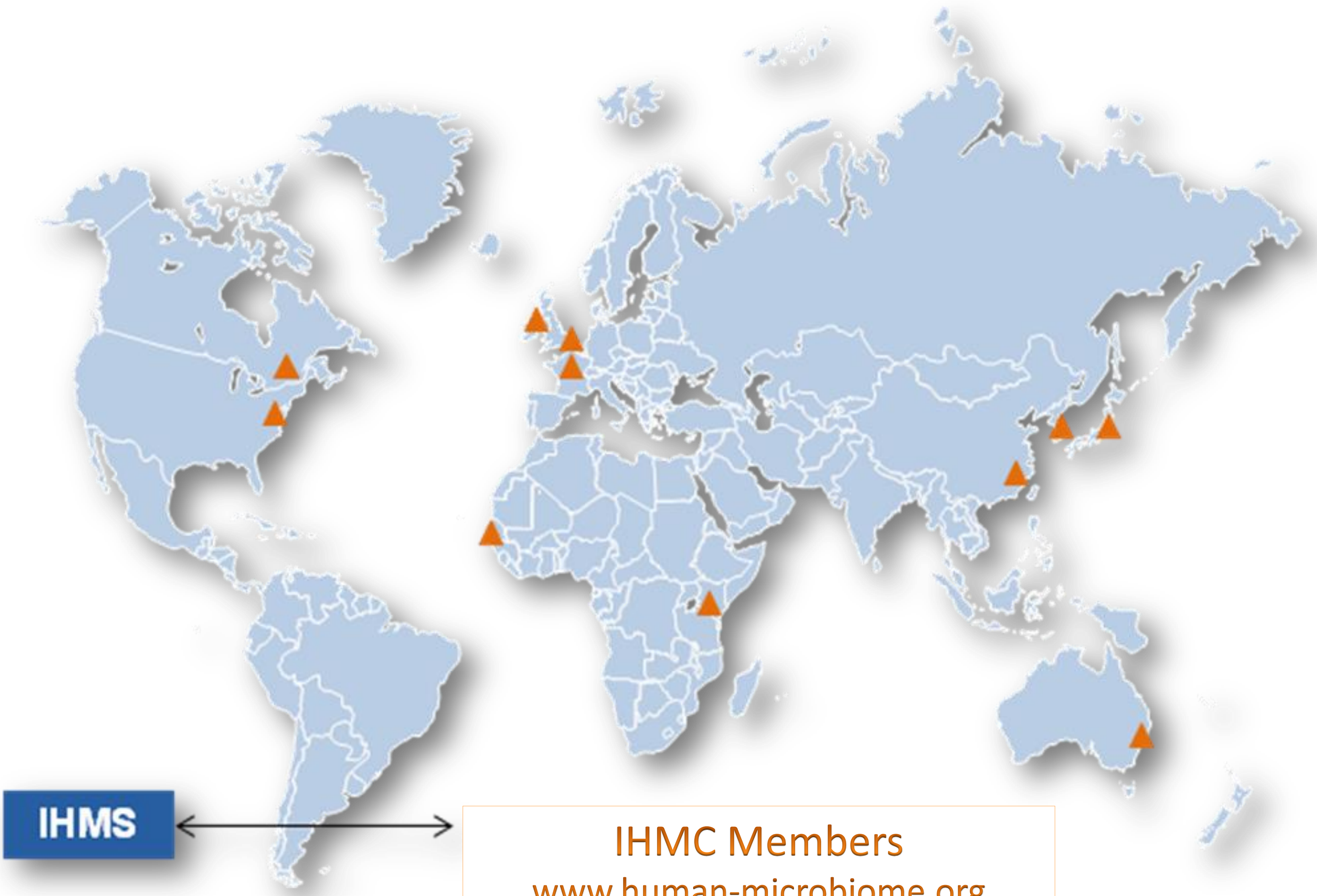
Metatranscriptomics

Metaproteomics

Metabonomics

Metagenomics

Zoetendal et al, Gut 2008



IHMS

IHMC Members

www.human-microbiome.org

Metagenomics of the Human Intestinal Tract

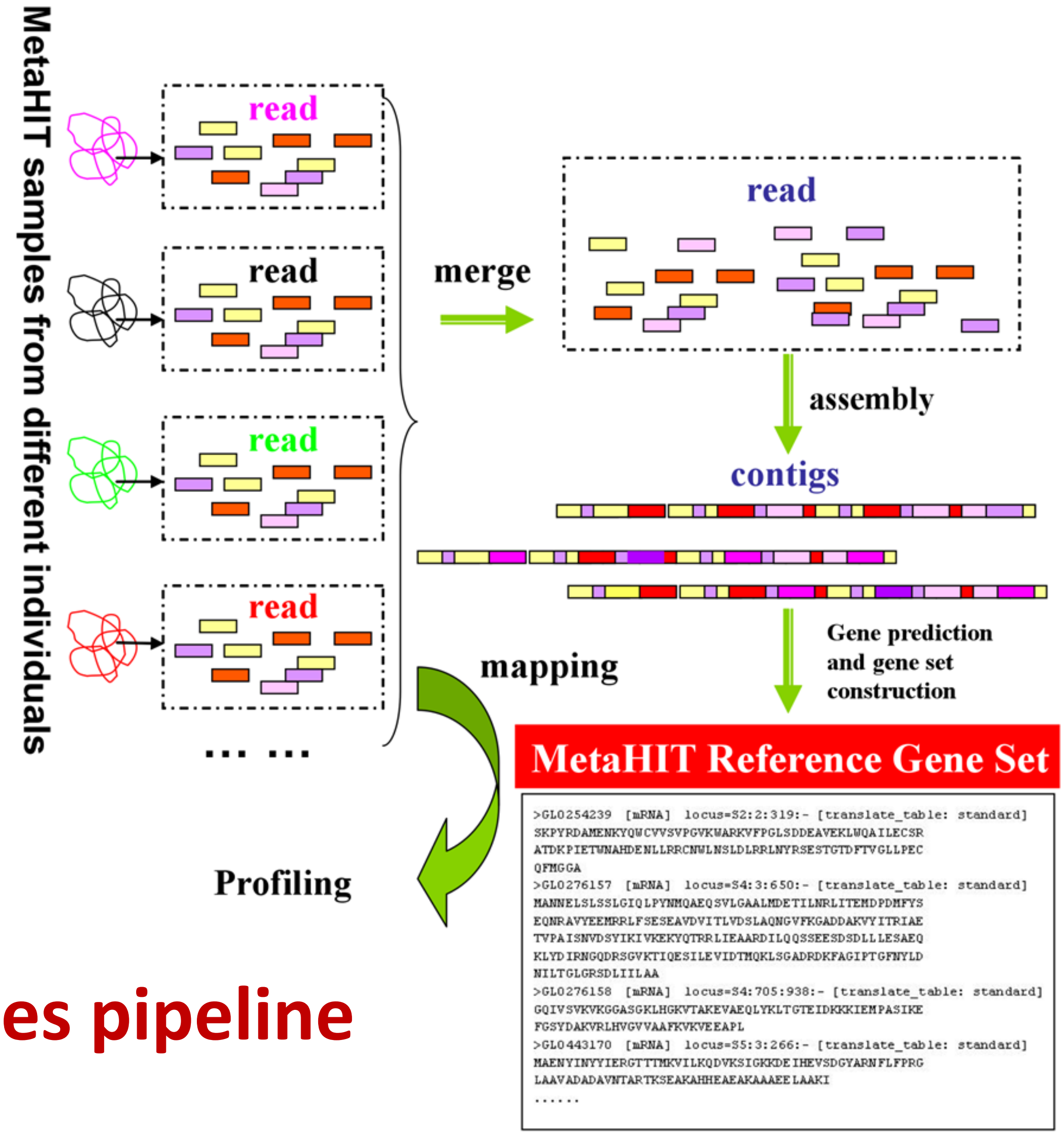
Meta
HIT

SEVENTH FRAMEWORK
PROGRAMME

http://www.metahit.eu/

Metagenome: collection of genes present in an ecosystem, consisting of the genomes of many individual organisms.

The pipeline of human intestinal metagenomics analysis using Solexa shotgun sequencing



```
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ATDKPIETWNAHDENLLRRCNWLNSLDLRRLNYRSESTGTDFTVGLLPEC
QFMGGA
>GL0276157  [mRNA]  locus=S4:3:650:- [translate_table: standard]
MANNELSLSSLGIQLPYNMQAEQSVLGAALMDETILNRLITEMDPDMFYS
EQNRAVYEEMRRLFSESEAVDVITLVDSLAQNGVFKGADDAKVYITRIAE
