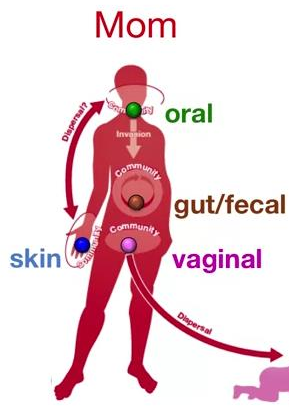


HOMO SAPIENS SYMBIOTICUS

Hervé M. Blottière

FInE lab « Functionality of the Intestinal Ecosystem »,
Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay
&
MetaGenoPolis
78350 Jouy en Josas

*Séance microbiome, Académie d'Agriculture
10 mai 2017, Paris*



adapted from Gonzalez et al. 2011, EMBO reports

Homo sapiens « symbioticus »

Une symbiose homme-microbes commençant dès la naissance

Maintien de la symbiose
= santé et bien-être

Maturation Immunitaire



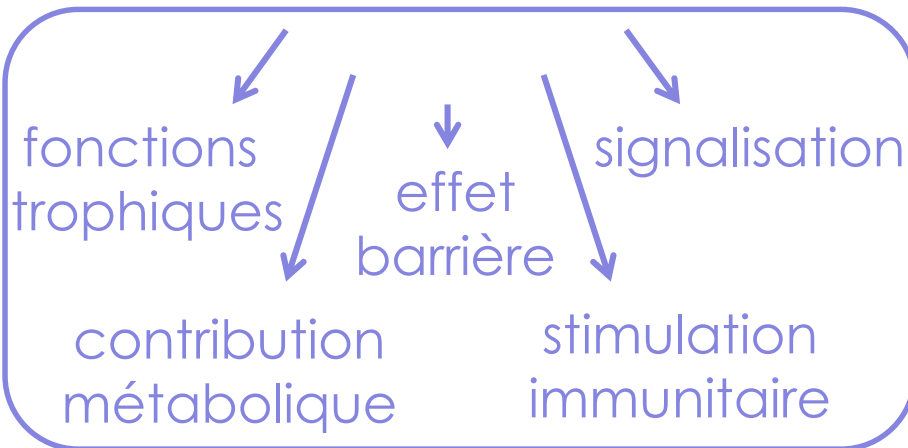
&



Une symbiose unique
Le microbiote est
reconnu comme
une composante du 'soi'

Rupture de
l'équilibre
écologique :
Risque
infectieux

Développement du microbiote



Rupture de la
tolérance :
Risque auto-immun
et désordres
inflammatoires

diète, aliments
fonctionnels,
antibiotiques,
microbiotes

LETTER

26 May 2016

OPEN

doi:10.1038/nature17645

Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation

Hilary P. Browne^{1*}, Samuel C. Forster^{1,2,3*}, Blessing O. Anonye¹, Nitin Kumar¹, B. Anne Neville¹, Mark D. Stares¹, David Goulding⁴ & Trevor D. Lawley¹

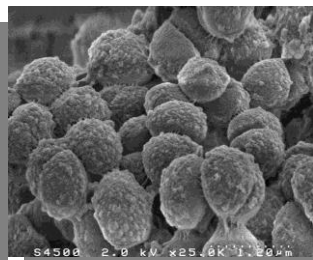
being » throughout all stages of our life »

- ✓ A key organ, interacting with food (fermentation,...); interacting with our cells (Immune & nervous systems,...); protecting against pathogens (barrier function);...



Faecalibacterium prausnitzii

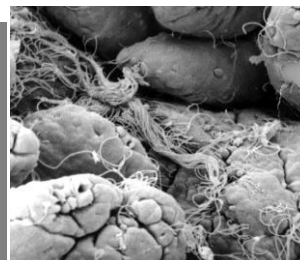
Photos INRA



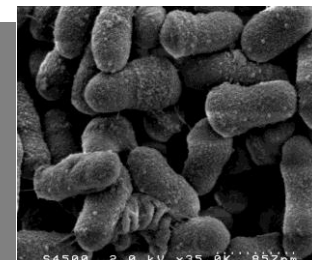
Ruminococcus spp



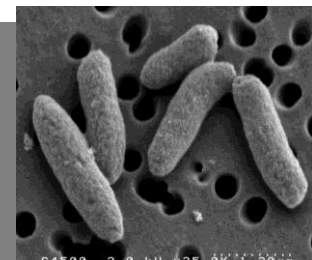
Clostridium difficile
From mice cecum



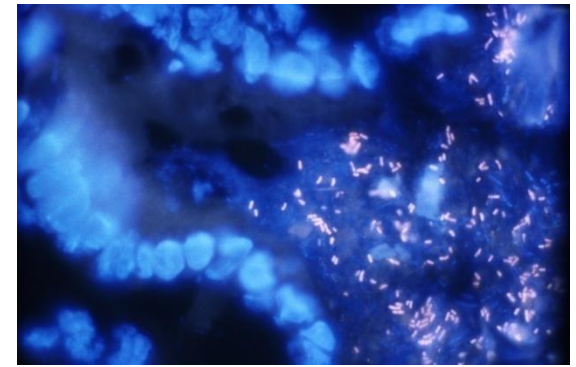
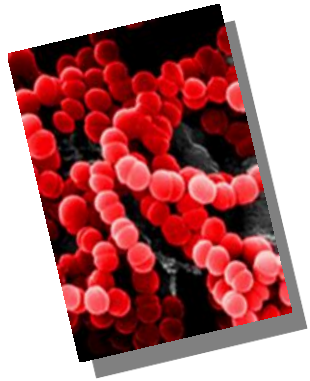
Bacteria anchored in a
Peyer patch
Mouse intestine



