Microbioma y enfermedad inflamatoria intestinal

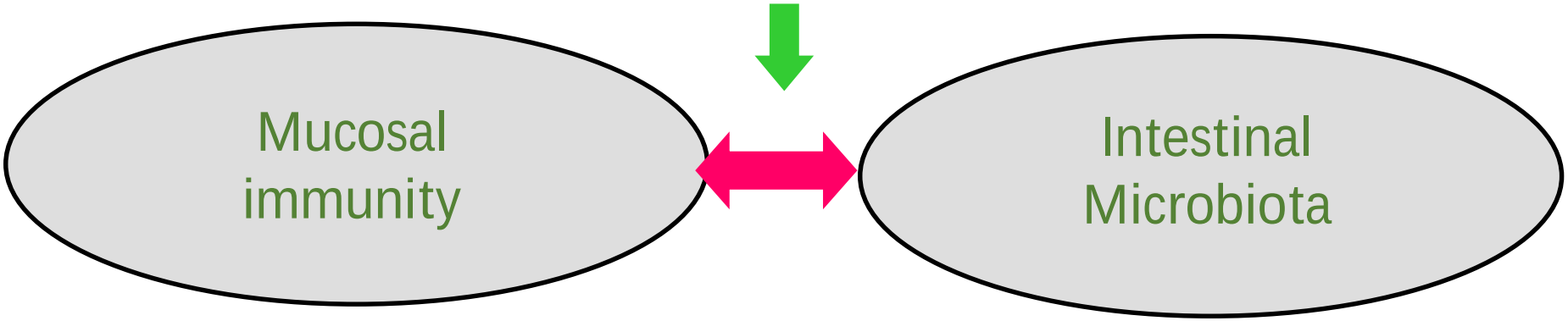
Natalia Borruel Sainz
Crohn's and Colitis Attention Unit
Digestive System Research Unit
Barcelona, Spain.

Simposi Paral·lel

MSD

ETIOPATHOGENESIS-Inflammatory bowel disease

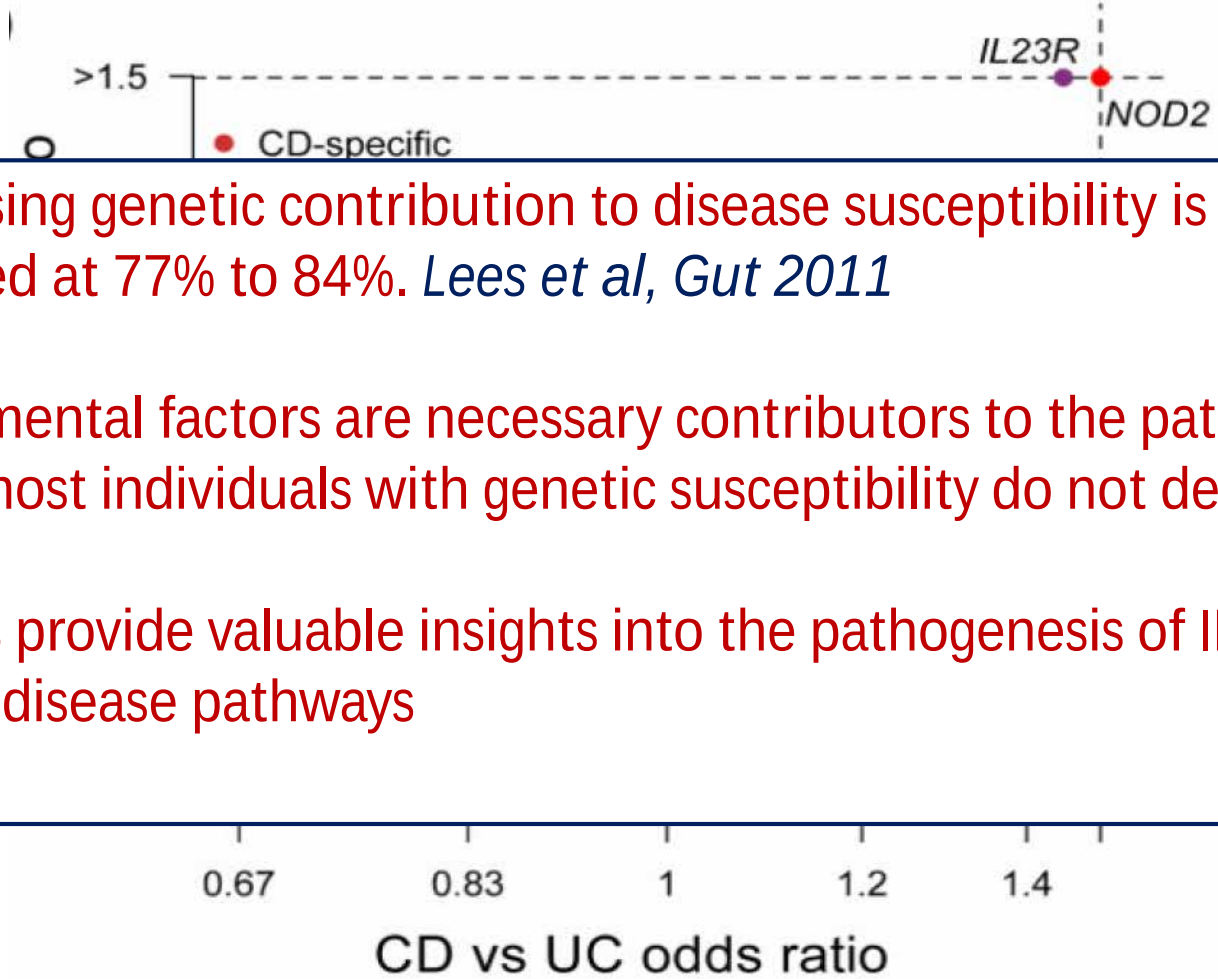
Genetic susceptibility



Abnormal communication or misbalance:
DISBIOSIS

Chronic intestinal inflammation

Genetic Susceptibility



The missing genetic contribution to disease susceptibility is presently calculated at 77% to 84%. *Lees et al, Gut 2011*

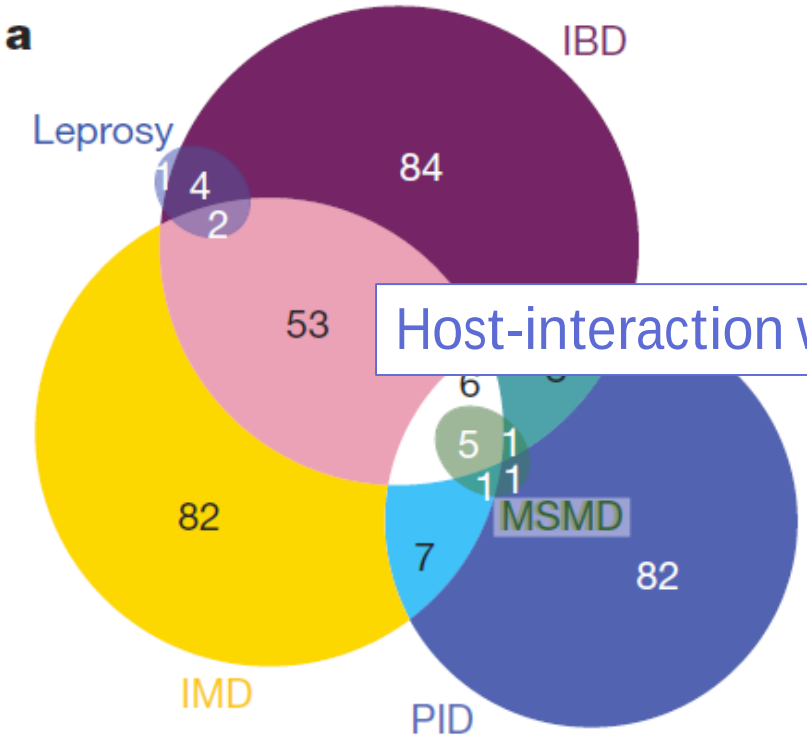
Environmental factors are necessary contributors to the pathogenesis of IBD: most individuals with genetic susceptibility do not develop the disease.

Genetics provide valuable insights into the pathogenesis of IBD and the relevant disease pathways

Meta-analysis of CD and UC genome-wide association studies identified a total of 163 IBD loci (>75,000 cases and controls) . Most loci contribute to both CD and UC.

Jostins et al, Nature 2012

Genes and relevant disease pathways



Host-interaction with bacteria

Gene enrichment

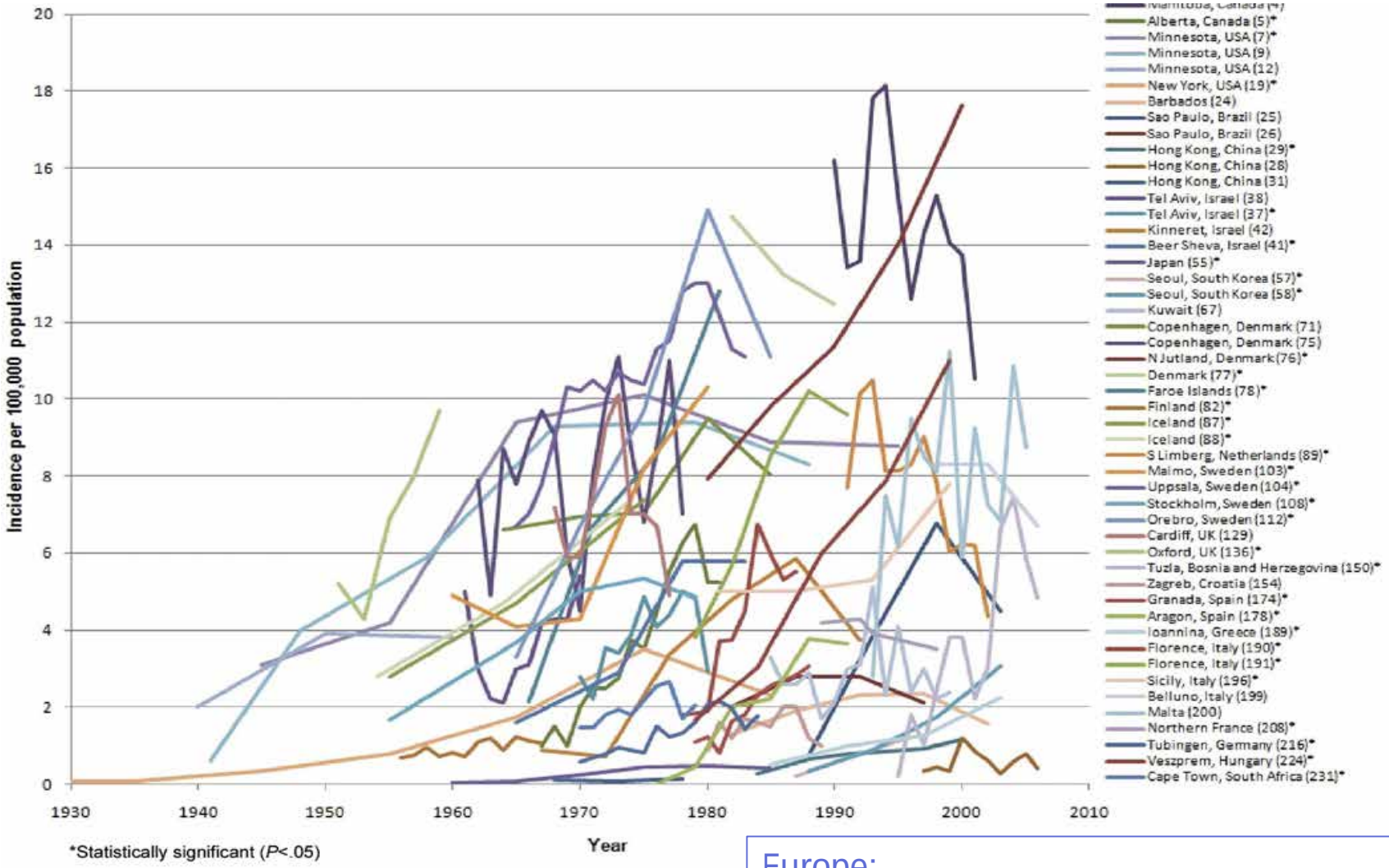
- Regulation of cytokine production (IFNγ, IL-12, IL-10, TNF)
- [illegible]activation
- Response to molecules of bacterial origin: NOD2, IL-17, IL-10, CARD-9
- Activation of dendritic cells