TVPAISNVDSYIKIVKEKYQTRRLIEAARDILQQSSEESDSDLLLESAEQ
KLYDIRNGQDRSGVKTIQESILEVIDTMQKLSGADRDKFAGIPTGFNYLD
NILTGLGRSDLIILAA
>GL0276158  [mRNA]  locus=S4:705:938:- [translate_table: standard]
GQIVSVKVKGGASGKLHGKVTAKEVAEQLYKLTGTEIDKKKIEMPASIKE
FGSYDAKVRLHVGVVAAFKVKVEEAPL
>GL0443170  [mRNA]  locus=S5:3:266:- [translate_table: standard]
MAENYINYYIERGTTTMKVILKQDVKSIGKKDEIHEVSDGYARNFLFPRG
LAAVADADAVNTARTKSEAKAHHEAEAKAAAEELAAKI
......
```

Multiple samples pipeline

Standard analysis: Ca 14 Mio ORFs, all-against-all blast

12.000 days on one CPU (ca 5 days real time at BSC)

Data transfer a major issue

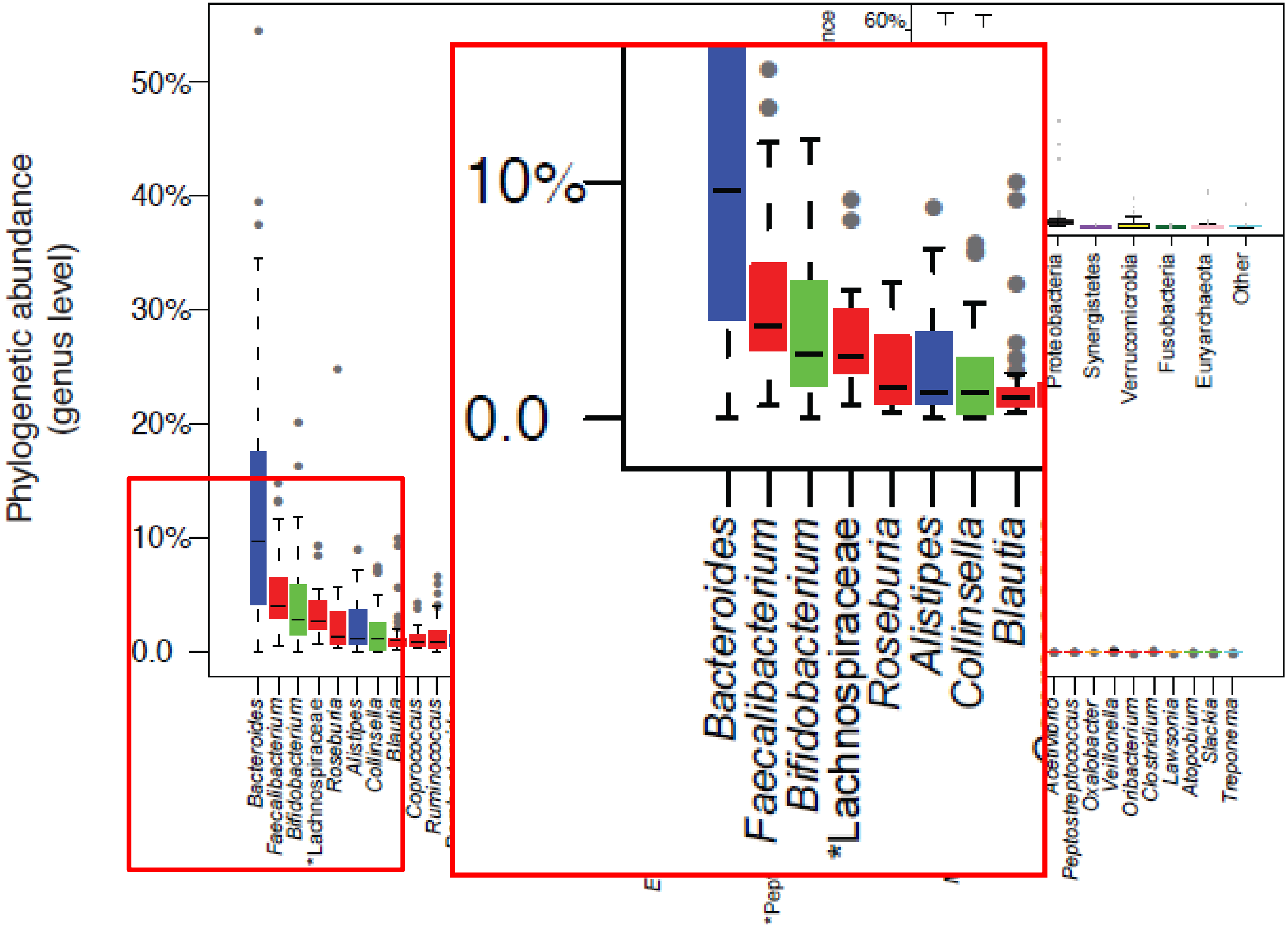
MareNostrum supercomputer Barcelona

The Human Gut Metagenome

	# of genes
Average gene set per individual	590,384
Common (>50% of individuals)	294,110
Rare (<20% of individuals)	2,375,655