Bacteroides dorei



Escherichia coli



CHAPITRE 1

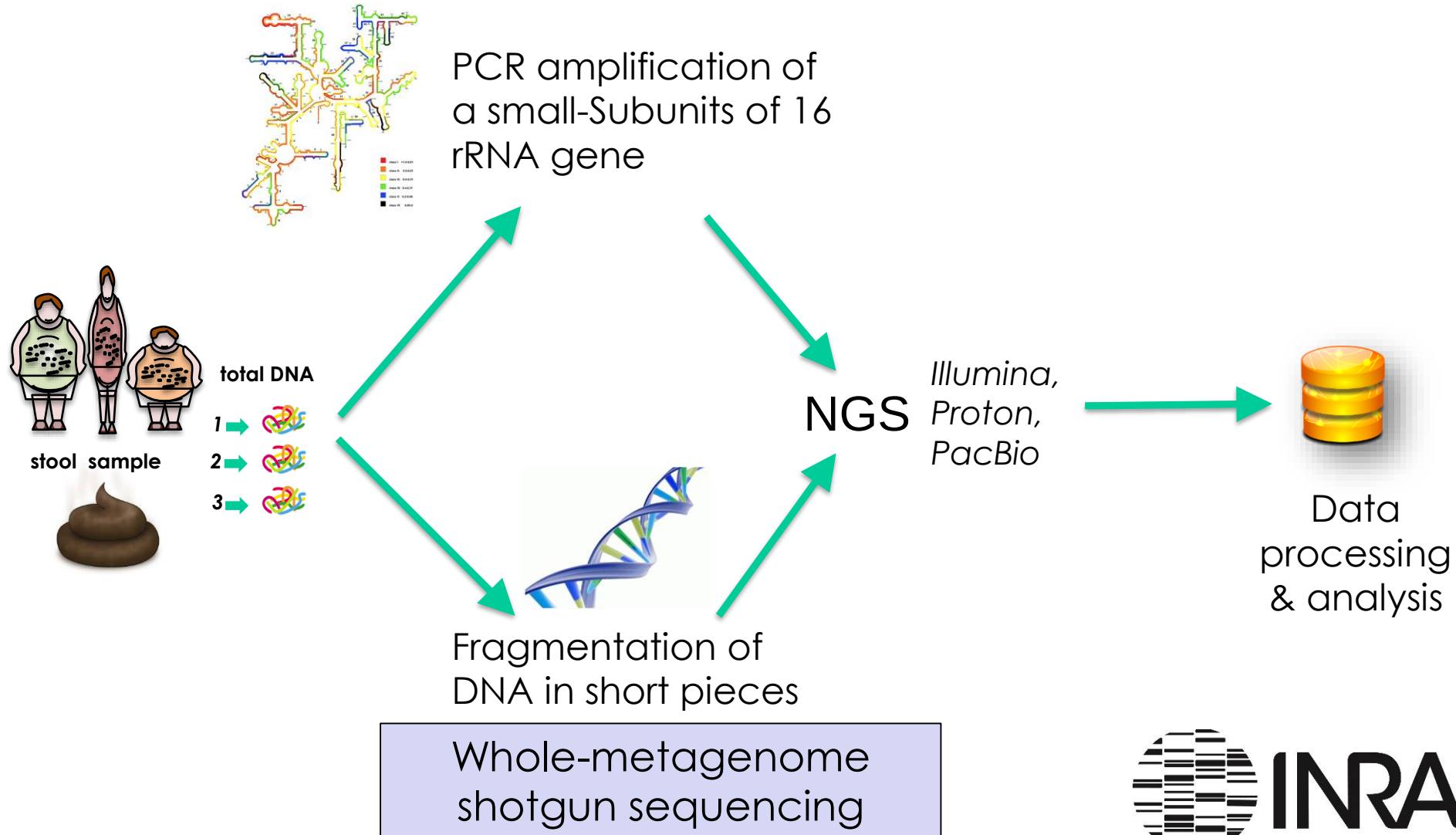
MICROBIOTE INTESTINAL,

DU MICROSCOPE AU MÉTAGÉNOME

MICROBIOTE & DYSBIOSES

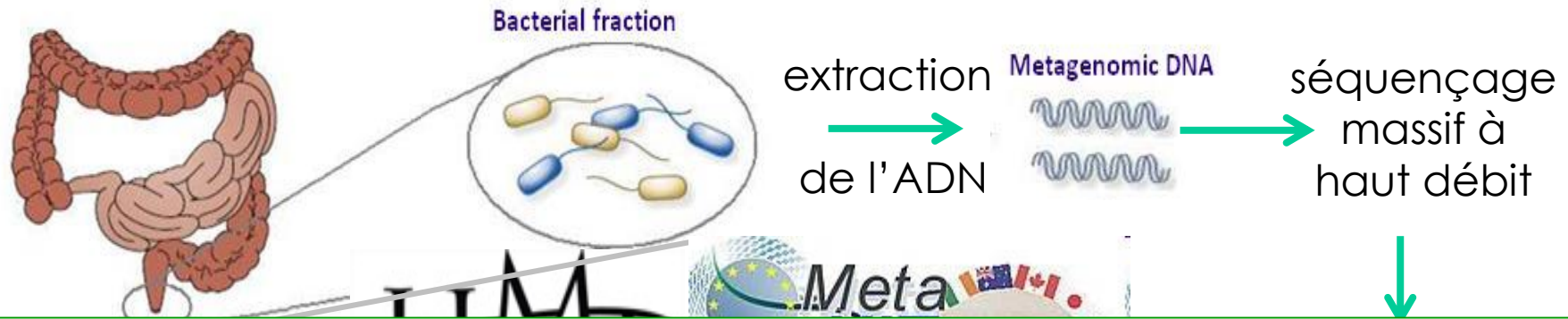
METAGENOMIC TOOLS TO CHARACTERIZE THE MICROBIOME

16S rRNA amplicon sequencing



METAGENOME: GENOMES DE TOUS LES MICROBES DOMINANTS

Une révolution technique développée au début du siècle



Premières leçons de la métagénomique du microbiote intestinal :
Un catalogue de référence qui souligne les éléments partagés
mais aussi une spécificité individuelle :

Qin Nature 2010 ; Li Nature Biotech 2014 ; Nielsen Nature Biotech 2014 ; Xiao Nature Biotech 2015

Trois paysages d'écologies intestinales chez l'Homme et une relative
robustesse de la symbiose :

Arumugam Nature 2011 ; Schloissnig Nature 2012 ; Shoaie Cell Metabol 2015

Une symbiose altérée dans les maladies chroniques (maladie de Crohn,
obésité, diabète, maladies du foie, maladies neurologiques) : *Sokol PNAS
2008, Qin Nature 2012 ;*

Le Chatelier Nature 2013 ; Qin Nature 2014 ; Cotillard Nature 2013

Des preuves de causalité par transfert de microbiote in vivo : *MICI Schaubek 2015,
Obésité & NASH Rabot FASEB 2010, Le Roy Gut 2013, cirrhose Llopis Gut 2016, dépression
Rabot comm pers.*