70% shared with other diseases
Most important: SpA, psoriasis

MSMD: Mendelian Susceptibility to Mycobacterial Diseases

Jostins L. Nature 2012

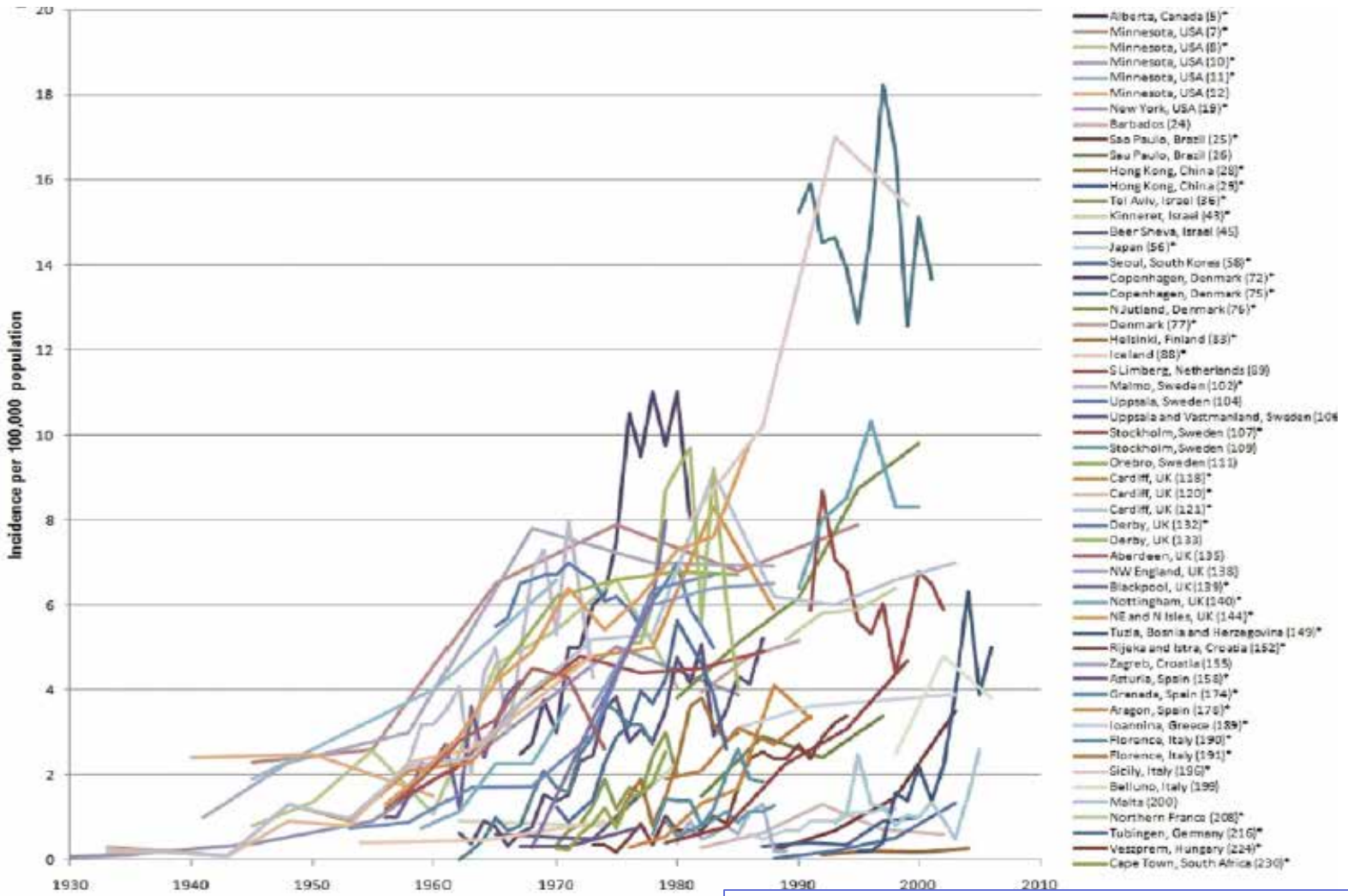
Increasing incidence and prevalence of UC



Molodecky et al, Gastroenterology 2012

Europe:
Incidence: 24.3 per 100,000 person-years
Prevalence: 505 per 100,000 persons

Increasing Incidence and Prevalence of CD



Molodecky et al, Gastroenterology 2012

Europe:
Incidence:12.7 per 100,000 person-years
Prevalence: 319 per 100,000 persons

Epidemiology of IBD

Risk factors (OR)

Childhood		
Hot water tap	**5.0**	**CD**
Separate bathroom	**3.3**	**CD**
Frequent antibiotic use	**2.1**	**UC/CD**
7 courses of antibiotic	7.3	CD
Urban living	**2.6**	**UC/CD**
Adult life		
Prisoner of war (Vietnam)	**0.4**	**CD**

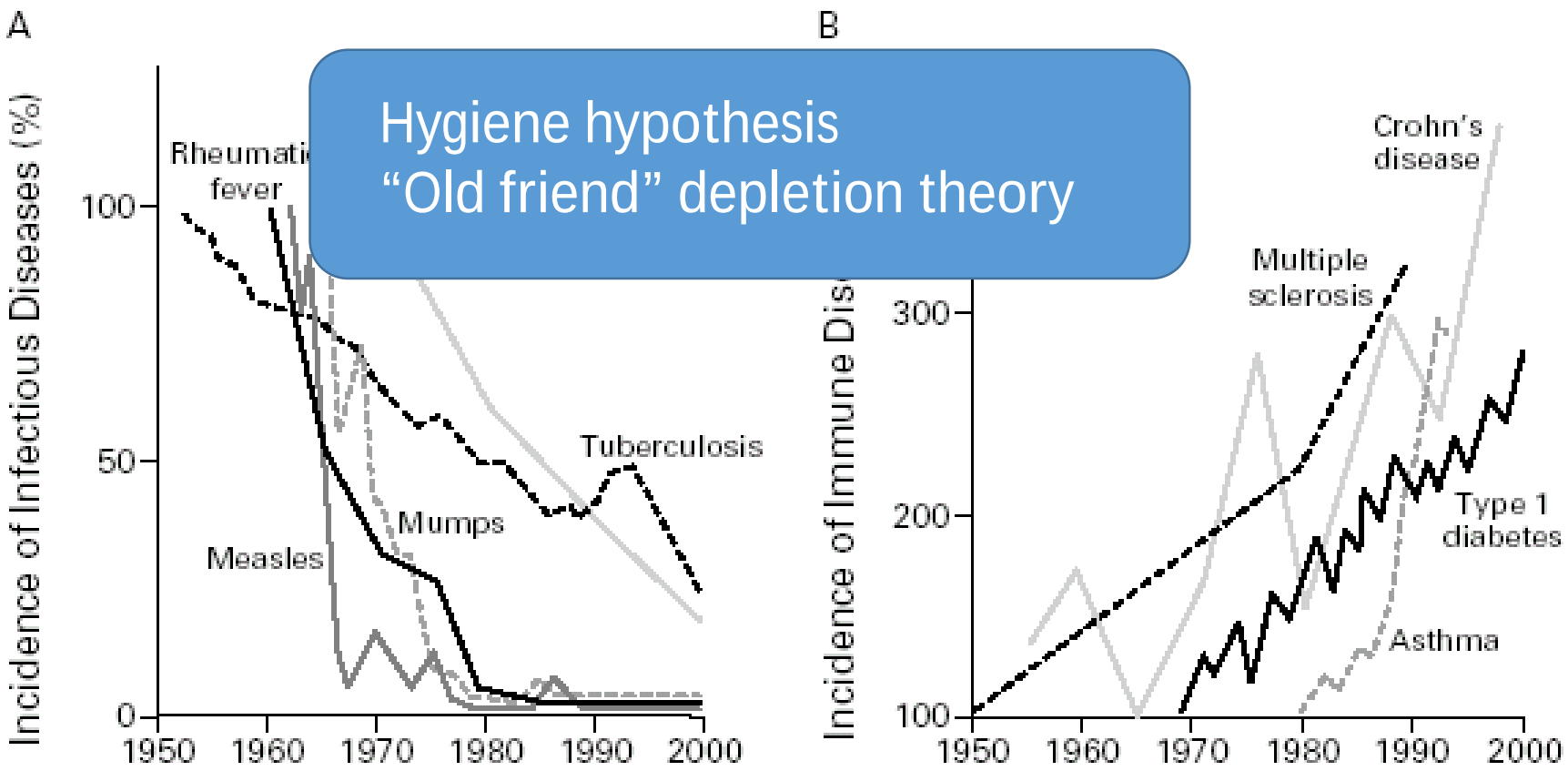
Gent et al, Lancet 1994
Wurzelmann et al, Dig Dis Sci 1994
Delco et al, Am J Gastroenterol 1998
Hiviid et al, Gut 2011

IBD risk factors are linked with altered microbial colonization

Modern life style	Traditional lifestye
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

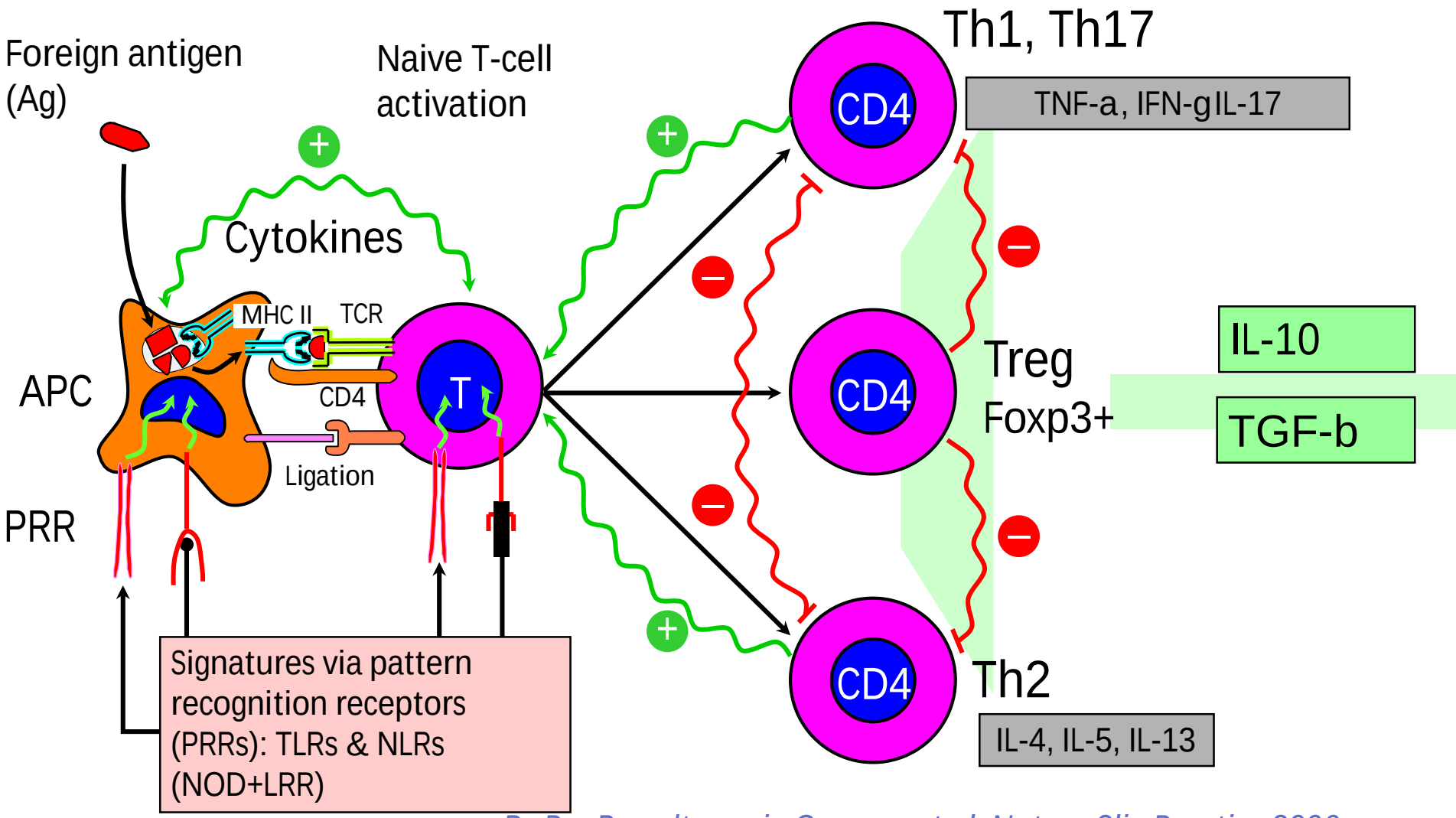
Manichanh et al, Nature Reviews Gastroenterology 2012

Inverse relation between the incidence of infectious diseases and immune disorders