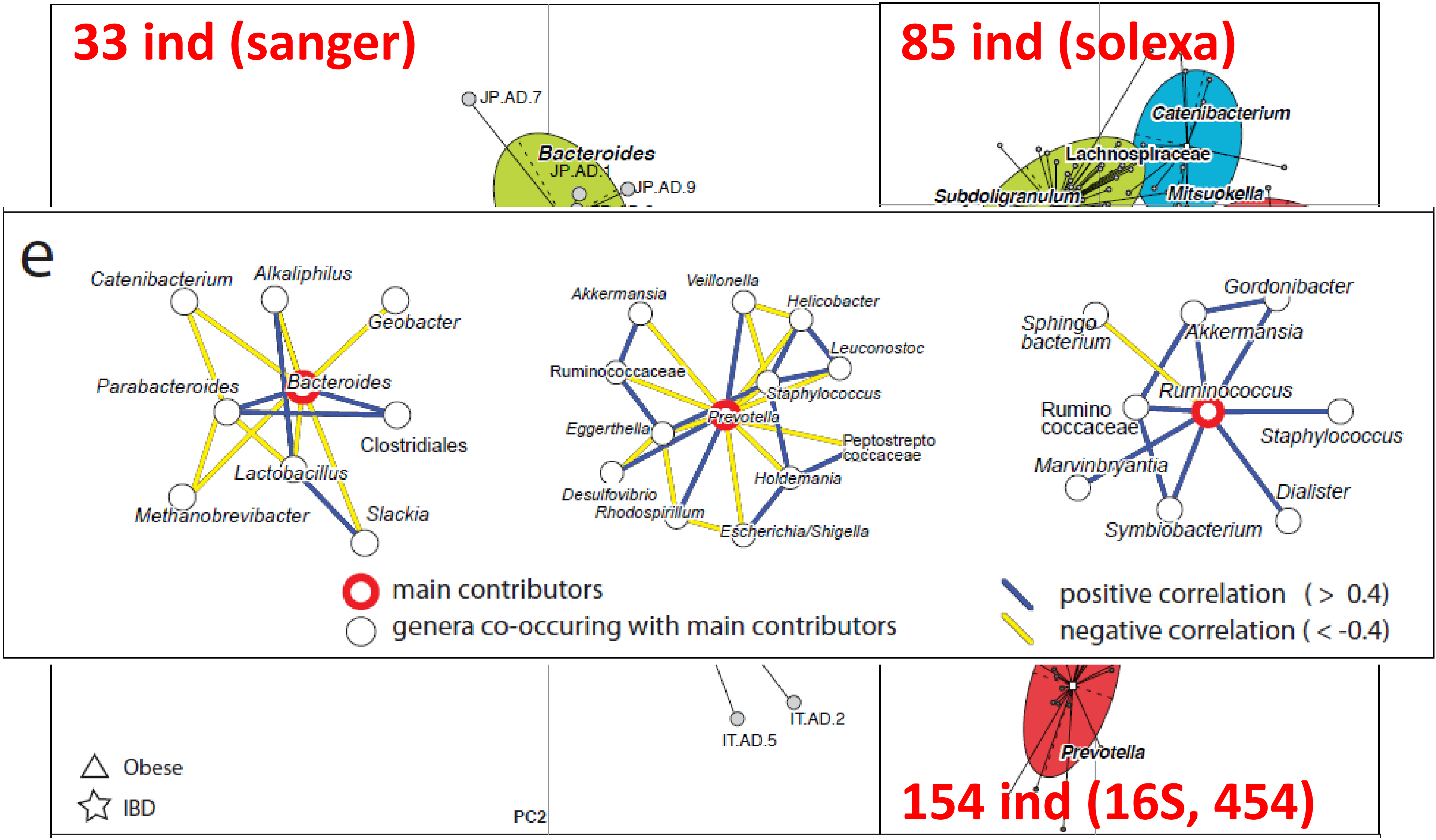
Qin et al, Nature 2010

Most abundant genera in the human gut microbiota



Arumugam, Raes et al, Nature 2011

Enterotypes



33 ind (sanger)

85 ind (solexa)

154 ind (16S, 454)