QUANTITATIVE METAGENOMIC PIPELINE AT



SAMBO-METAQUANT-INFOBIOSTAT

sample
collection

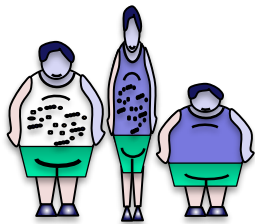
sequencing

reference
construction

gene profiling

bioinformatics
/statistics analyses

4000 samples/yr



stool sample

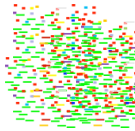
total DNA



library preparation
SOLiD/Illumina/Proton
sequencing



short sequences
30-50 million



The prime way to
characterize a
microbiome

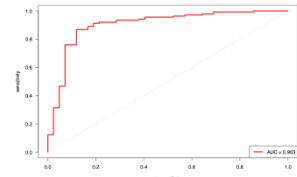
with
data



Identify clinically
relevant groups

Re	15	0	0	0	0	0	95	0
16	1250	6002	468	607	492	141	8023	
17	0	0	0	0	0	0	0	
18	0	9	108	0	0	55	0	
19	0	0	0	3	0	0	0	
3300000	0	36	2	0	43	106	1250	

preprocessing /
normalization &
dimension reduction



build and test
prediction models

reference gene
catalogue



catalogue
annotation

catalogue
structuration



Known
genome

SOP Downloads

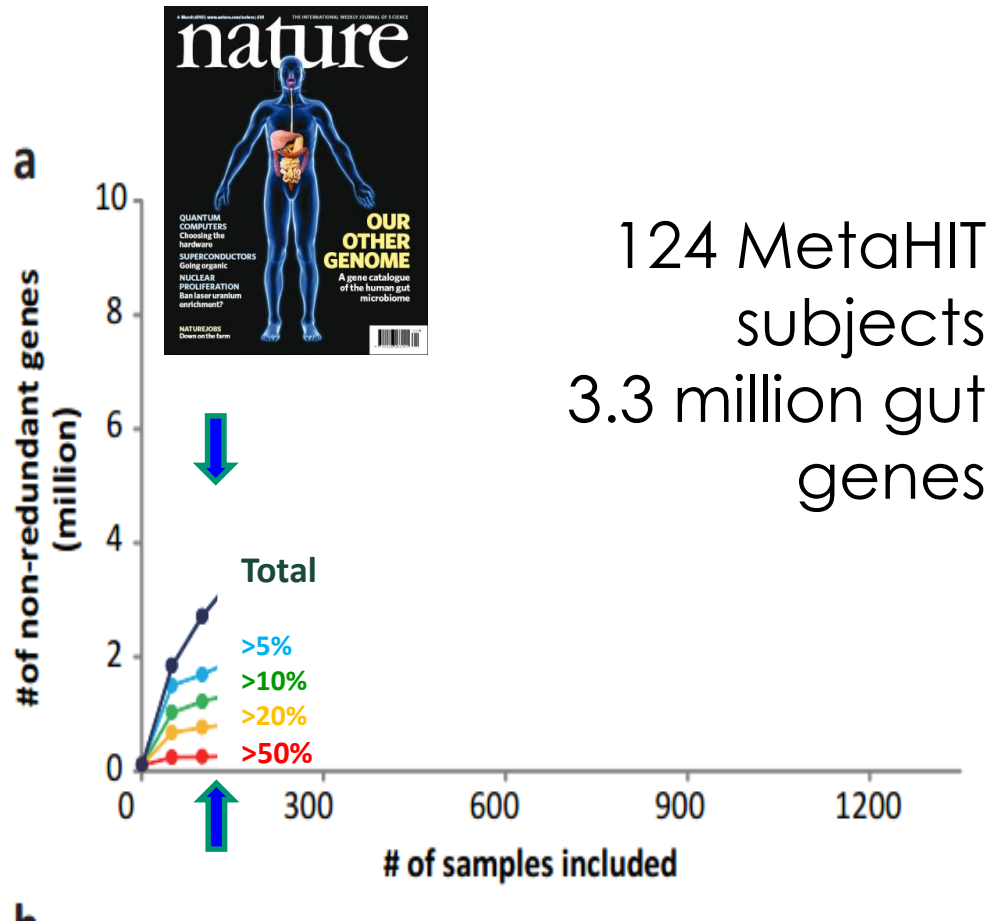


Standardization is critical
<http://www.microbiome-standards.org/#SOPS>



INRA
SCIENCE & IMPACT

A REFERENCE CATALOG OF 3.3 MILLION GUT GENES



Rare genes are increasing
Transient species ?
Strain differences ?



Common genes are not
May be clinically useful for
Diagnosis and follow up ?

Individuals from MetaHIT, Chinese and HMP studies, n=1267

Qin, Nature 2010

Li et al. Nature Biotech, 2014

HUMANS SHARE A CORE MICROBIOME, AND YET DIFFER AT THE METAGENOMIC LEVEL

On average, each individual carries ~540 000 genes of the initial 3.3 million genes catalog

Similarity:

Core metagenome genes :
~50 % of an individual's genes
are shared by at least 50 % of
individuals of the cohort

Yet, individuality:

Rare genes :
genes shared by less than 20 %
of individuals
= 2.4 million genes

We are all rather similar ! but not identical!!