Hygiene hypothesis
"Old friend" depletion theory

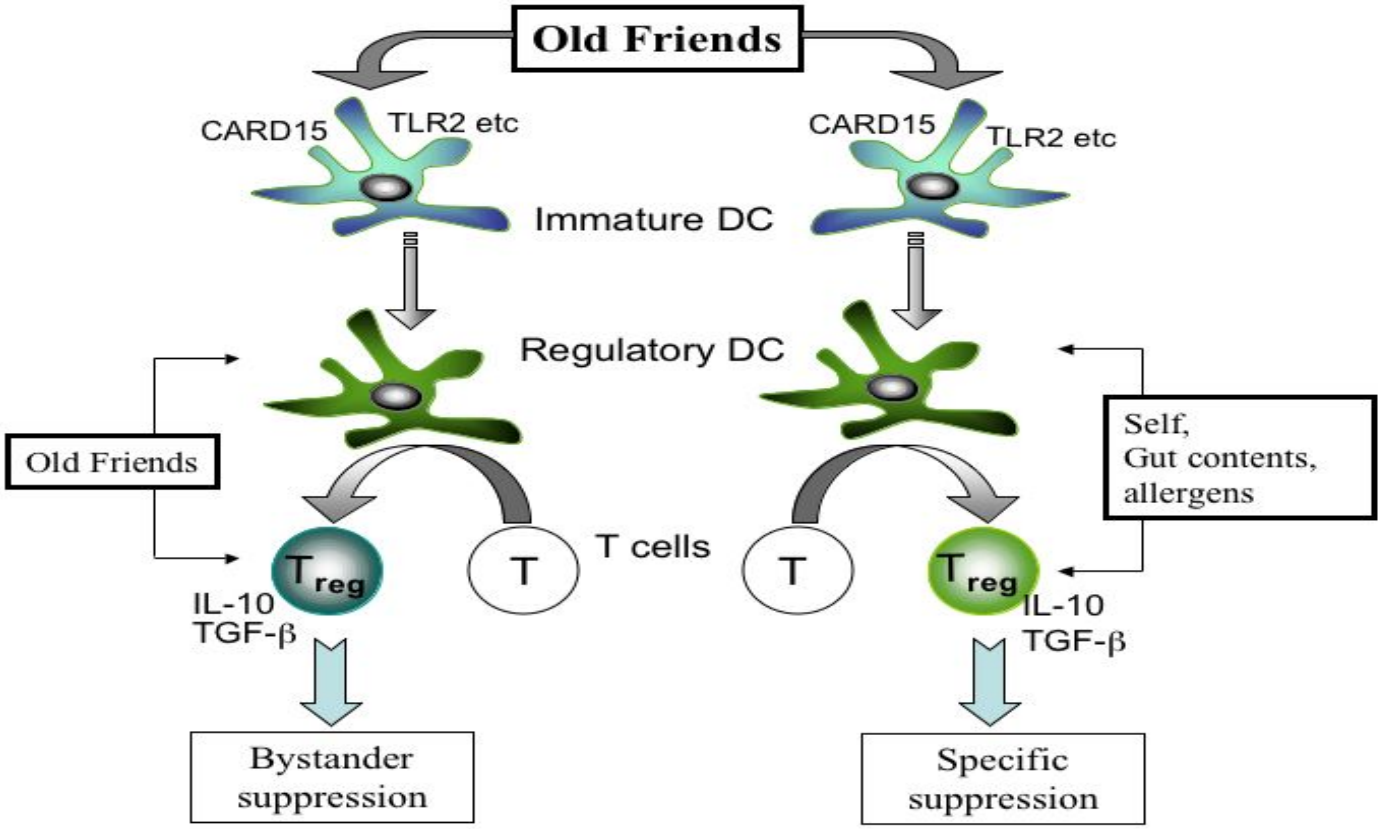
Decision making in the adaptive (acquired) immune system



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

The ‘ Old friends’ hypothesis

Harmless organisms(helminths, bacteria, mycobacteria, etc) have been depleted from the modern human microenvironment



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

The enemy is within the fecal stream

VOL 338: SEPT 28, 1991 THE LANCET

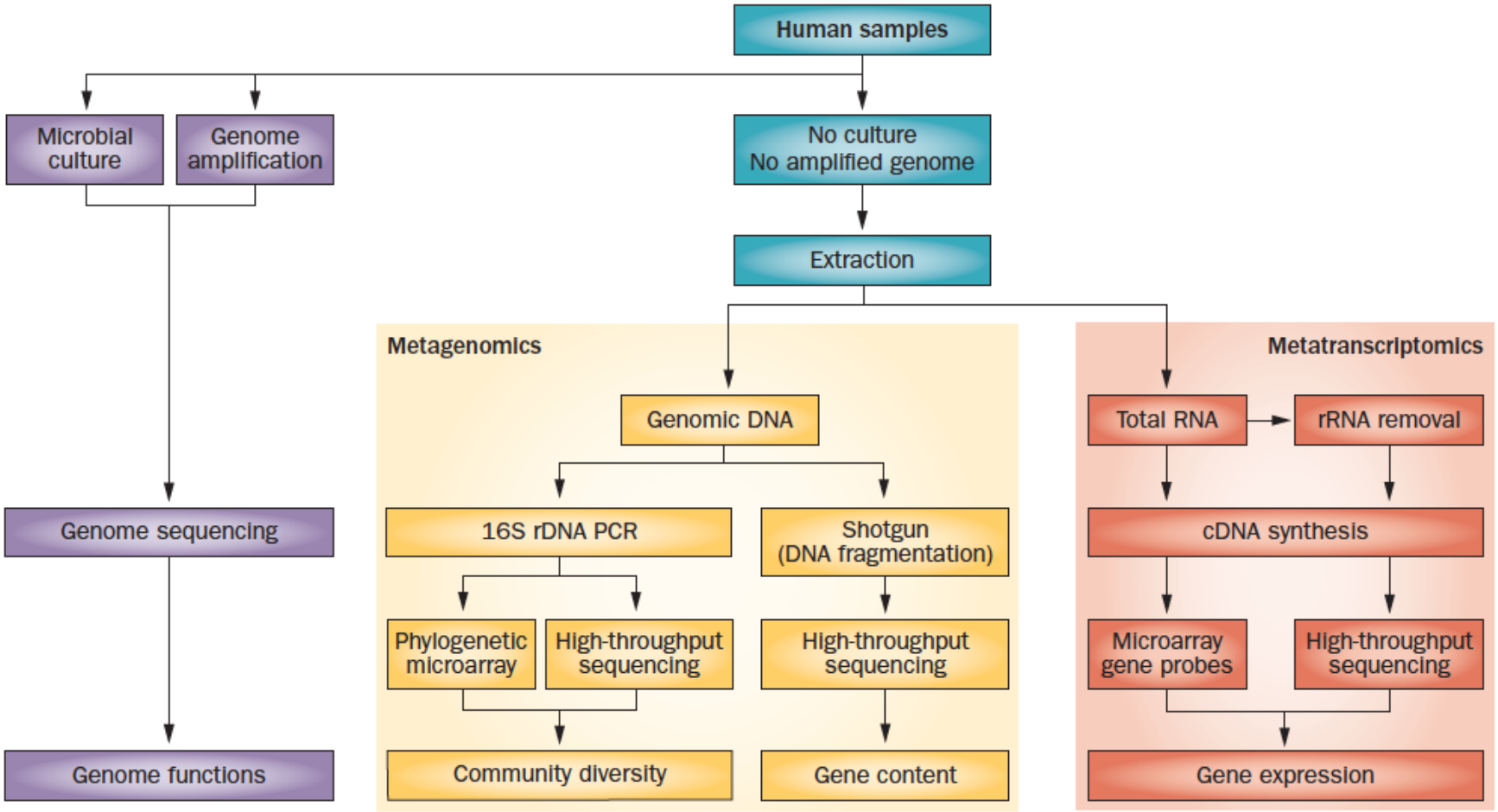
Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum

P. RUTGEERTS K. GOBOES M. PEETERS M. HIELE
F. PENNINCKX R. AERTS R. KERREMANS G. VANTRAPPEN

Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998;114:262–267

D'Haens GR, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P.