Particular constellations of bacterial species in individuals

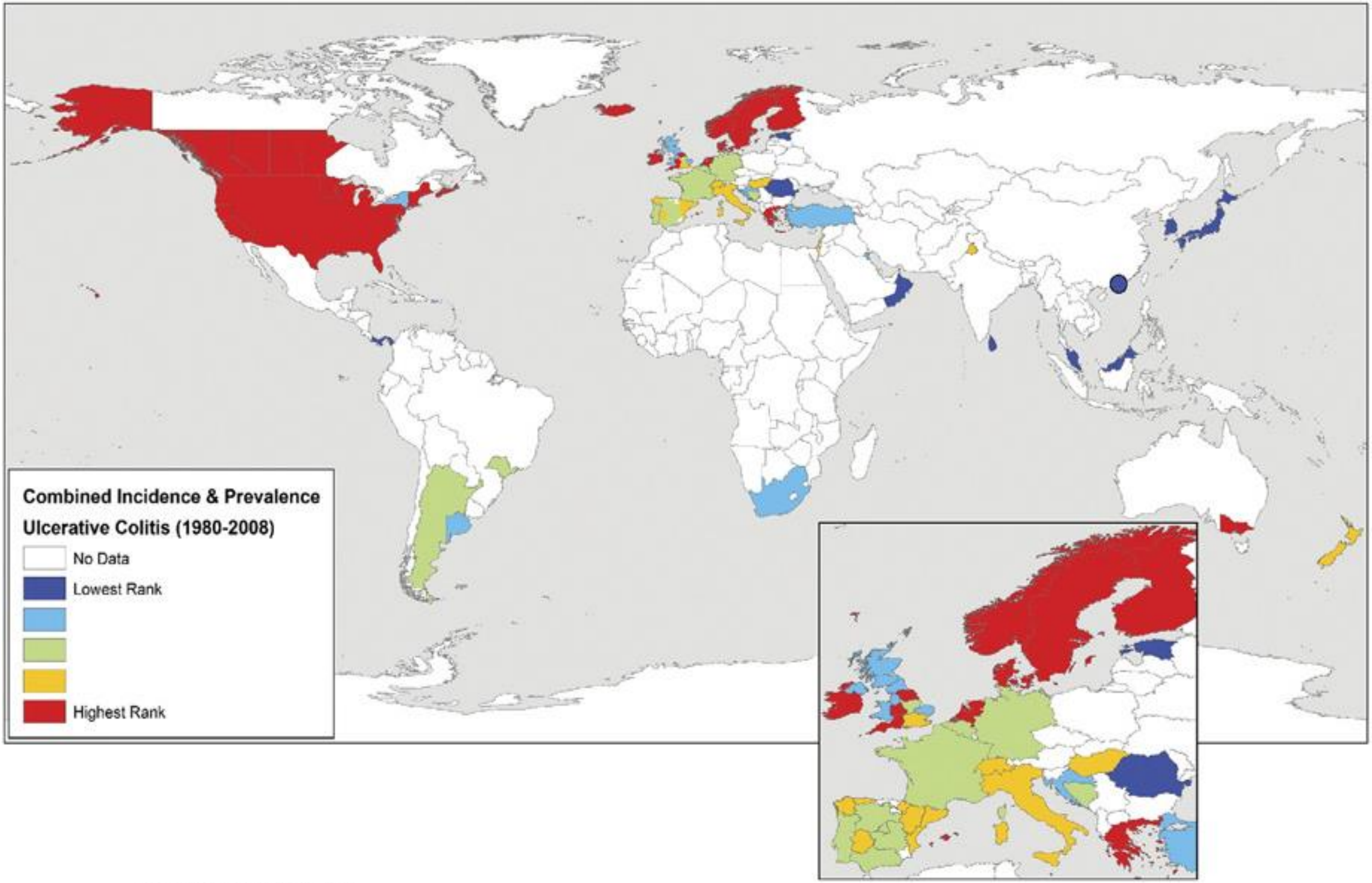
Arumugam et al, Nature 2011

Modern lifestyle linked with alterations of gut microbial colonization

Modern life style	Traditional life style
Birth in the hospital; increasing rate of caesarean delivery	Vaginal delivery at home
Small family size	Large family size, crowding
Life on concrete	Life in contact with soil microorganisms
Sanitation of living spaces: environment colonized by resistant micro-organisms (including resistant bacteria, fungii, acari)	Ancestral colonization of the living environment
Antibiotic usage early in life	No antibiotics in infant life
Daily body wash with hot water and soap	Limited access to hot water and soap
Low rate of H. pylori colonization	High rate of H. pylori colonization
Decline in endemic parasitism	Common carriage of parasitic worms
Food conserved by refrigeration	Food conserved by microbial fermentation
Consumption of processed foods	Consumption of natural foods

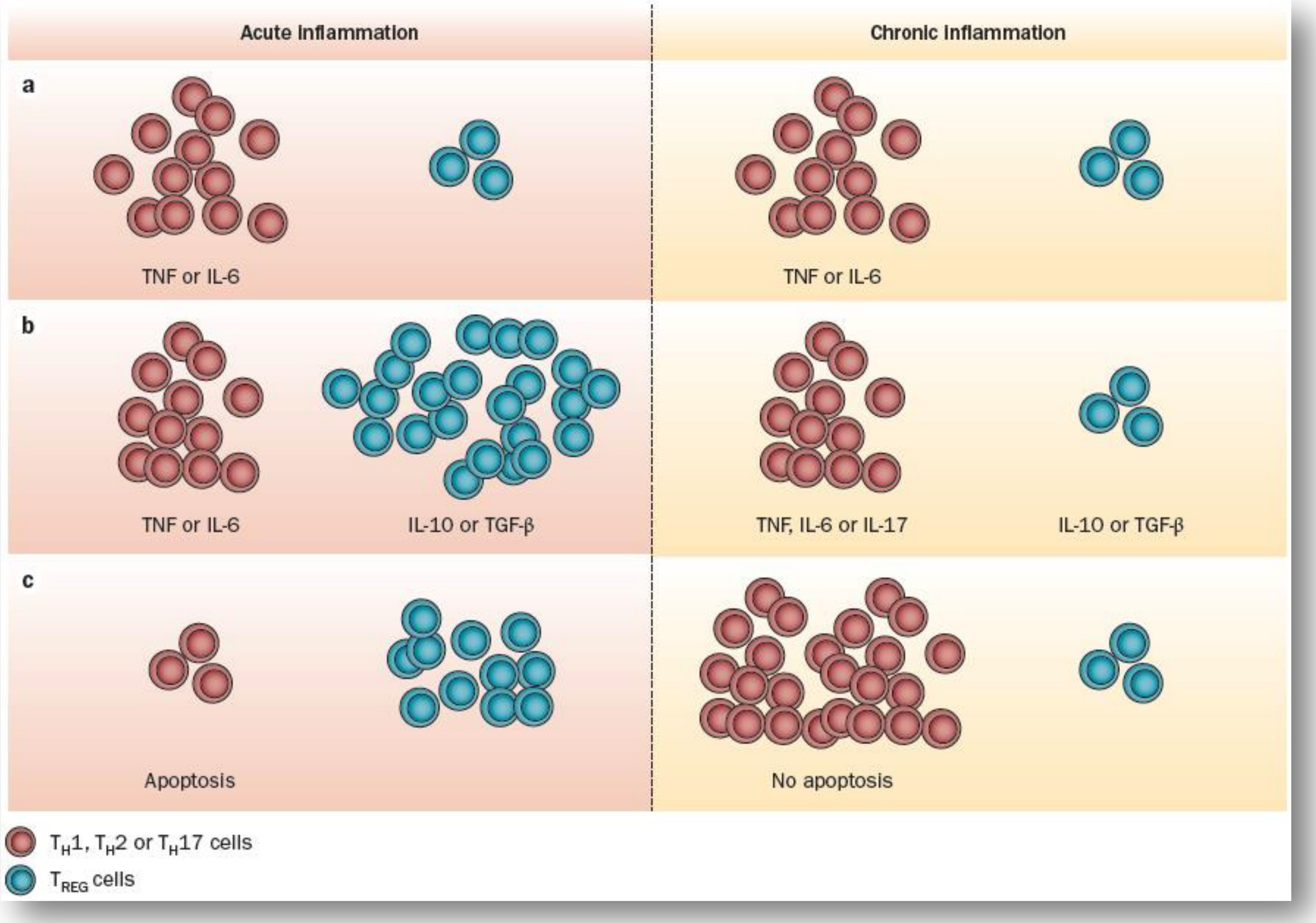
Manichanh et al, Nature Reviews Gastroenterology 2012