Very different from mice : 4% shared genes in the 2 catalogues

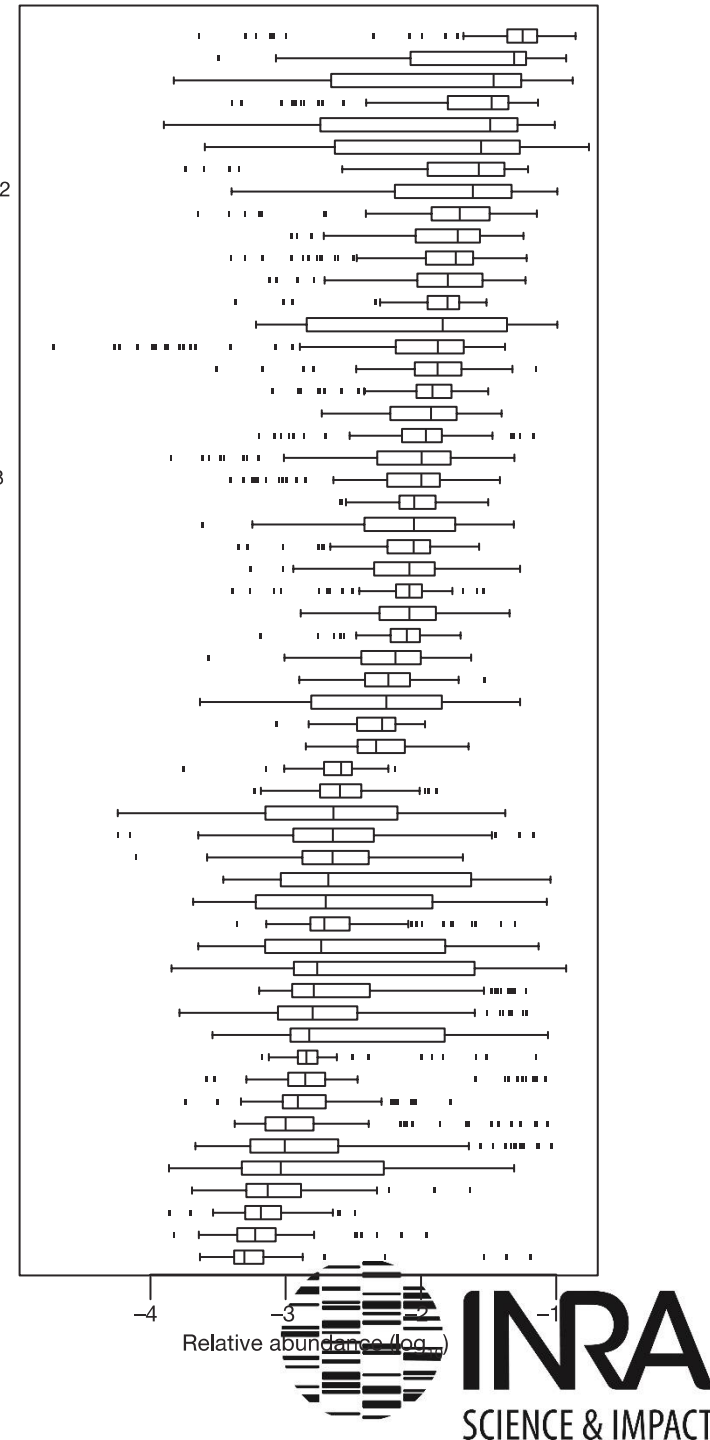
Qin, Raes et al, Nature 2010

Mouse catalogue : Xiao et al, Nature Biotech, 2015

A METAGENOMIC CORE

57 species found in
more than 90% of the
124 individuals

Bacteroides uniformis
Alistipes putredinis
Parabacteroides merdae
Dorea longicatena
Ruminococcus bromii L2-63
Bacteroides caccae
Clostridium sp. SS2-1
Bacteroides thetaiotaomicron VPI-5482
Eubacterium hallii
Ruminococcus torques L2-14
Unknown sp. SS3 4
Ruminococcus sp. SR1 5
Faecalibacterium prausnitzii SL3 3
Ruminococcus lactaris
Collinsella aerofaciens
Dorea formicigenerans
Bacteroides vulgatus ATCC 8482
Roseburia intestinalis M50 1
Bacteroides sp. 2_1_7
Eubacterium siraeum 70 3
Parabacteroides distasonis ATCC 8503
Bacteroides sp. 9_1_42FAA
Bacteroides ovatus
Bacteroides sp. 4_3_47FAA
Bacteroides sp. 2_2_4
Eubacterium rectale M104 1
Bacteriodes xylanisolvens XB1A
Coprococcus comes SL7 1
Bacteroides sp. D1
Bacteroides sp. D4
Eubacterium ventriosum
Bacteroides dorei
Ruminococcus obeum A2-162
Subdoligranulum variabile
Bacteroides capillosus
Streptococcus thermophilus LMD-9
Clostridium leptum
Holdemania filiformis
Bacteroides stercoris
Coprococcus eutactus
Clostridium sp. M62 1
Bacteroides eggerthii
Butyrivibrio crossotus
Bacteroides finegoldii
Parabacteroides johnsonii
Clostridium sp. L2-50
Clostridium nexile
Bacteroides pectinophilus
Anaerotruncus colihominis
Ruminococcus gnavus
Bacteroides intestinalis
Bacteroides fragilis 3_1_12
Clostridium asparagiforme
Enterococcus faecalis TX0104
Clostridium scindens
Blautia hansenii

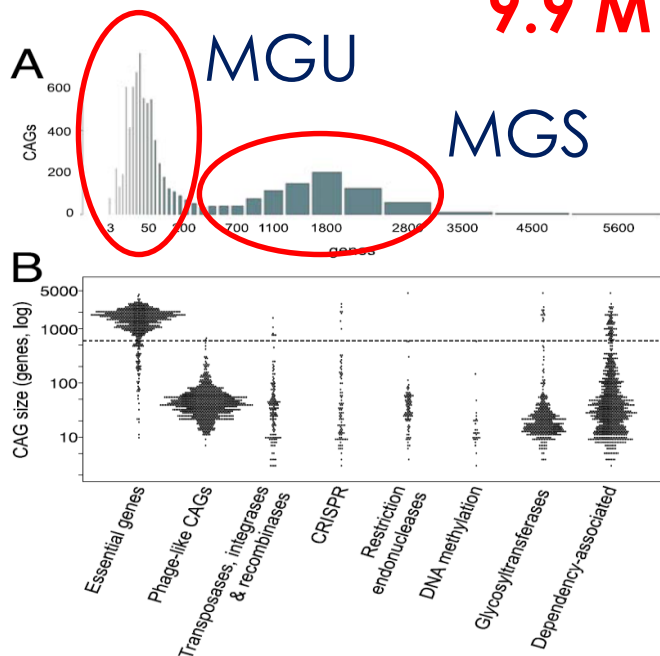


Qin et al, Nature, 2010

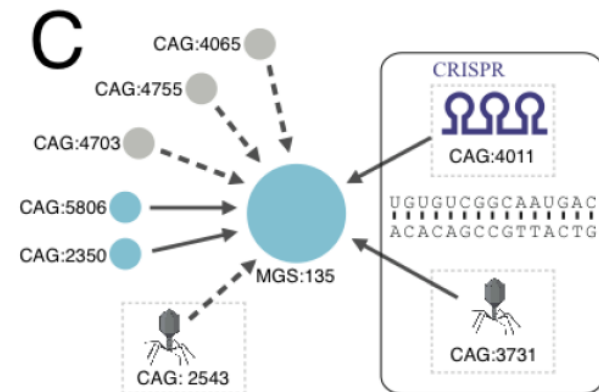
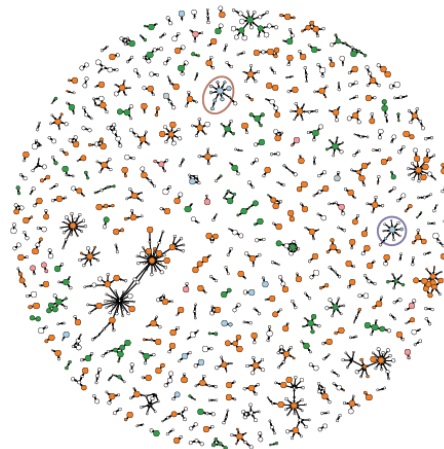
GENE CATALOG CLUSTERED IN METAGENOMIC UNITS BY CO-ABUNDANCE BINNING

741 large MGU (>700 Genes) correspond to bacterial species (MetaGenomic Species; 85% previously unknown)
238 high quality genomes reconstructed
6640 small MGU (phages, plasmids, CRISPR...)