Profiling the Human Microbiome



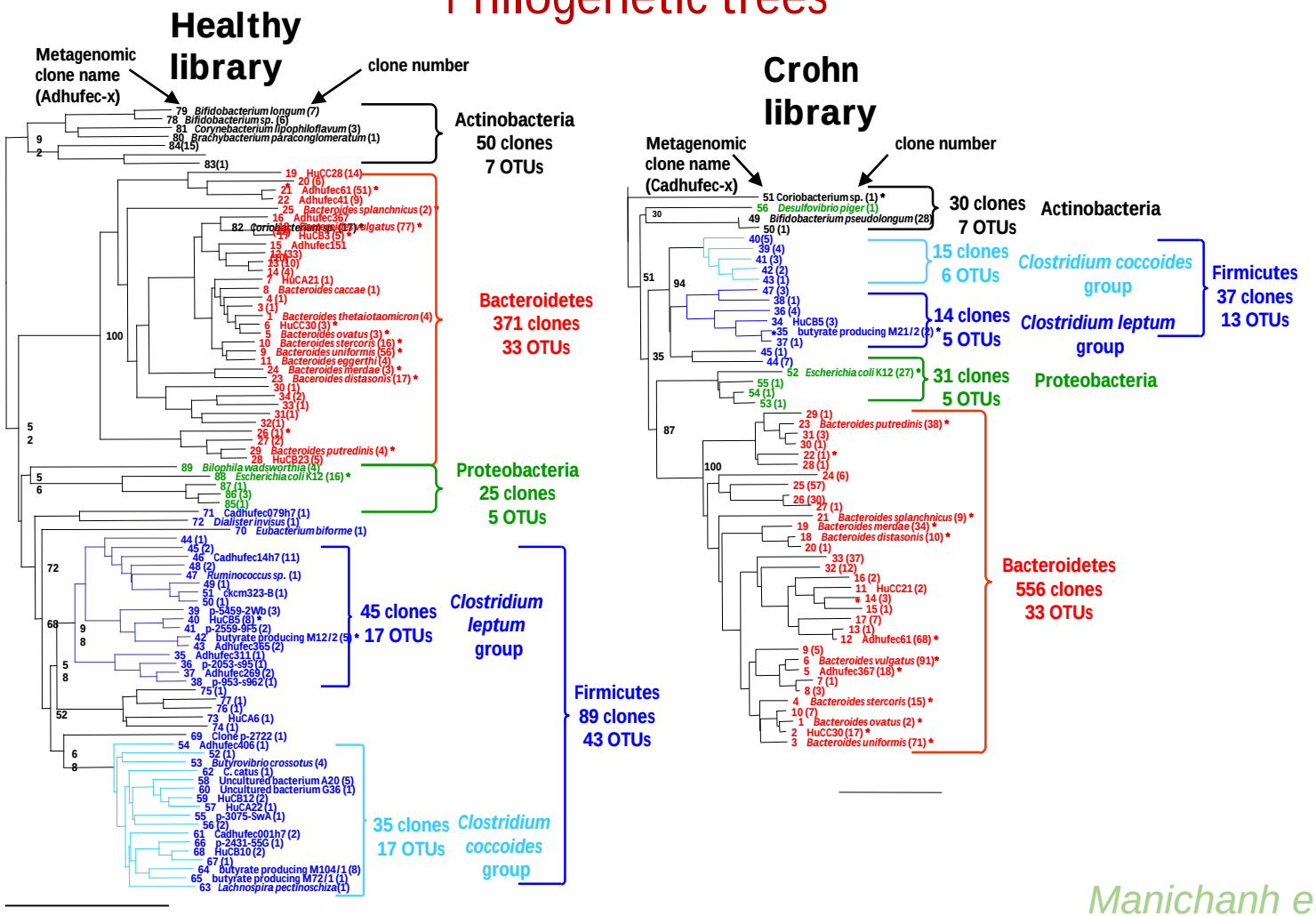
TAXONOMY

WHOLE GENOME

Manichanh et al, Nat Rev GH 2012

Low bacterial diversity in IBD

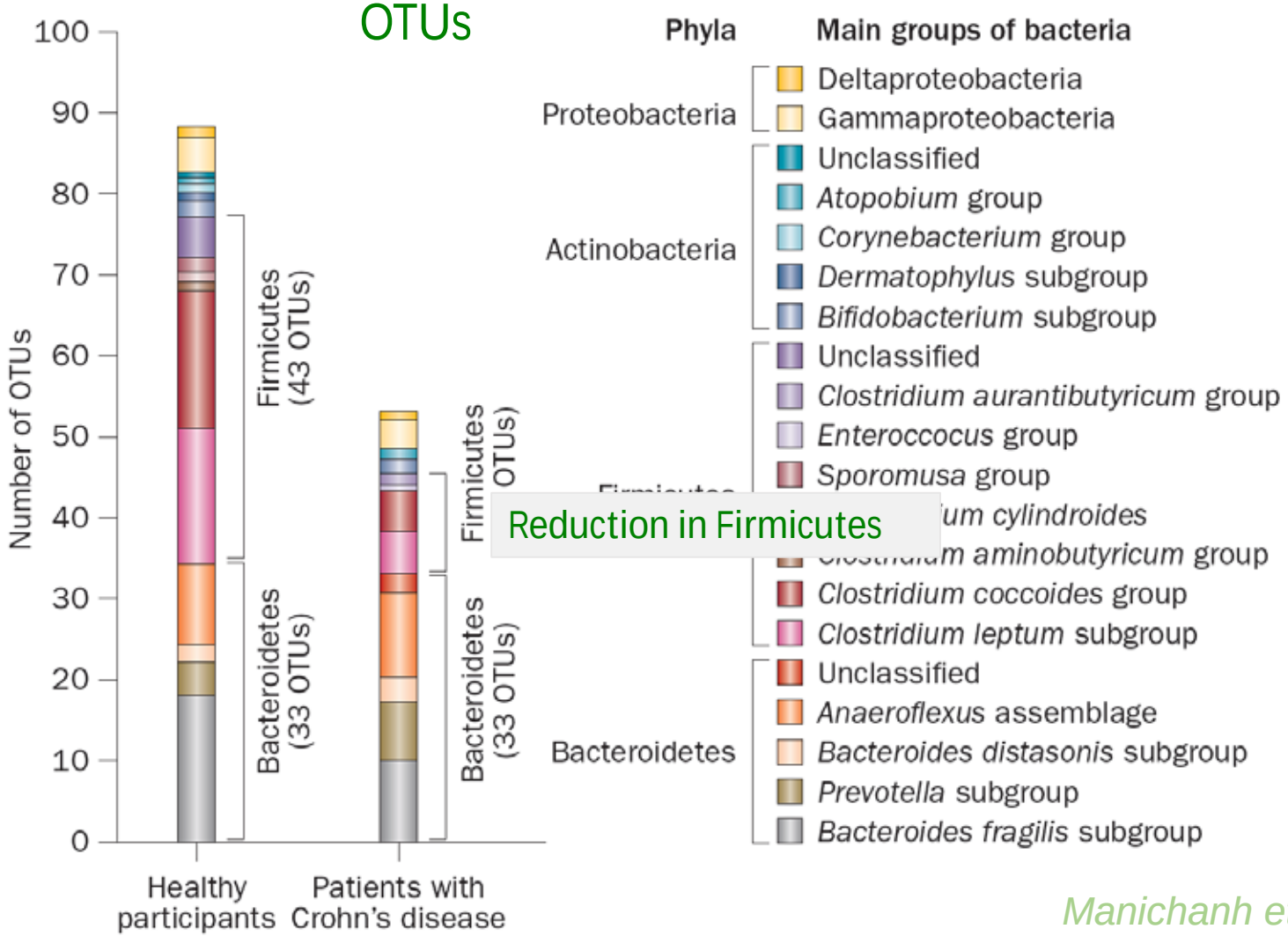
Philogenetic trees



Manichanh et al, Gut 2006

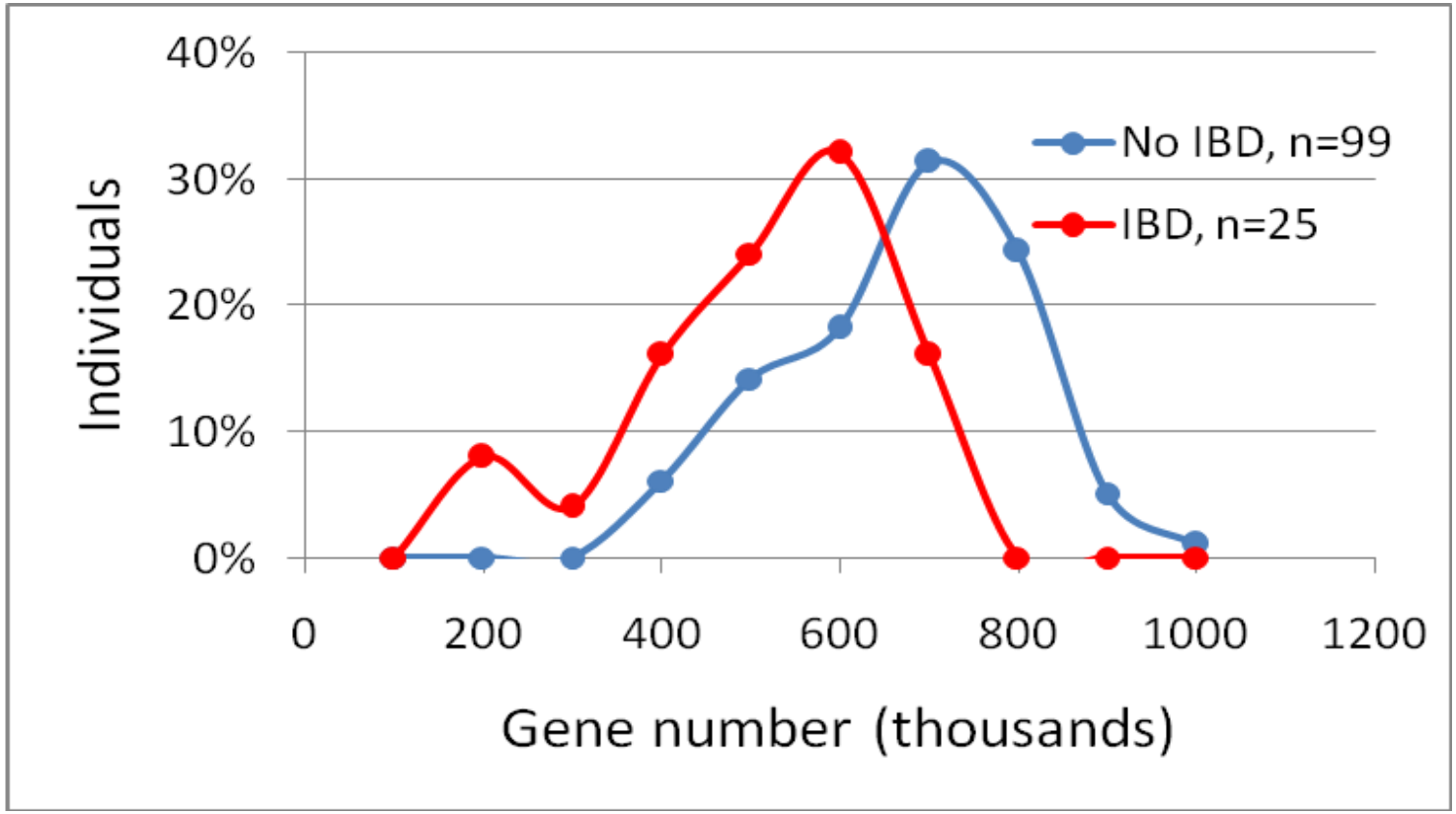
Reduction in diversity in Crohn's disease