Increasing Incidence and Prevalence of UC



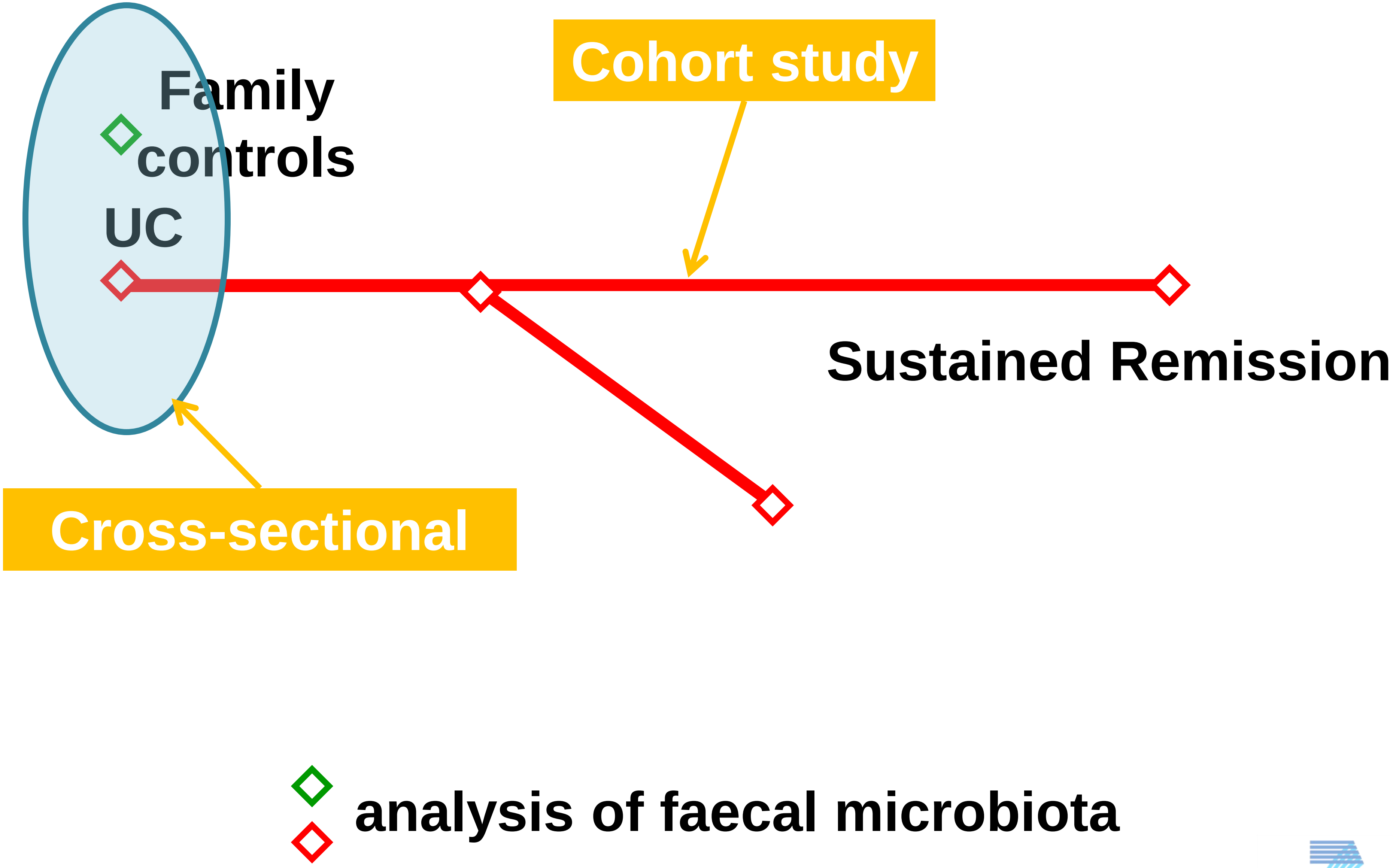
Molodecky et al, Gastroenterology 2012

Perpetuation of intestinal inflammation in IBD



Manichanh et al, Nature Reviews Gastroenterology 2012

UC studies in MetaHIT



Family controls

UC

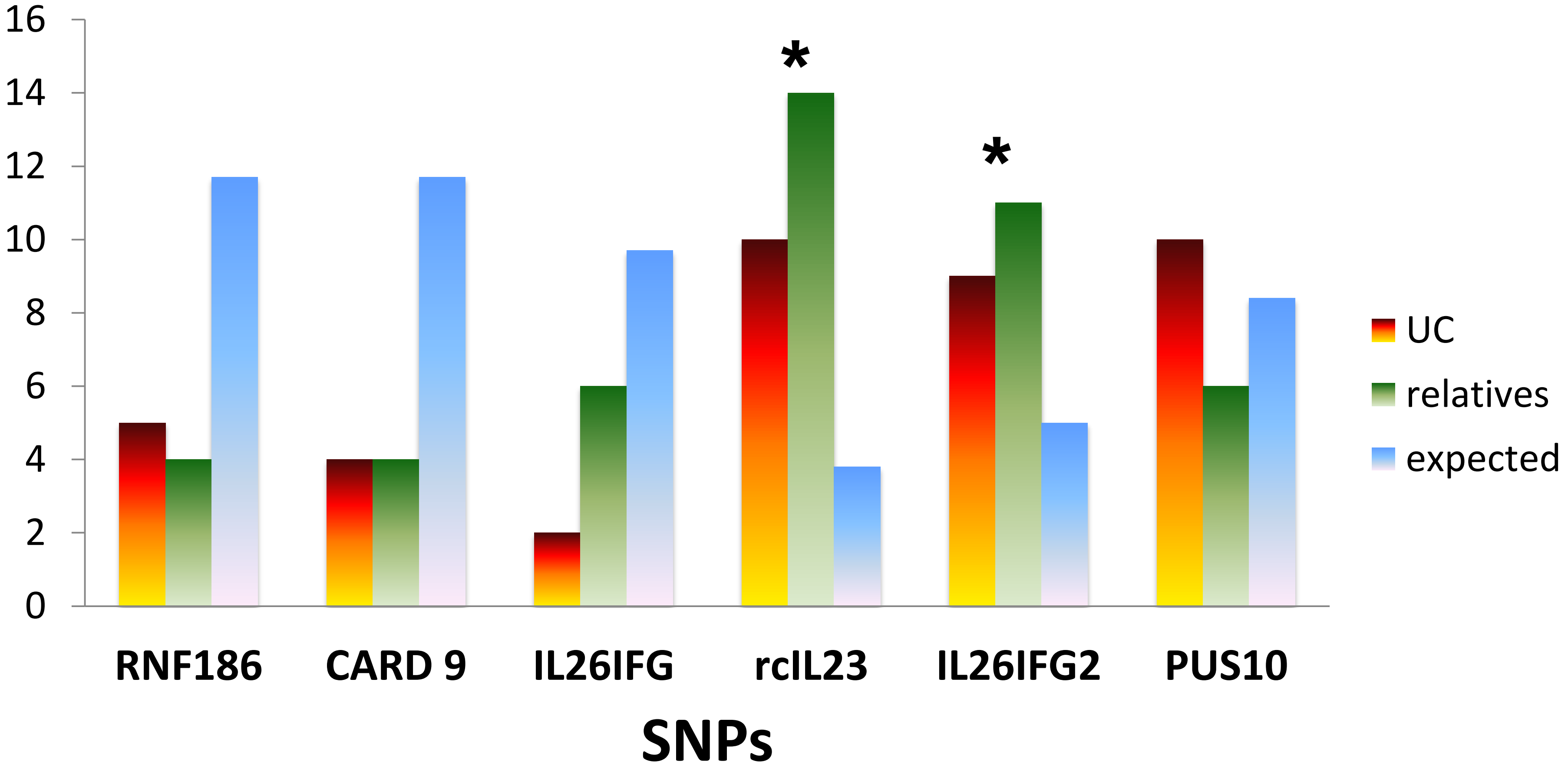
Cohort study

Sustained Remission

Cross-sectional

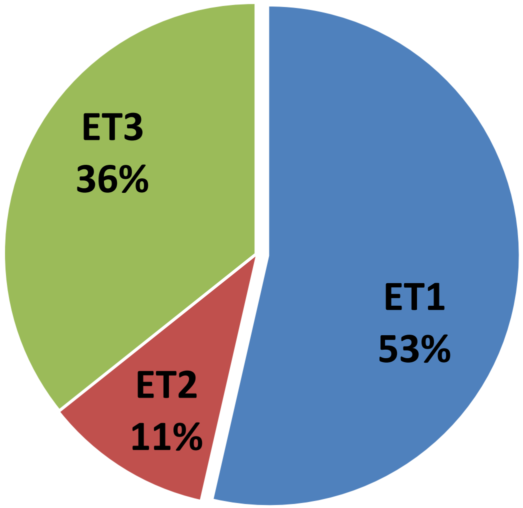
◇ ◇ analysis of faecal microbiota

Homozygous carriage of risk SNPs for UC