9.9 M MetaHit: ~1 500 MGS & ~12 000 MGU



Interaction network



CAG: Co-abundant Gene Group

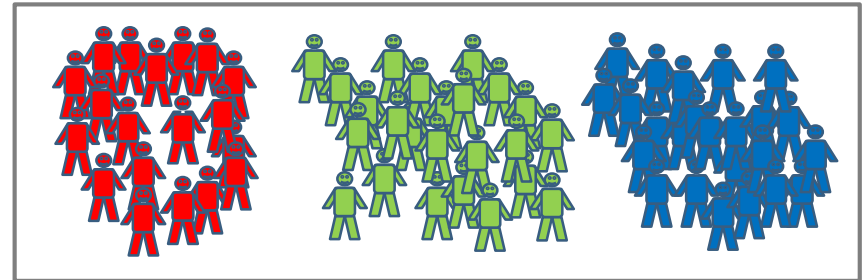
HUMANS MICROBIOMES DIFFER AT THE LEVEL OF ECOLOGICAL ARRANGEMENT : THE ENTEROTYPES



May 2011

In a attempt to characterize the intestinal microbiome of 'the average human', we observed ...
an organization of intestinal metagenomes in 3 assemblages of genes and microbial taxa :

order in chaos !?!



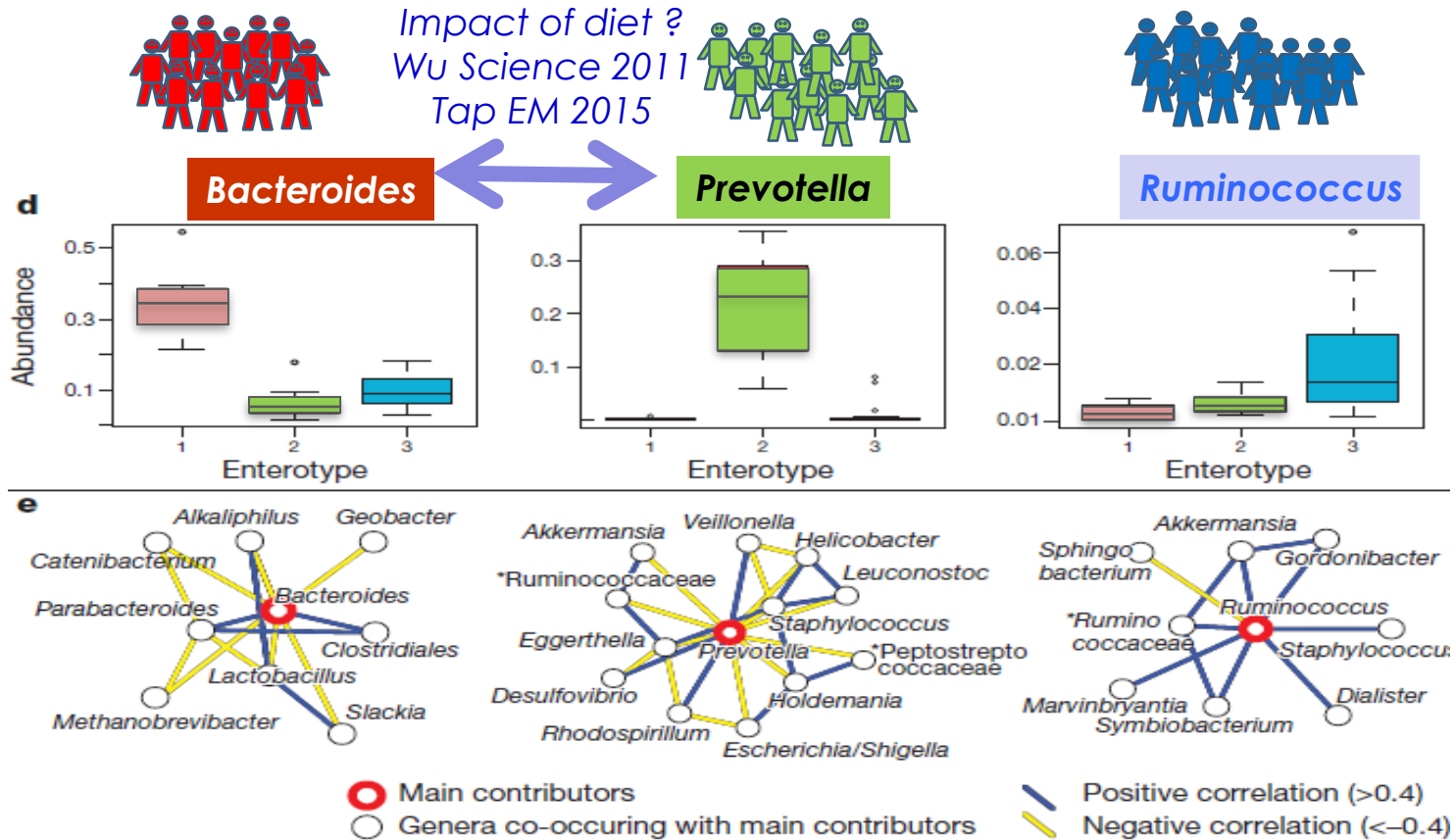
the *enterotypes*

Qin et al, Nature 2010

Arumugam, Raes et al, Nature 2011

Enterotypes of the human gut microbiome

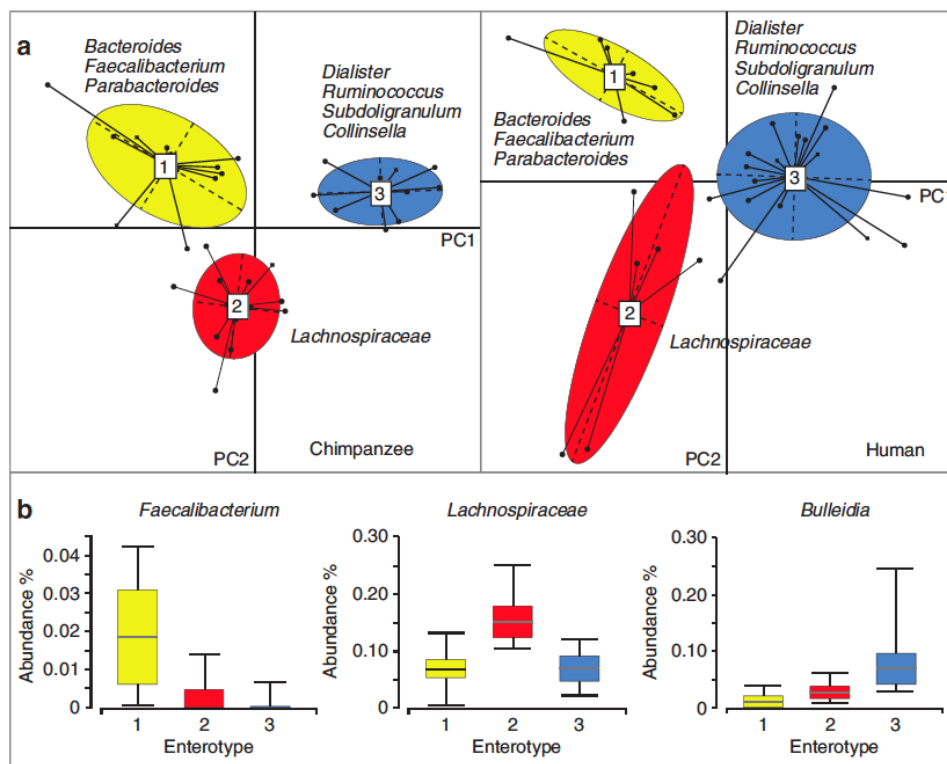
Manimozhayan Arumugam^{1*}, Jeroen Raes^{1,2*}, Eric Pelletier^{3,4,5}, Denis Le Paslier^{3,4,5}, Takuji Yamada¹, Daniel R. Mende¹, Gabriel R. Fernandes^{1,6}, Julien Tap^{1,7}, Thomas Bruls^{3,4,5}, Jean-Michel Batto⁷, Marcelo Bertalan⁸, Natalia Borrueal⁹, Francesc