Taxonomy of the dominant OTUs



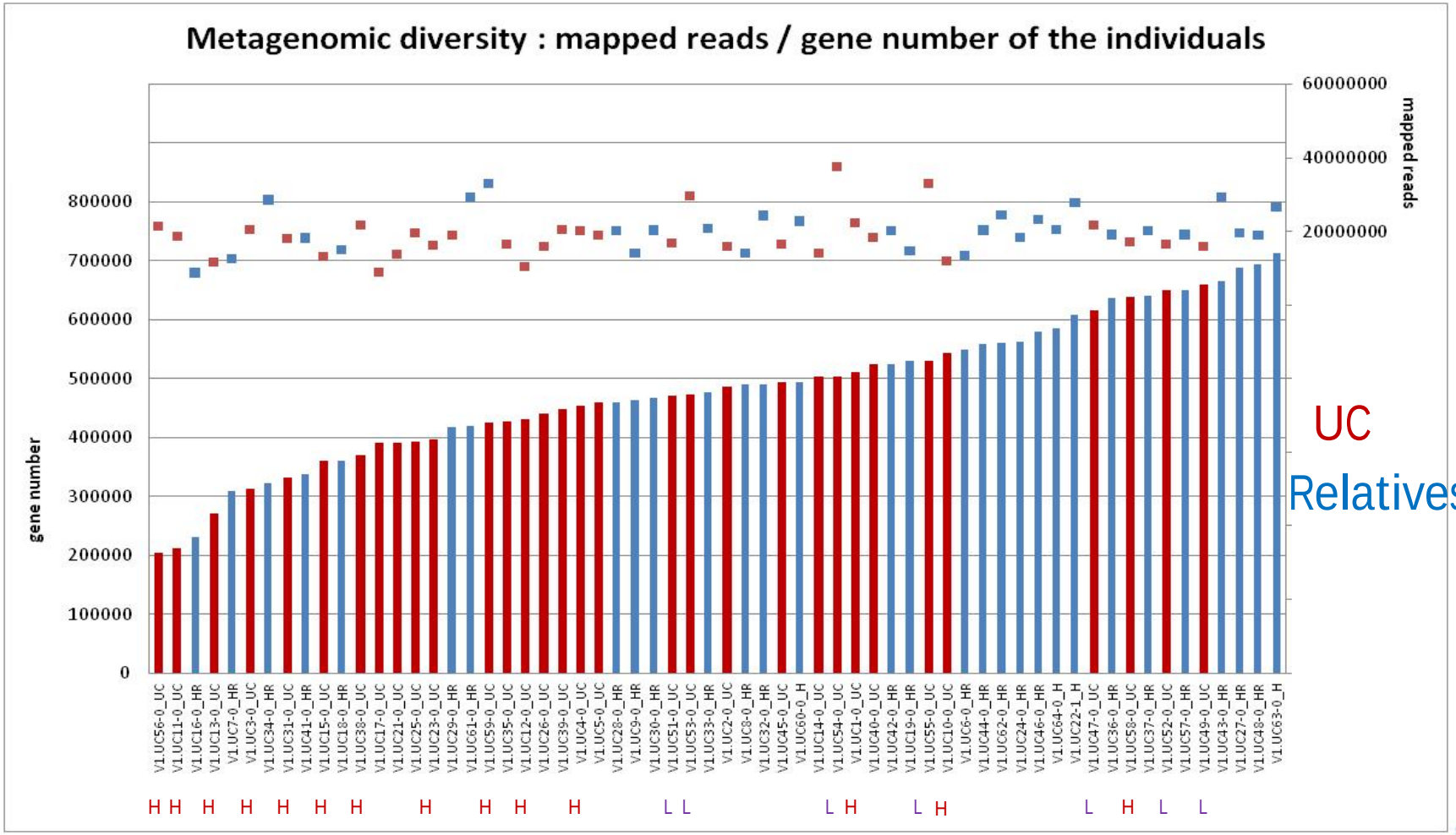
Manichanh et al, Gut 2006

Reduced Microbial Gene Diversity in IBD

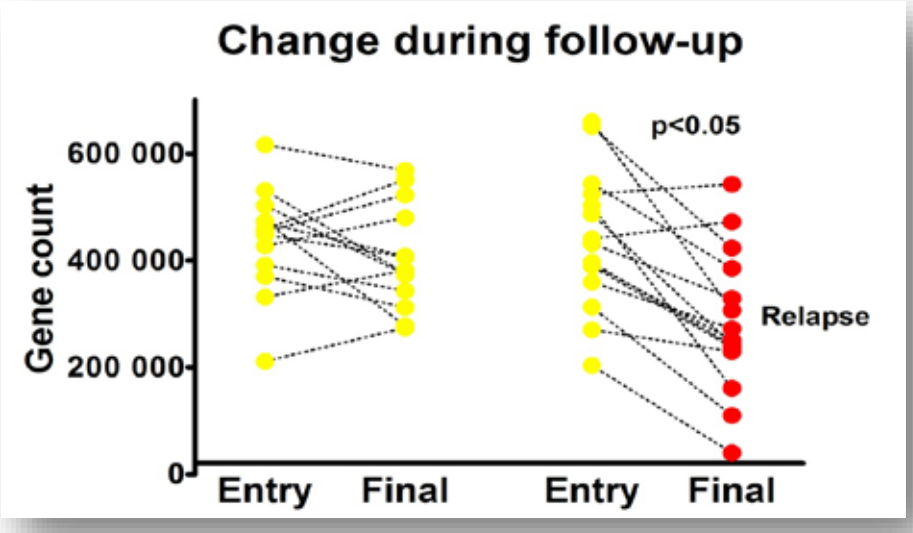


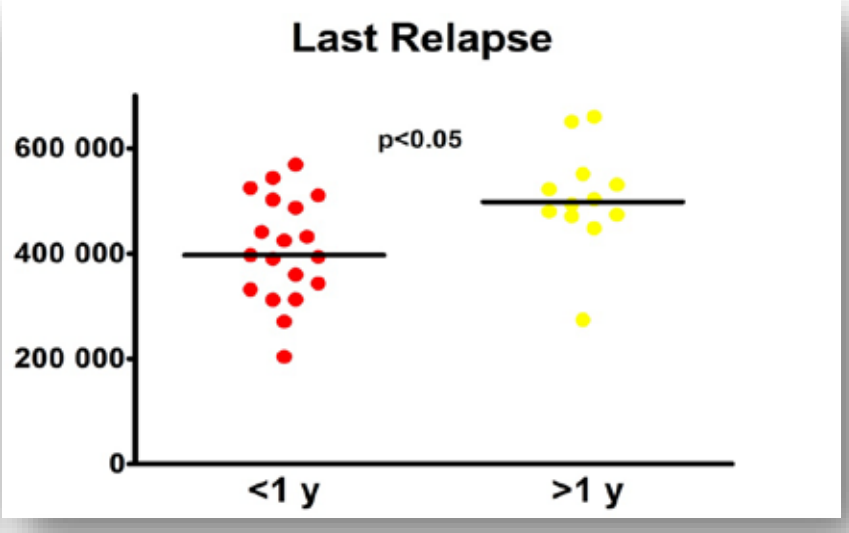
Qin et al, Nature 2010

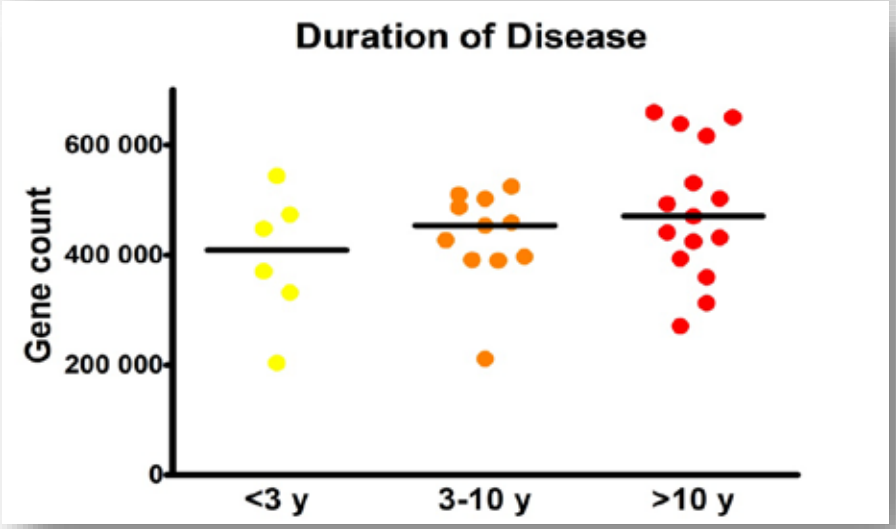
Low Gene-count in UC during remission

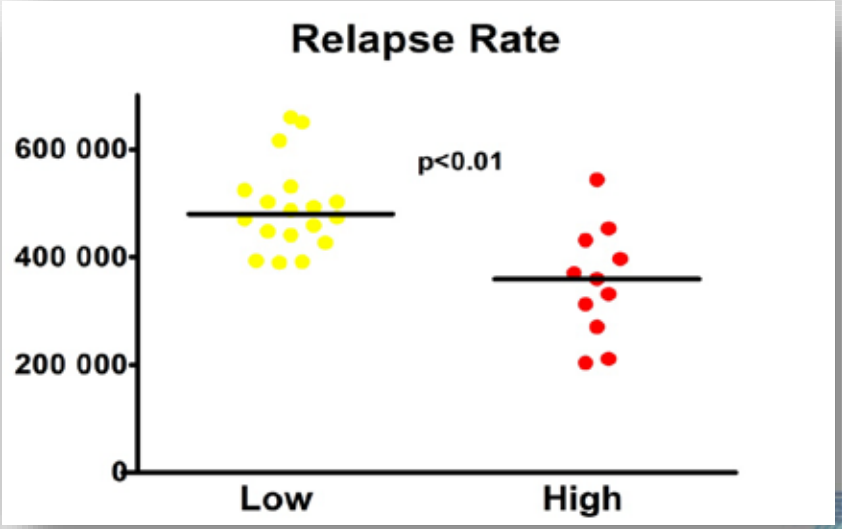


Low Gene-count in Ulcerative colitis

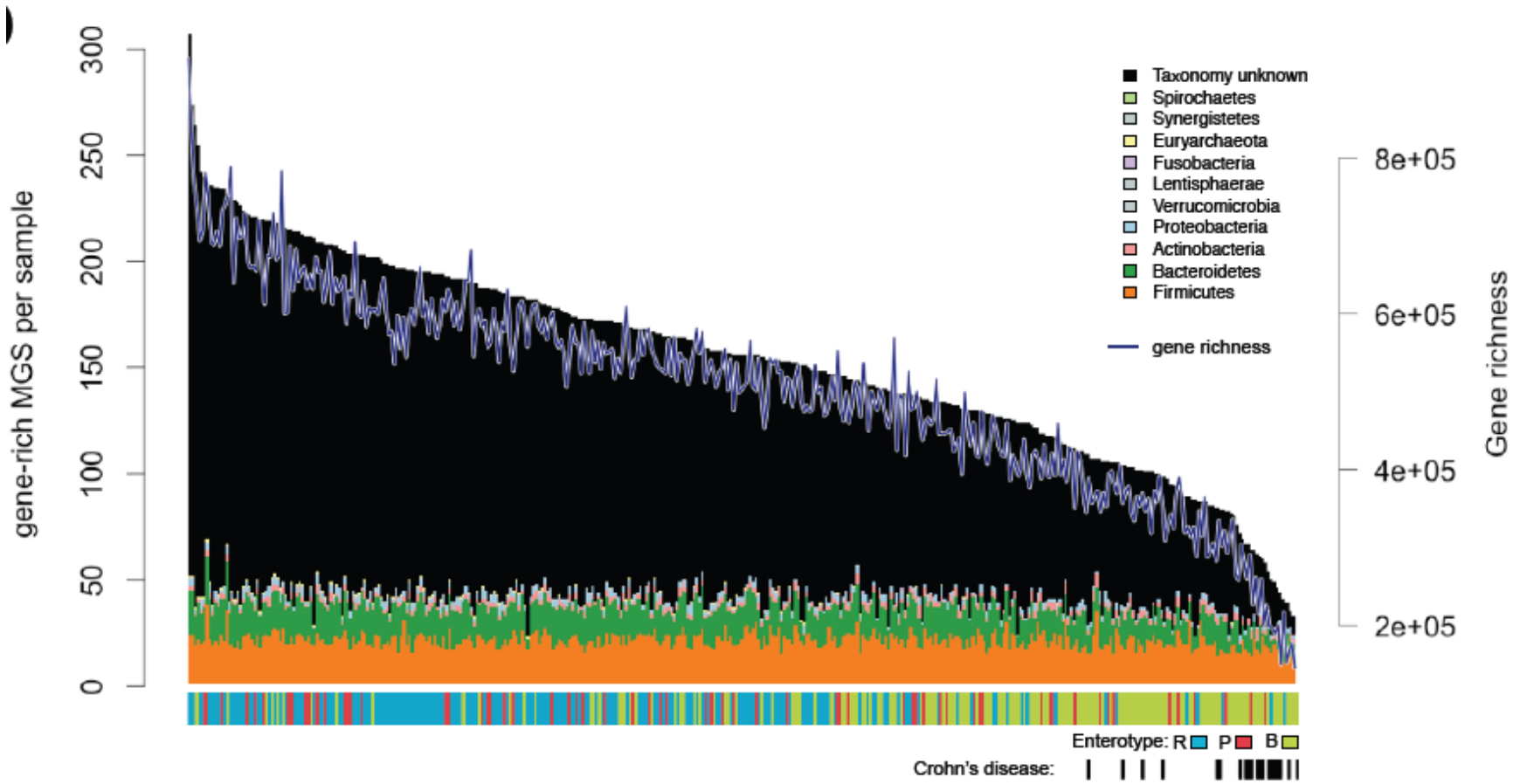




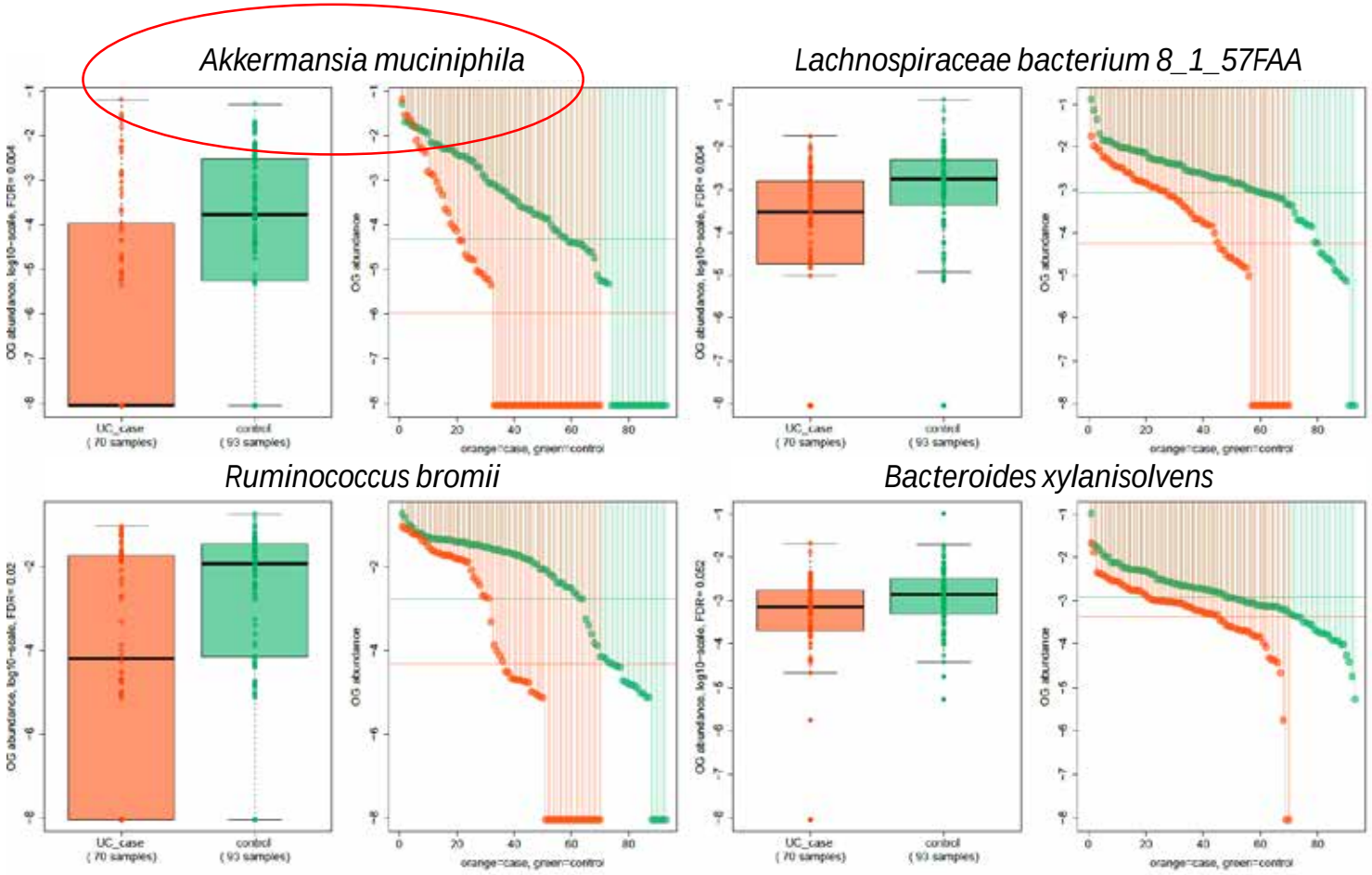




Metagenomic species and gene richness across 381 samples



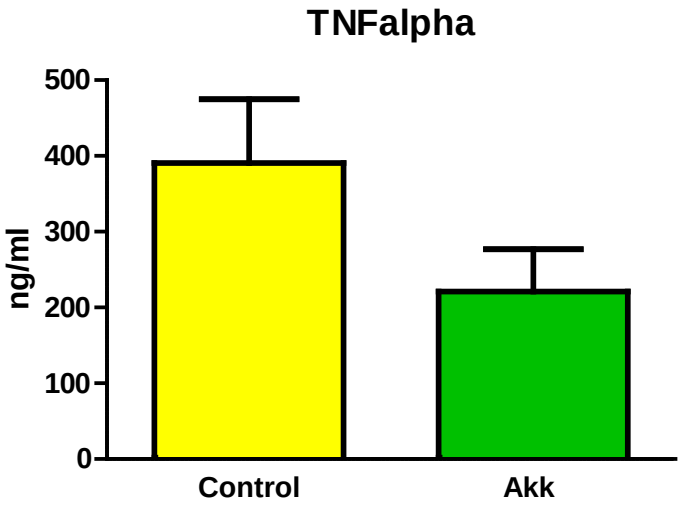
4 species depleted in UC



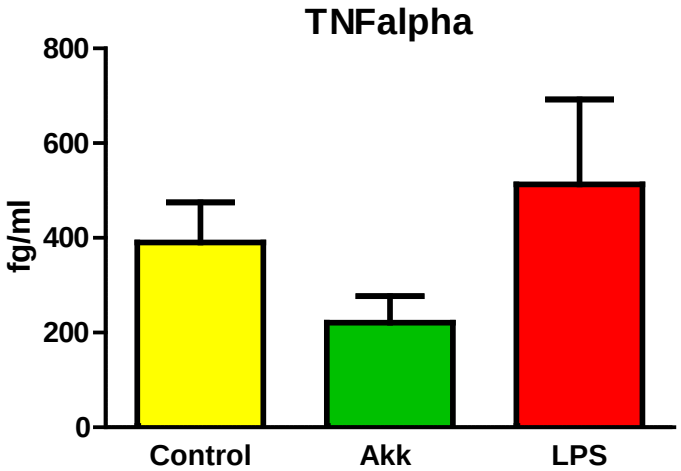
Sunagawa et al, EMBL

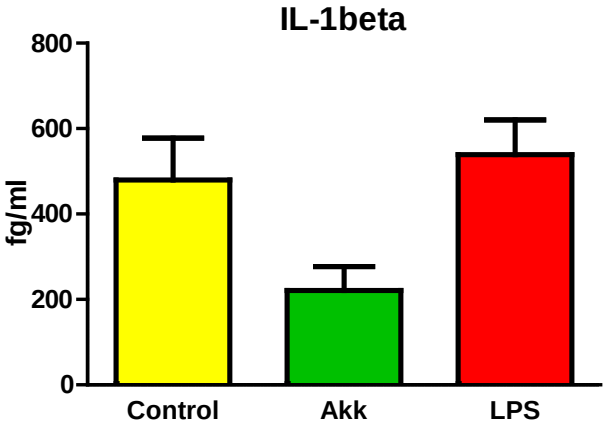
Antiinflammatory effects of *Akkermansia muciniphila*

Inflamed colon explants



Intestinal DC cells





Antolin, Llopis et al, HUVH

Akkermansia muciniphila depletion in UC

	Akk +	Akk -
UC remission	39	67 (63%)
Healthy relatives**	21	6 (21%)
Control**	37	8 (17%)

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 faeces as predictor of stable remission in UC

Akkermansia muciniphila status in UC patients at entry	Remission[1]	Relapse[1]
Detected	33	6
Not detected	40	27
Fisher's exact test	P=0.009	
Sensitivity[2]	45.2 [33 – 57]	
Specificity[2]	81.8 [64 – 93]	