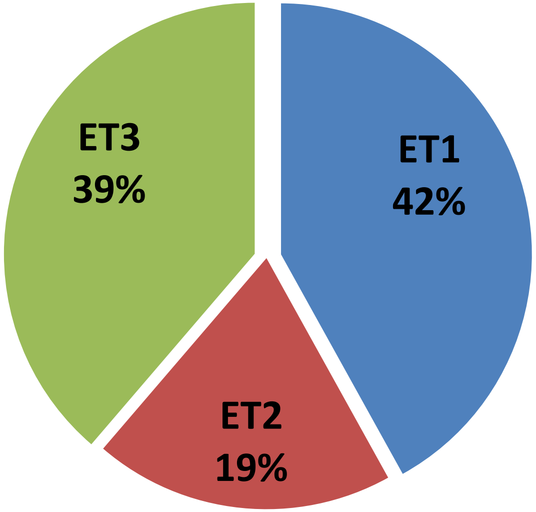


Robles et al, HUVH

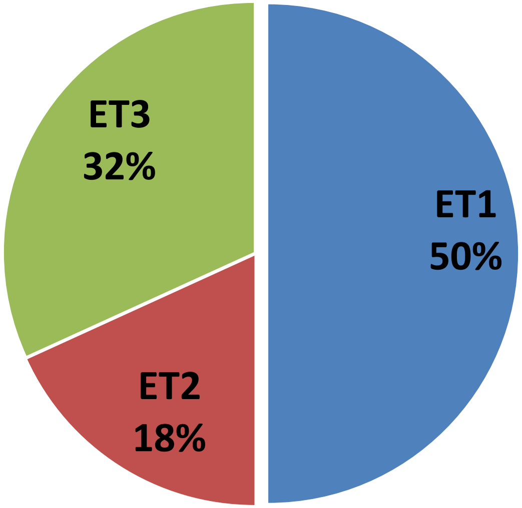
Enterotypes



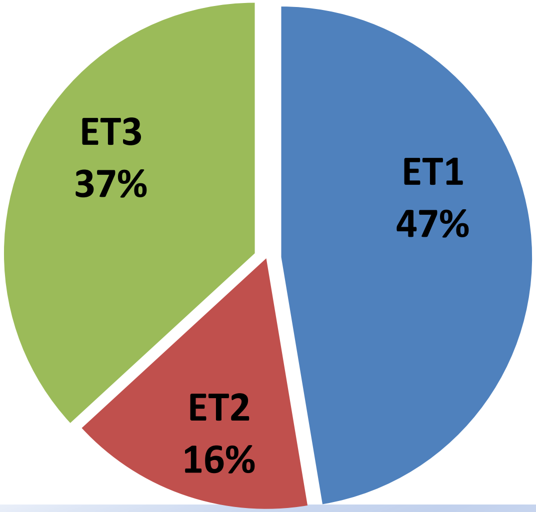
Healthy relatives



UC patients

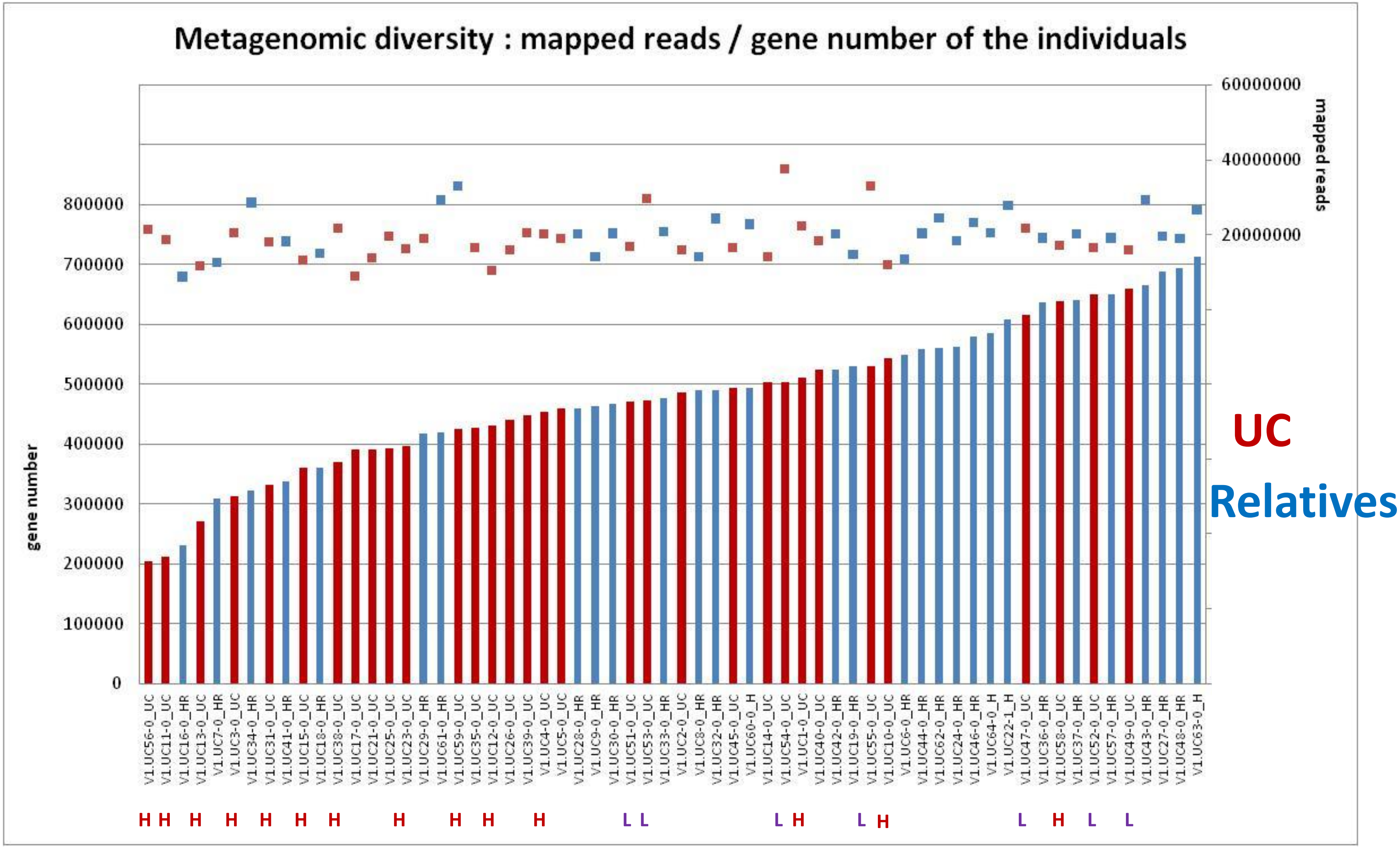


rcl IL23



IL26IFG2

Low Gene-count in UC during remission



Derrien, Le Chatellier et al, Danone Research -INRA

Akkermansia muciniphila in UC

	Akk +	Akk -
UC remission	39	67
Healthy relatives**	21	6
Control**	37	8

qPCR with specific primer for 16SrRNA region.