Casellas⁹, Leyden Fernandez¹⁰, Laurent Gautier⁸, Torben Hansen^{11,12}, Masahira Hattori¹³, Tetsuya Hayashi¹⁴, Michiel Kleerebezem¹⁵, Ken Kurokawa¹⁶, Marion Leclerc⁷, Florence Levenez⁷, Chaysavanh Manichanh⁹, H. Bjørn Nielsen⁸, Trine Nielsen¹¹, Nicolas Pons⁷, Julie Poulain³, Junjie Qin¹⁷, Thomas Sicheritz-Ponten^{8,18}, Sebastian Tims¹⁵, David Torrents^{10,19}, Edgardo Ugarte³, Erwin G. Zoetendal¹⁵, Jun Wang^{17,20}, Francisco Guarner⁹, Oluf Pedersen^{11,21,22,23}, Willem M. de Vos^{15,24}, Søren Brunak⁸, Joel Doré⁷, MetaHIT Consortium†, Jean Weissenbach^{3,4,5}, S. Dusko Ehrlich⁷ & Peer Bork^{1,25}



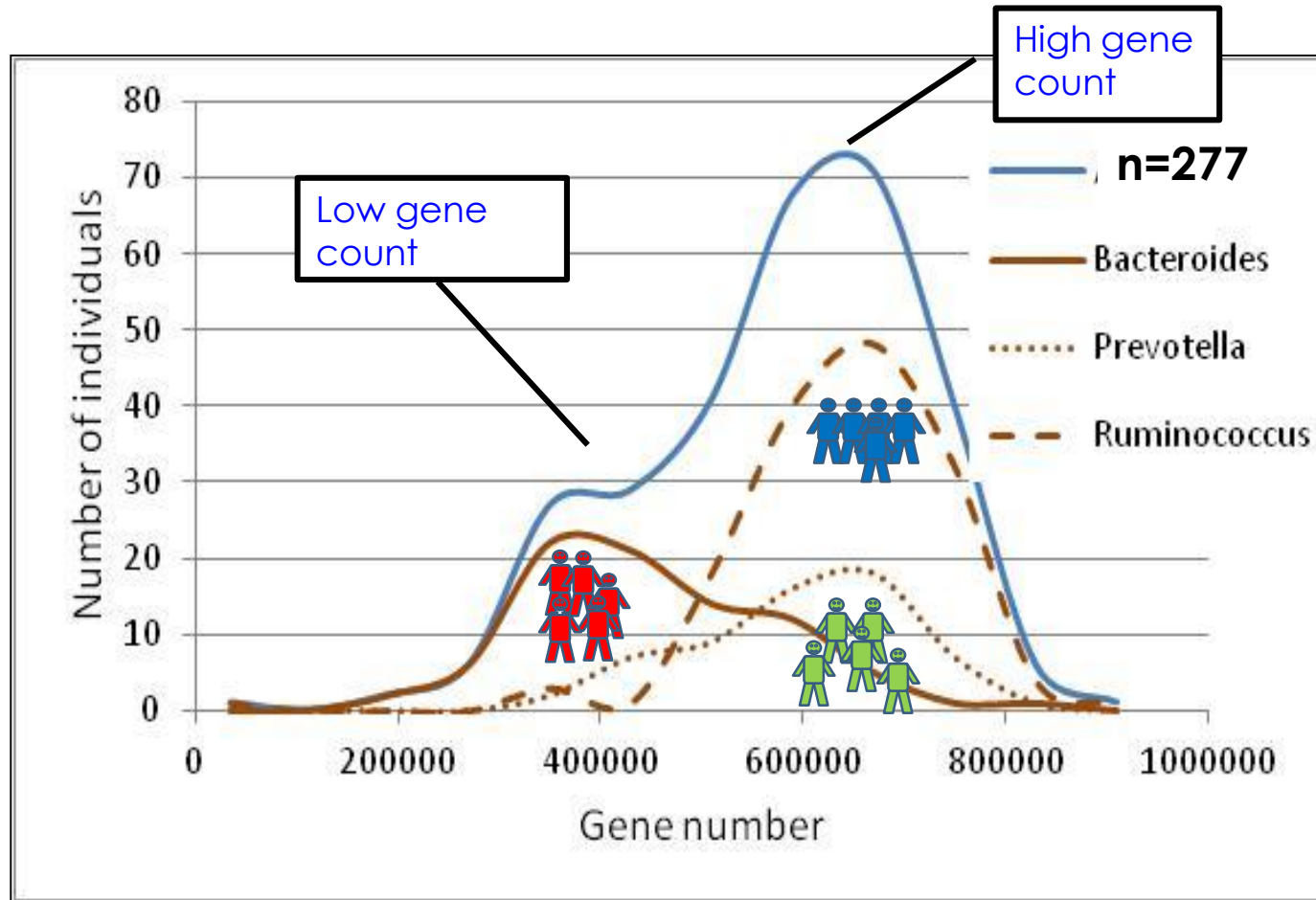
Ecology underlying enterotypes should be better understood

Chimpanzees and humans harbour compositionally similar gut enterotypes

Andrew H. Moeller¹, Patrick H. Degnan¹, Anne E. Pusey², Michael L. Wilson^{3,4}, Beatrice H. Hahn⁵ & Howard Ochman¹