Positive predictive value[2]	84.6 [69 – 94]	
Negative predictive value[2]	40.3 [28 – 53]	

[1] Cases in remission for at least one year, and cases suffering relapse within the follow-up period of one year.
[2] Expressed as percentage and 95% confidence interval.

Manichanh, Varela et al, HUVH

CENTERFORBIOLOGICALSEQUENCEANALYSIS CBS

Species enriched among differentially abundant genes for CD

MetaHIT

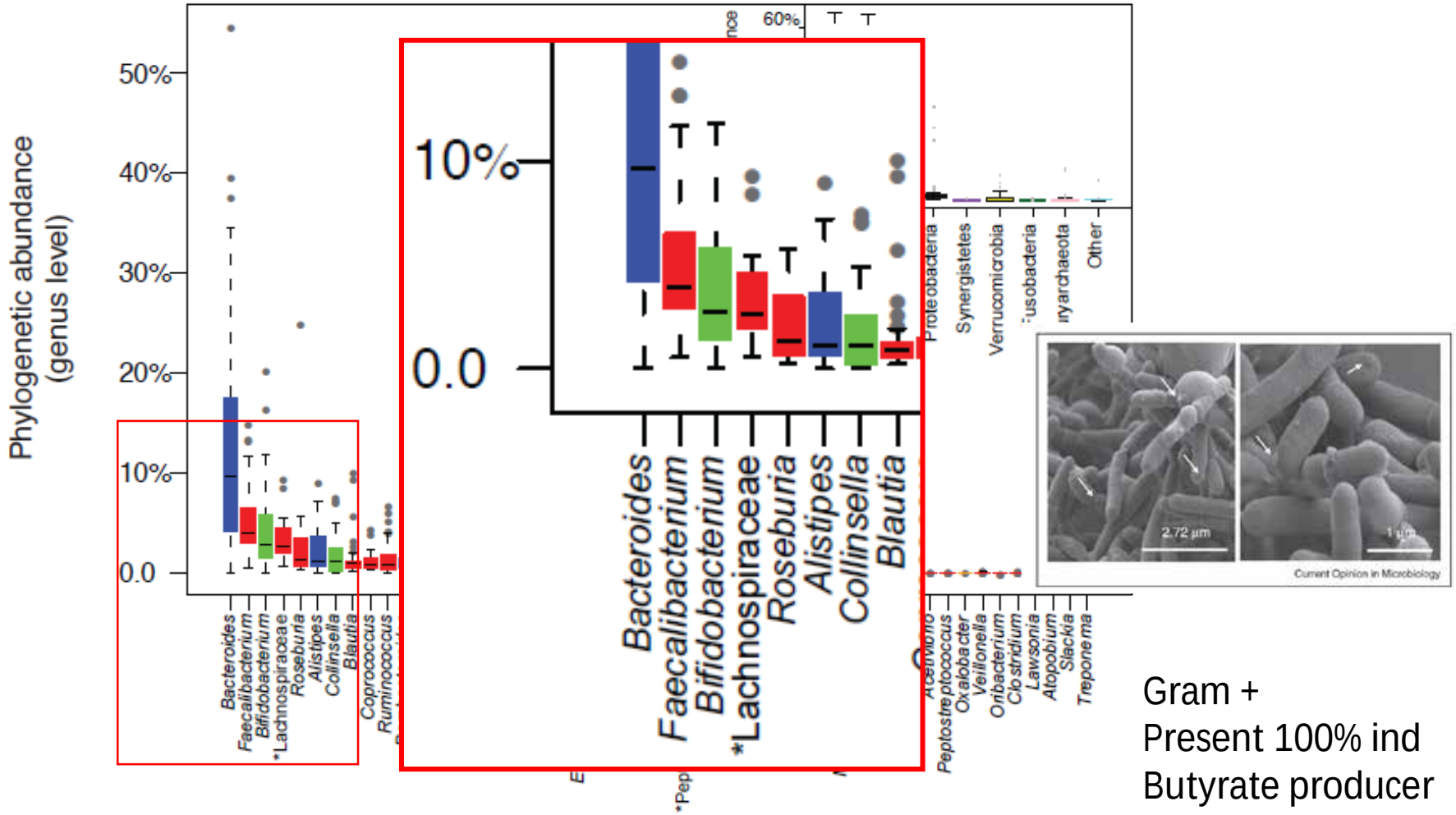
Species, fc ↑	p-value (<1e-50)
Bacteroides_sp._4_3_47FAA	0.00
Bacteroides_vulgatus	0.00
Clostridium_bolteae*	0.00
Clostridium_nexile*	0.00
Clostridium_ramosum*	0.00
Coprobacillus_sp._D7	0.00
Ruminococcus_lactaris*	4.02e-223
Ruminococcus_torques*	1.54e-145
Blautia_hansenii*	9.02e-117
Bacteroides_dorei	5.90e-105

*among top 13 most significant for arrays

Species, fc ↓	p-value (<1e-50)
Eubacterium_eligens*	0.00
Faecalibacterium_prausnitzii_A*	0.00
Faecalibacterium_prausnitzii_M*	0.00
Roseburia_intestinalis*	0.00
Ruminococcus_obeum*	0.00
Eubacterium_rectale*	1.60e-259
Ruminococcus_sp._5_1_39BFAA	7.17e-249
Eggerthella_lenta	1.56e-244
Bacteroides_pectinophilus	9.84e-83
Coprococcus_comes	7.93e-70
Alistipes_putredinis	1.00e-59
Clostridium_asparagiforme	2.57e-59

*among top 10 most significant for arrays

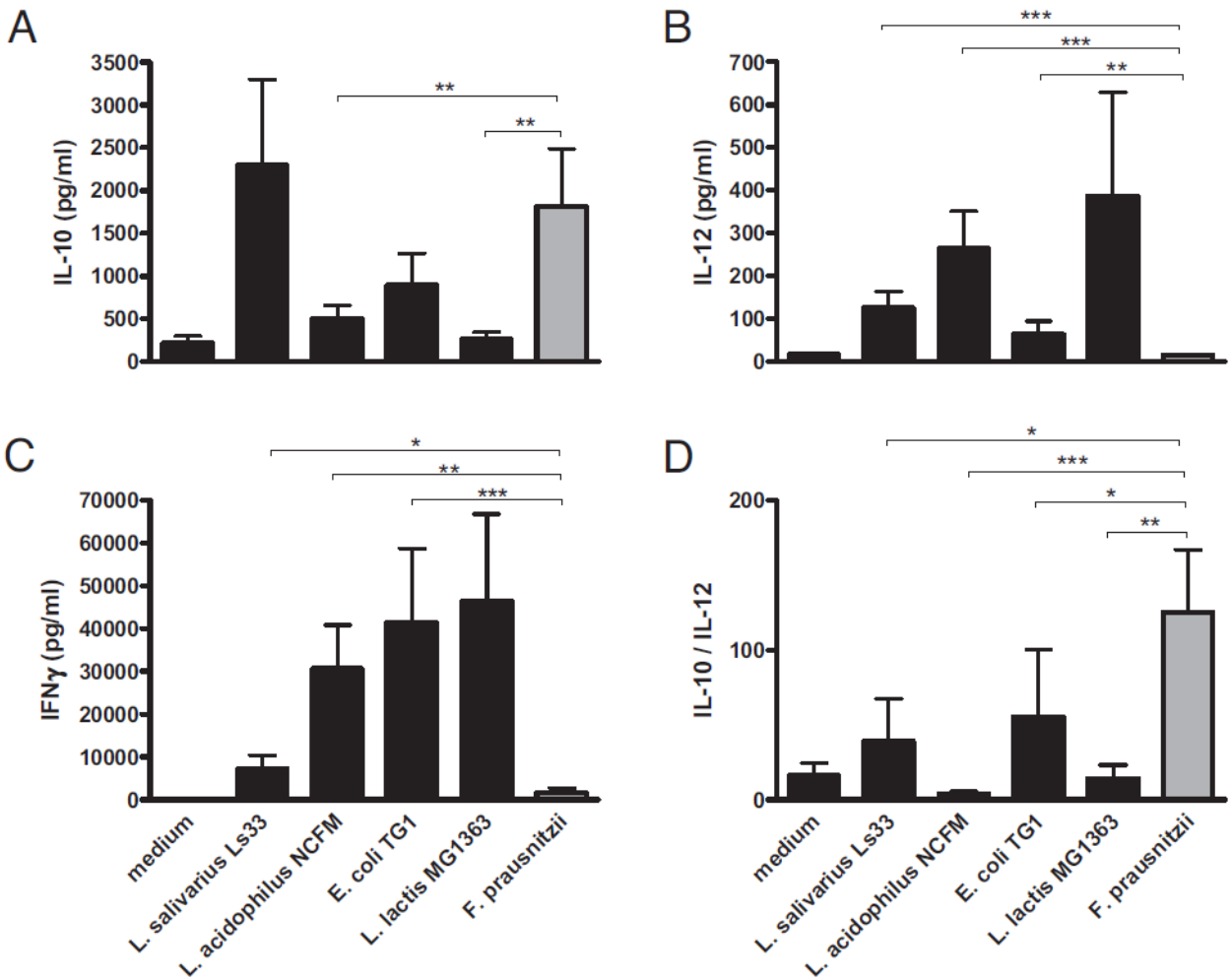
Most abundant genera in the human gut microbiota