** Two sided p value <0.001 vs. UC remission (Fisher’s exact test).

Manichanh, Varela et al, HUVH

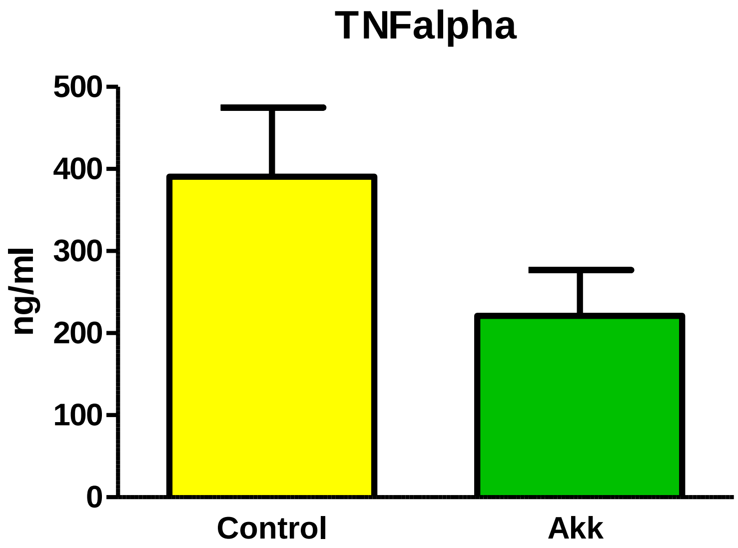
Akkermansia muciniphila in UC

Multivariate analysis

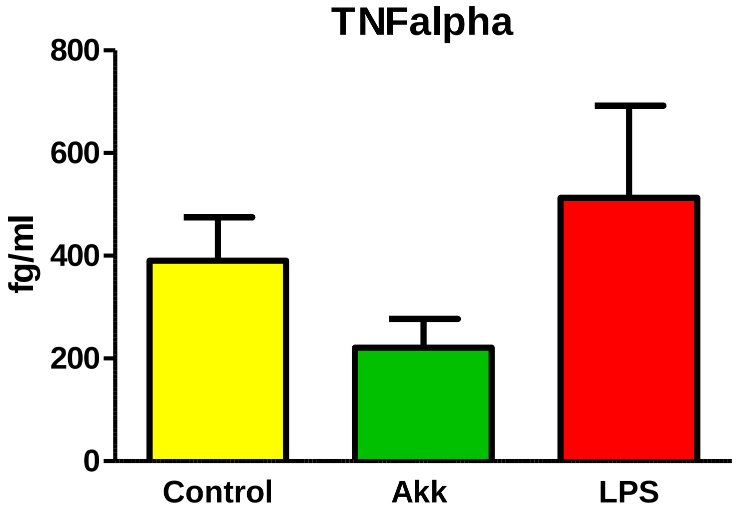
	Akk +	Akk -	P value
N	39	67	
Male/Female	16 / 23	31 / 36	
Median age	44 [35.0 – 51.0]	38 [31.0 – 46.0]	
Extended colitis	29.3 %	70.7 %	0.03
Relapses per year	0.5 [0.2 – 0.9]	1.0 [0.5 – 1.0]	0.01
Time on remission (mo)	34.5 [3.0 – 94.2]	3.0 [3.0 – 30.5]	0.01