HUMAN GUT MICROBIOMES DIFFER AT THE LEVEL OF GENE RICHNESS (DIVERSITY)



LGC
≈ 15 % of lean
individuals

≈ 40%
Overweight-
moderate obese

≈ 75%
Morbid obese
before bypass
surgery

58 « species » significantly linked to gene count

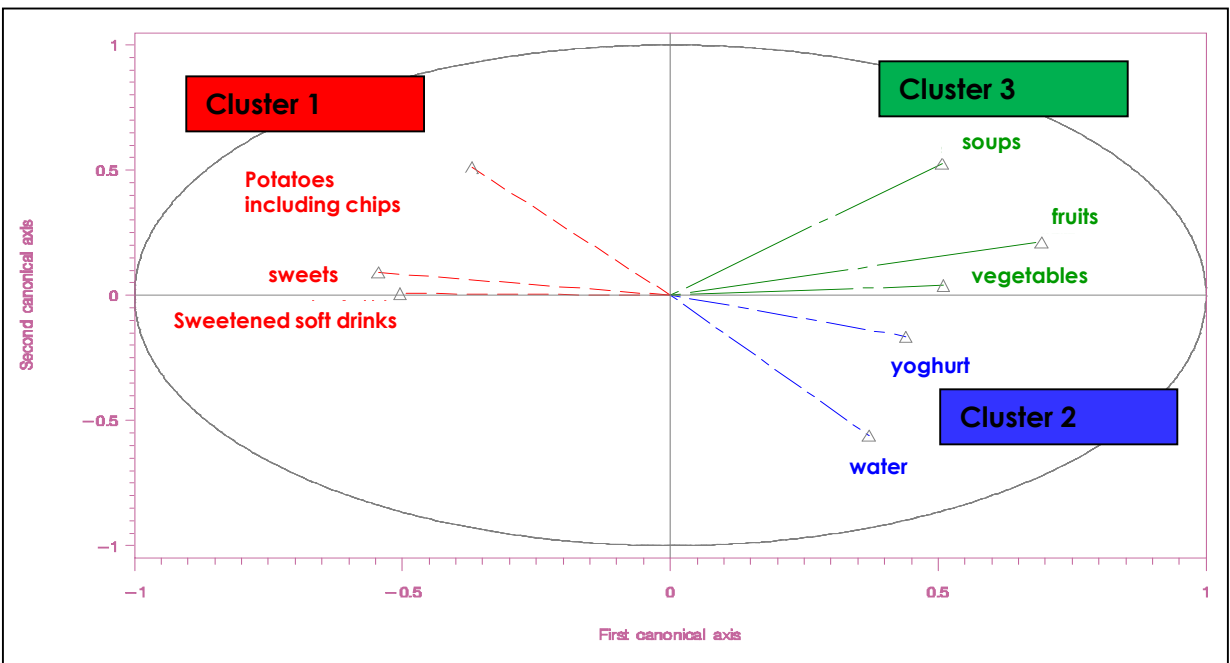
Le Chatelier, et al, Nature 2013; Cottillard et al, Nature, 2013

HABITUDES ALIMENTAIRES ET DIVERSITÉ DU MICROBIOTE

Karine Clément, ICAN,
& Danone Research

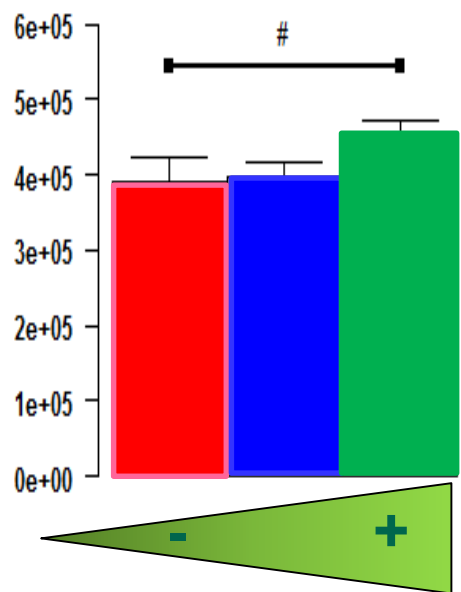


3 types de prises alimentaire sont observés,
pour 26 catégories d'aliments et sur la base de relevés
complets de 7 jours



Canonical discriminant analysis

Richesse en gènes



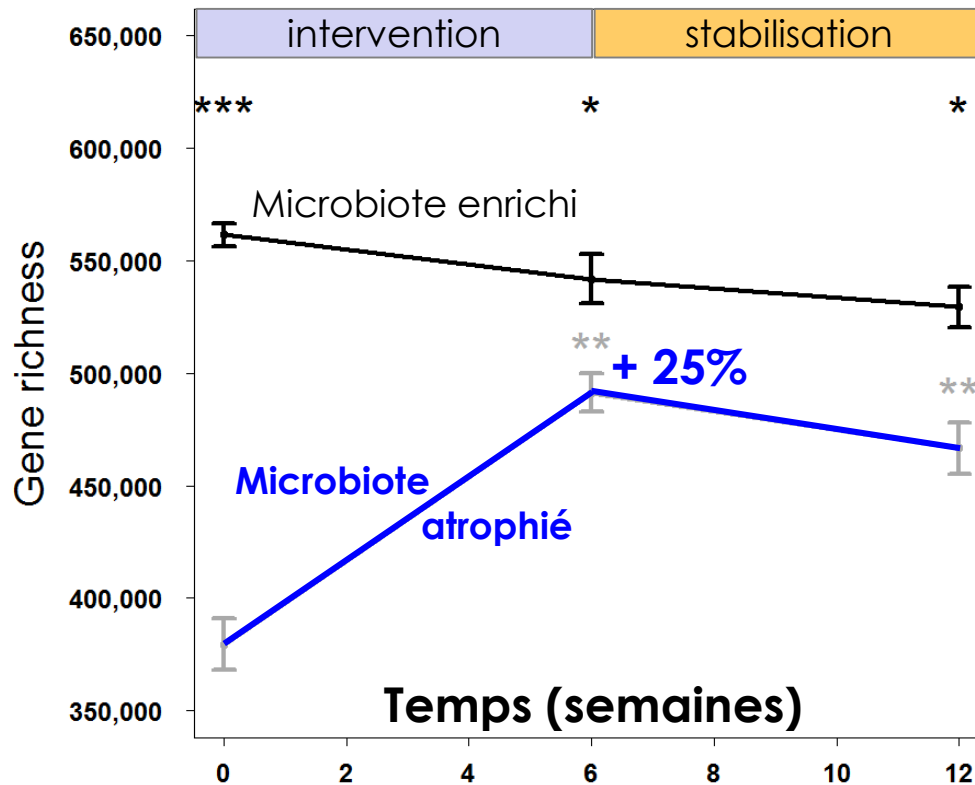
'richesse en fibres'

A un régime plus riche en fibres correspond un microbiote plus diversifié.