Gram +
Present 100% ind
Butyrate producer

Arumugam et al, Nature 2011

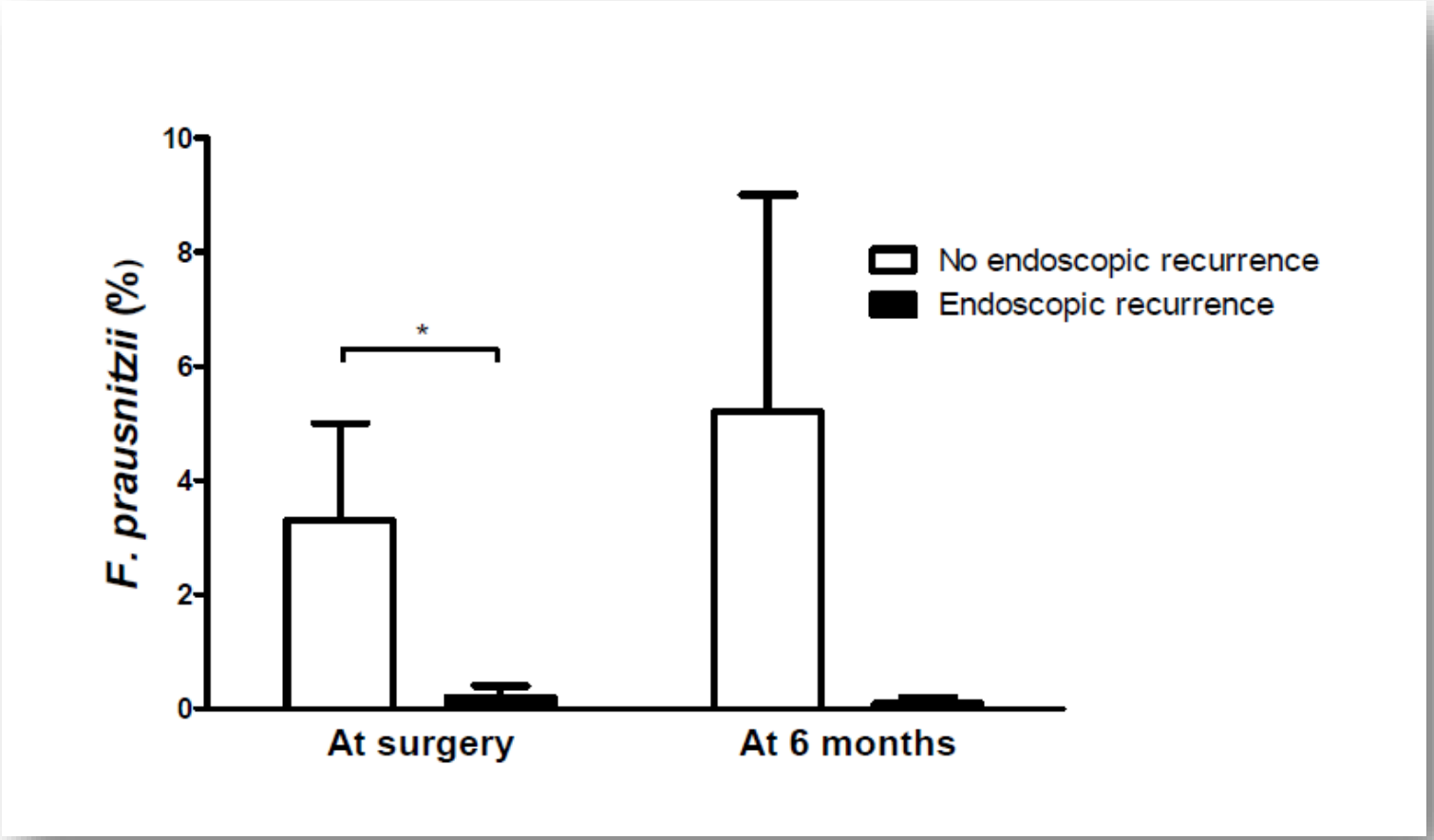
Faecalibacter prausnitzii antiinflammatory effects



Stimulation of PBMC

Sokol et al, PNAS 2008

Faecalibacter prausnitzii in Crohn's disease



FISH in ileal mucosa

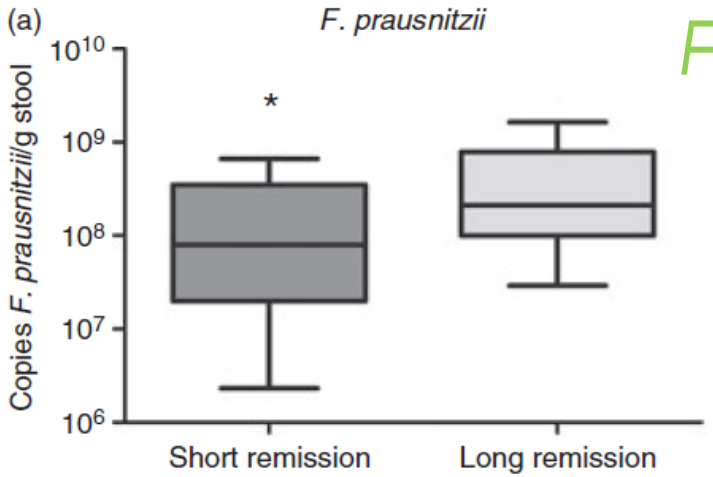
Sokol et al, PNAS 2008

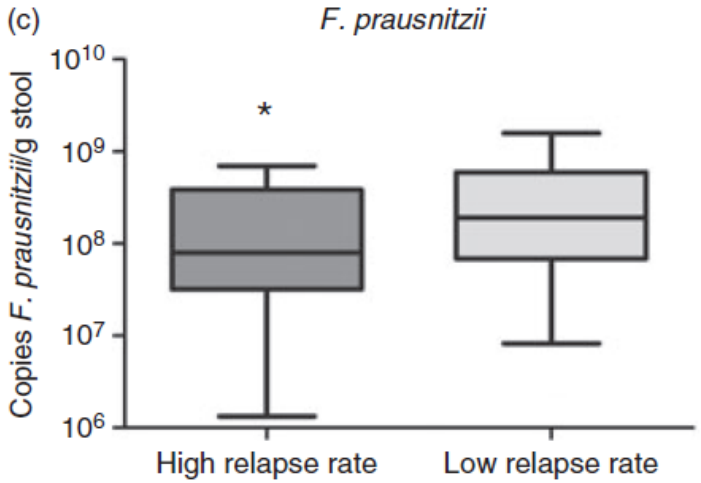
AP&T Alimentary Pharmacology and Therapeutics

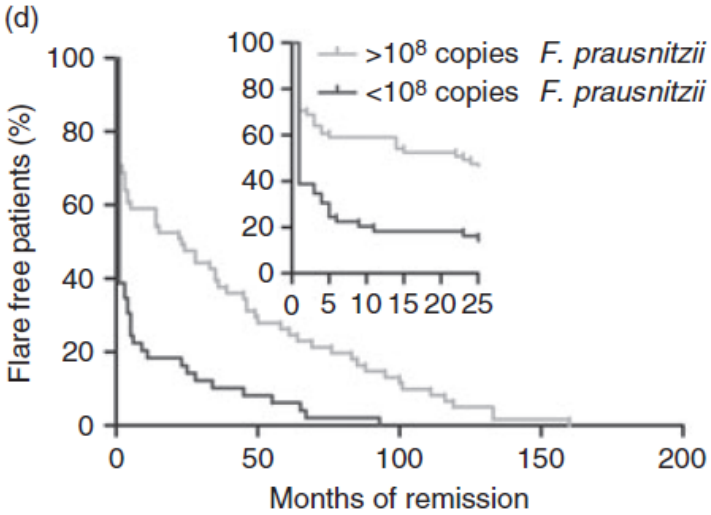
Colonisation by *Faecalibacterium prausnitzii* and maintenance of clinical remission in patients with ulcerative colitis

E. Varela, C. Manichanh, M. Gallart, A. Torrejón, N. Borruel, F. Casellas, F. Guarner & M. Antolin

Faecalibacter prausnitzii and remission



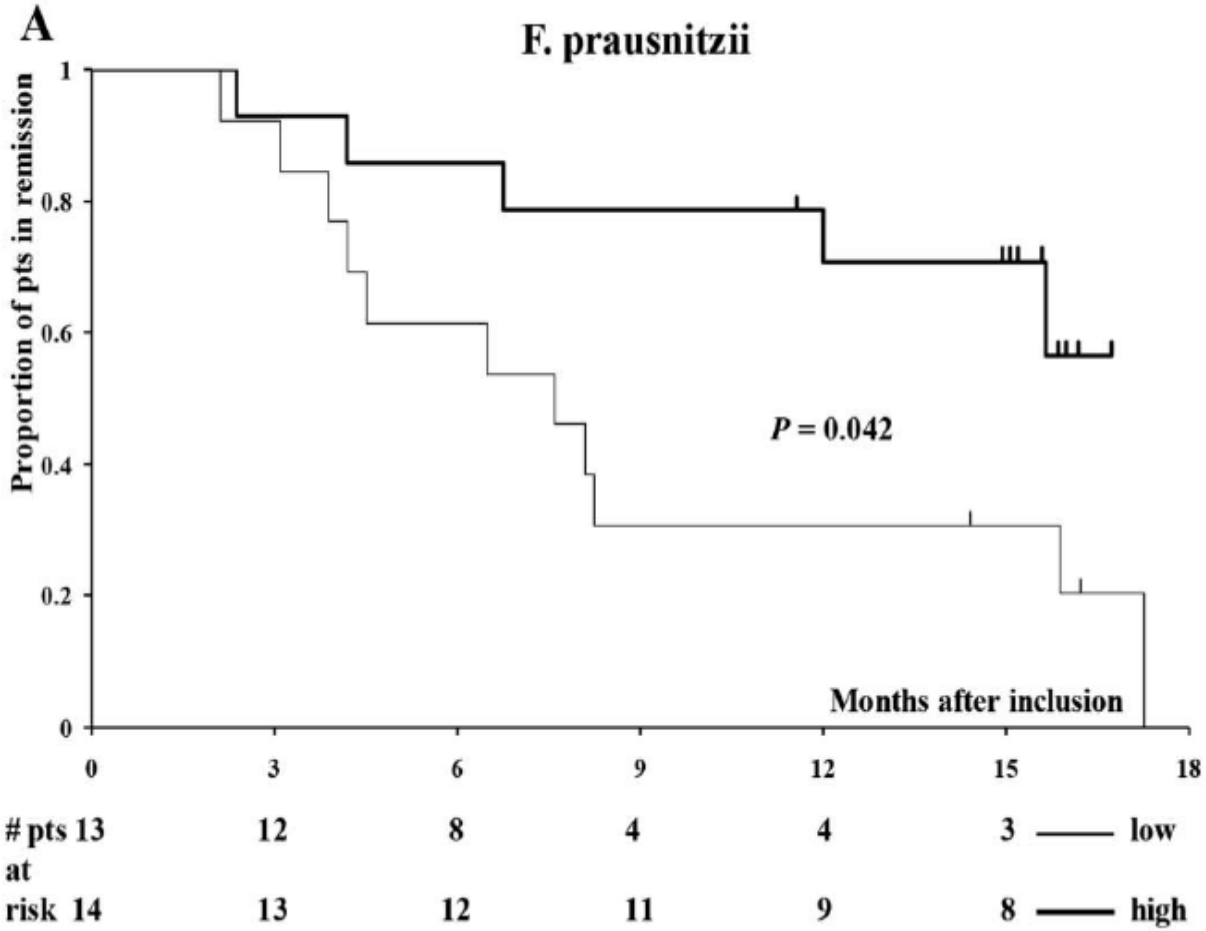




Faecalibacter prausnitzii as a predictor of relapse

ORIGINAL ARTICLE

Alt ictor
of I



l study

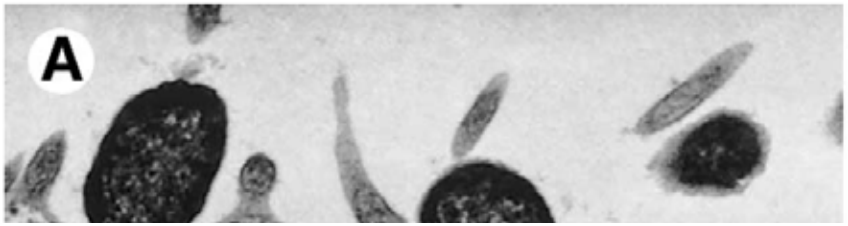
arison between
sers and non-
ser after
rawal of
mab
microbiota

Adherent-Invasive *Escherichia coli* in Crohn's disease

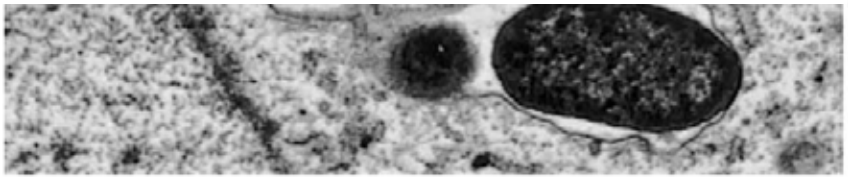
GASTROENTEROLOGY 2004;127:412–421

High Prevalence of Adherent-Invasive *Escherichia coli* Associated With Ileal Mucosa in Crohn's Disease