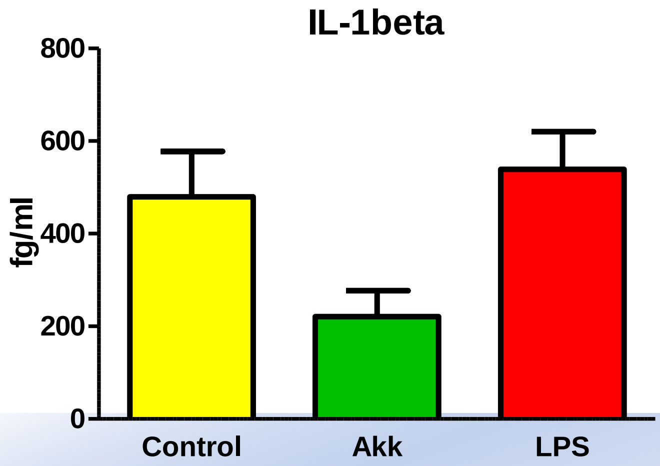
Antiinflammatory effects of *Akkermansia muciniphila*

Inflamed colon explants



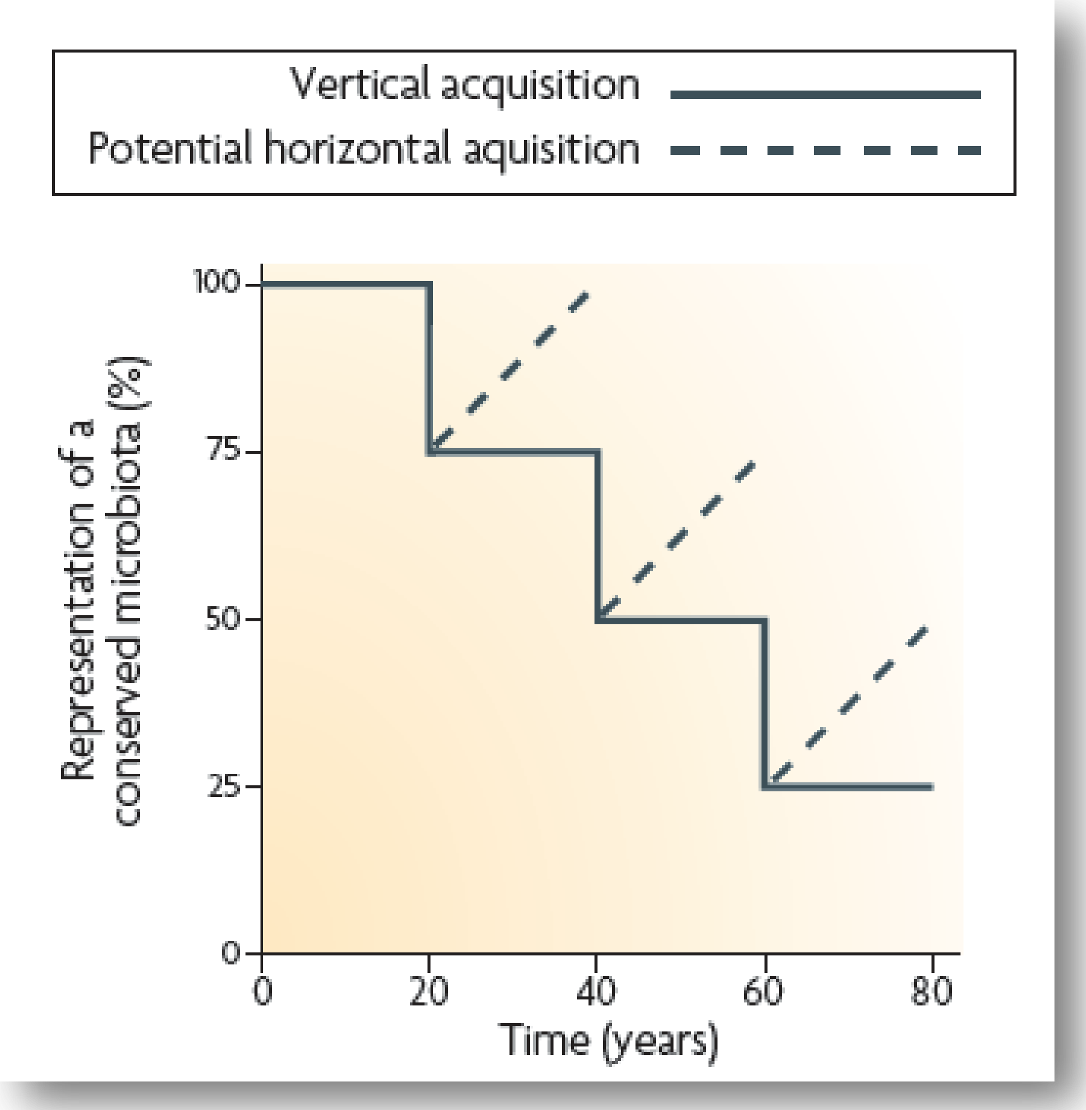
Intestinal DC cells





Antolin, Llopis et al, HUVH

Progressive loss of vertically transmitted microorganisms



Blaser & Falkow, Nature Rev Microbiol 2009

Conclusions

1. There is a permanent distortion of the gut microbial ecosystem in UC patients, even during remission periods, characterized by reduction in microbial gene counts.
2. Low gene counts in the gut microbiome is associated with a severe clinical course.
3. A small number of Meta-Genomic species are diagnostic for UC.
4. Species with potential anti-inflammatory activity, like *A. muciniphila* are missing in patients with severe clinical course.

Meta HIT

Acknowledgments

MetaHIT Consortium

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GENOSCOPE
INSTITUT MERIEUX
Vall d'Hebron Institut de Recerca
华大基因 BGI SHENZHEN
wellcome trust sanger institute
IEO European Institute of Oncology
WAGENINGEN UNIVERSITY WAGENINGEN UR
DTU
UNIVERSITY OF COPENHAGEN
novo nordisk®
DANONE RESEARCH
ucb
EMBL

INRA: **E. Le Chatelier, M. Almeida, N. Pons, J.M. Batto, P. Renault.**

HUVH: **Ch. Manichanh, E. Varela, M. Antolín, N. Borruel, A. Torrejón, F. Casellas.**

CBS-DTU: **A. Juncker, H. Bjorn Nielsen.**

Danone Research: **M. Derrien.**

SEVENTH FRAMEWORK PROGRAMME