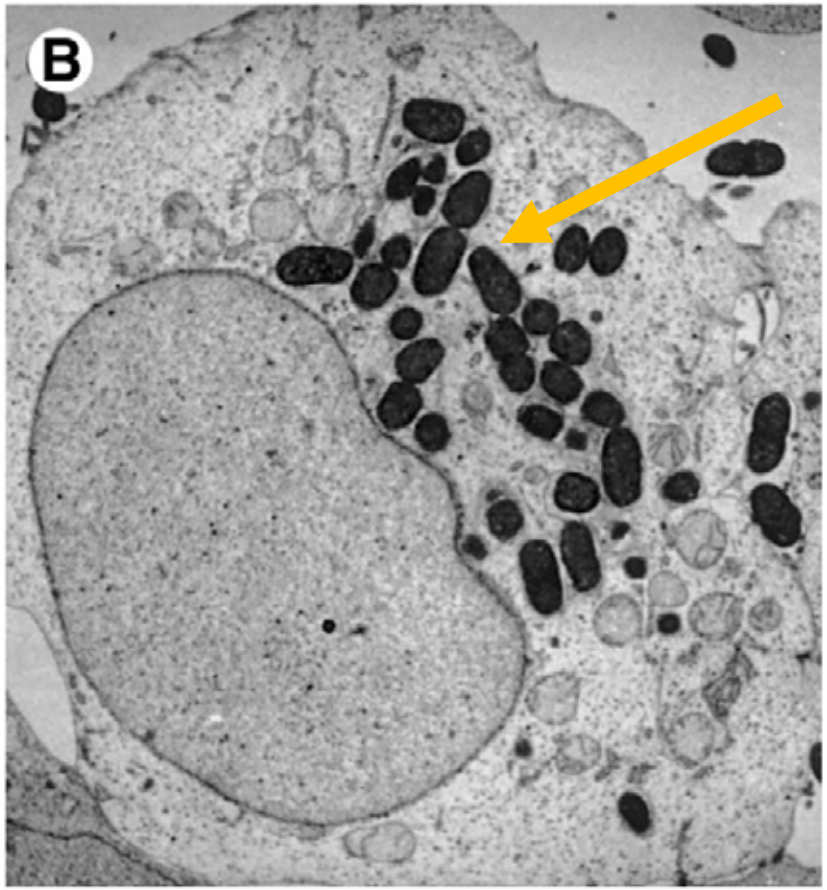
ARLETTE DARFEUILLE–MICHAUD,* JÉRÔME BOUDEAU,* PHILIPPE BULOIS,† CHRISTEL NEUT,§ ANNE–LISE GLASSER,* NICOLAS BARNICH,* MARIE–AGNÈS BRINGER,* ALEXANDER SWIDSINSKI,¶ LAURENT BEAUGERIE,‖ and JEAN–FRÉDÉRIC COLOMBEL†



Origin of the strains	Total no. of subjects	No. of subjects (%) positive		
		Invasive *E. coli*[a]	AIEC[b]	*P* value[c]
Ileal specimens of				
CD patients with chronic lesion	23	7 (30.4)	5 (21.7)	0.196
CD patients with early lesion	22	8 (36.4)	8 (36.4)	0.034[d]
CD patients with healthy mucosa	18	4 (22.2)	4 (22.2)	0.206
Controls	16	1 (6.2)	1 (6.2)	
Colonic specimens of				
CD patients	27	1 (3.7)	1 (3.7)	0.508
UC patients	8	1 (12.5)	0 (0)	0.859
Controls	102	4 (3.9)	2 (1.9)	



Adherent-Invasive *Escherichia coli* in Crohn's disease



- AIEC adhere to epithelial cells, survive and replicate in macrophages and induces an inflammatory response with cytokine secretion like TNFα origina mation "in vitro".
- They posses long fimbria that allow them to cross mucosal barrier and to access to lymphoid cells.
- In animal models, they colonize intestinal tissue stimulating Th1 and Th17 reactions inducing inflammation and fibrosis similar to that found in Crohn's disease.

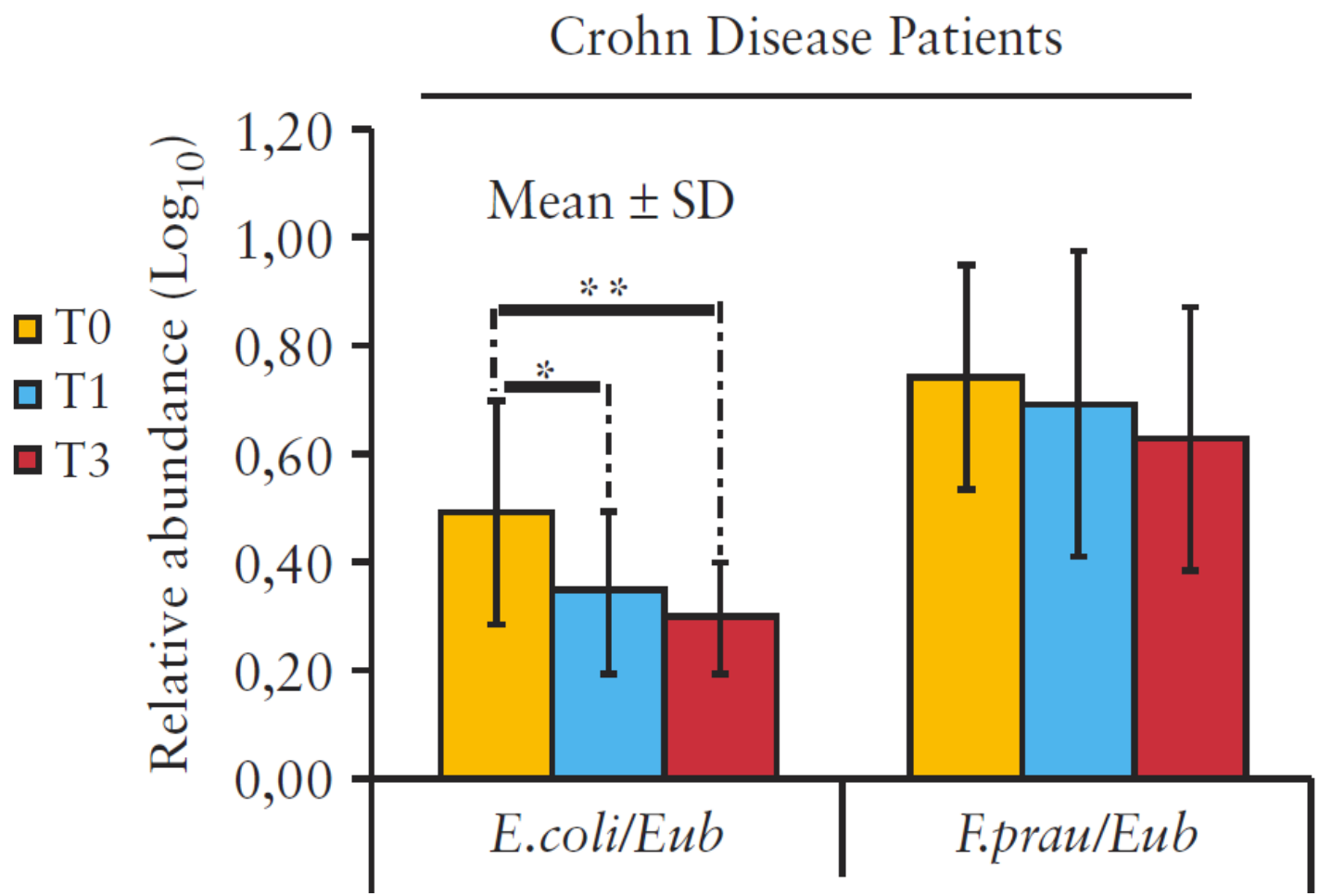
Pathobiont

Disbiosis as a monitoring biomarker

Original Article

Anti-tumour Necrosis Factor Treatment with Adalimumab Induces Changes in the Microbiota of Crohn's

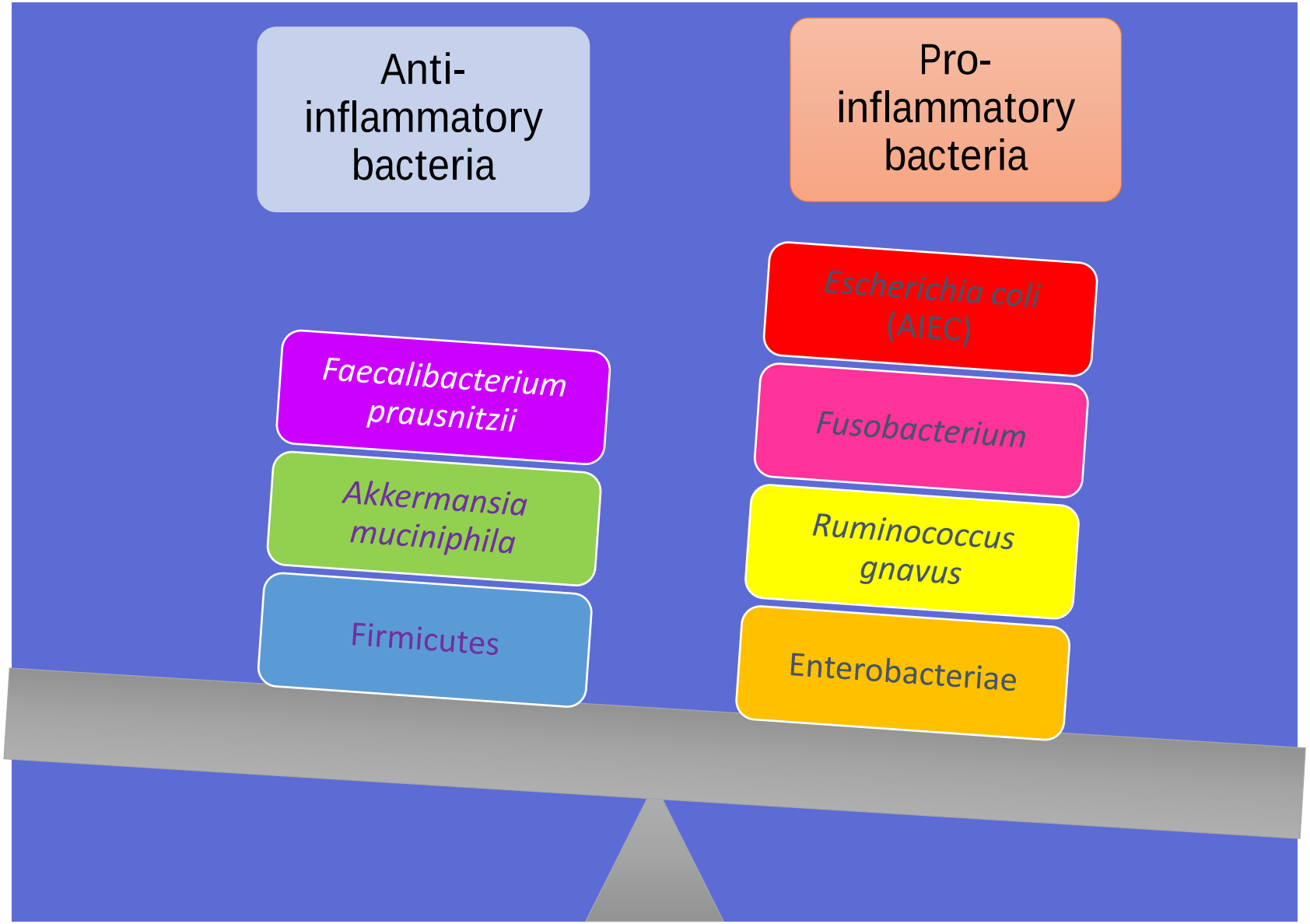
David Busquet
Margarita Mar
Josep Oriol M



Disbiosis in inflammatory bowel disease

1. There is a permanent distortion of the gut microbial ecosystem in IBD patients, even during remission periods, characterized by reduction in microbial diversity and instability.
2. Low diversity in the gut microbiome is associated with a severe clinical course (high relapse rate, surgery..)
3. Species with potential anti-inflammatory activity, like *A. muciniphila* and *Faecalibacterium prausnitzii* are missing in patients with severe clinical course.

Disbiosis in inflammatory diseases



Disbiosis as a therapeutic target

- The Grand Challenge in IBD is prevention of relapse (and perhaps cure?)

- Approach: To increase diversity –microbial gene richness- of the gut microbiota

- Tools:
  - Faecal Microbiota Transplant (FMT)
  - Bacterial Consortium Transplant (targeted restoration)
  - Nutritional intervention: prebiotics, fibers
  - New probiotics: *Faecalibacter prausnitzii, Akkermansia*, etc