Kong et al, PLoS one 2014



INTERVENTION NUTRITIONNELLE ET DIVERSITÉ DU MICROBIOTE



intervention : apports enrichis en protéines, peu gras et enrichi en sucres à faible index glycémique, **apportant une grande diversité de fibres**

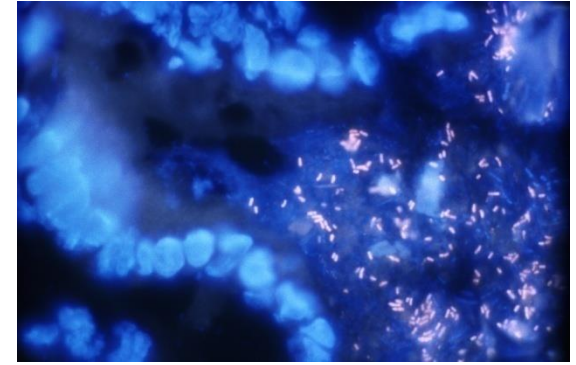
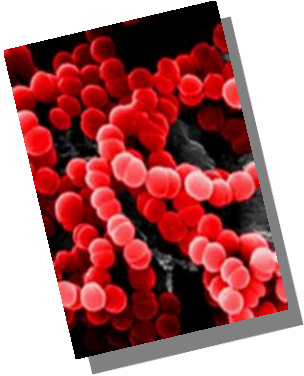
(régime KOT calibré pour apporter 1200 à 1500 Kcal)



Un régime riche en fibres peut diversifier un microbiote dominant atrophié

Un microbiote dominant atrophié prédit une moindre réponse au régime

Cotillard et al, Nature 2013



CHAPTER 2

MICROBIOTE INTESINAL,

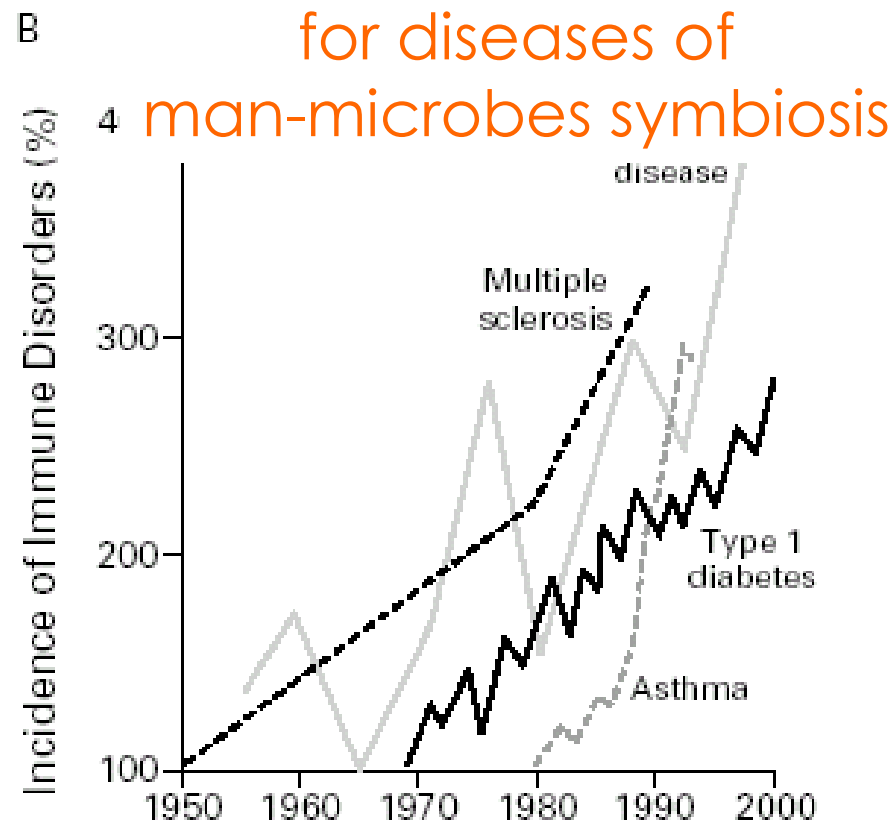
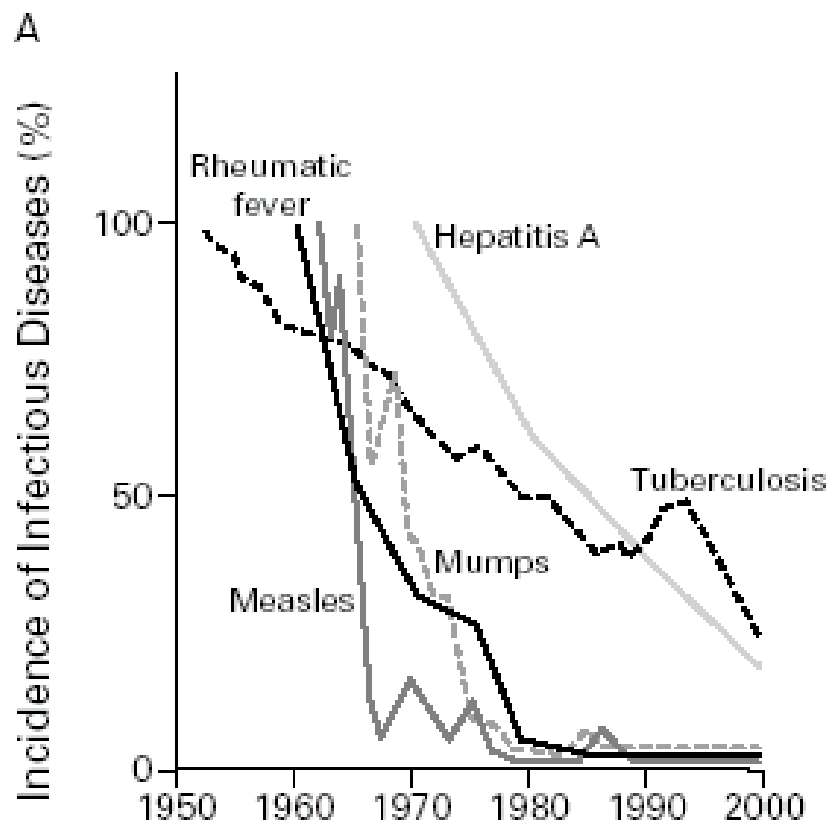
DU MICROSCOPE AU MÉTAGÉNOME

MICROBIOTE & MALADIES

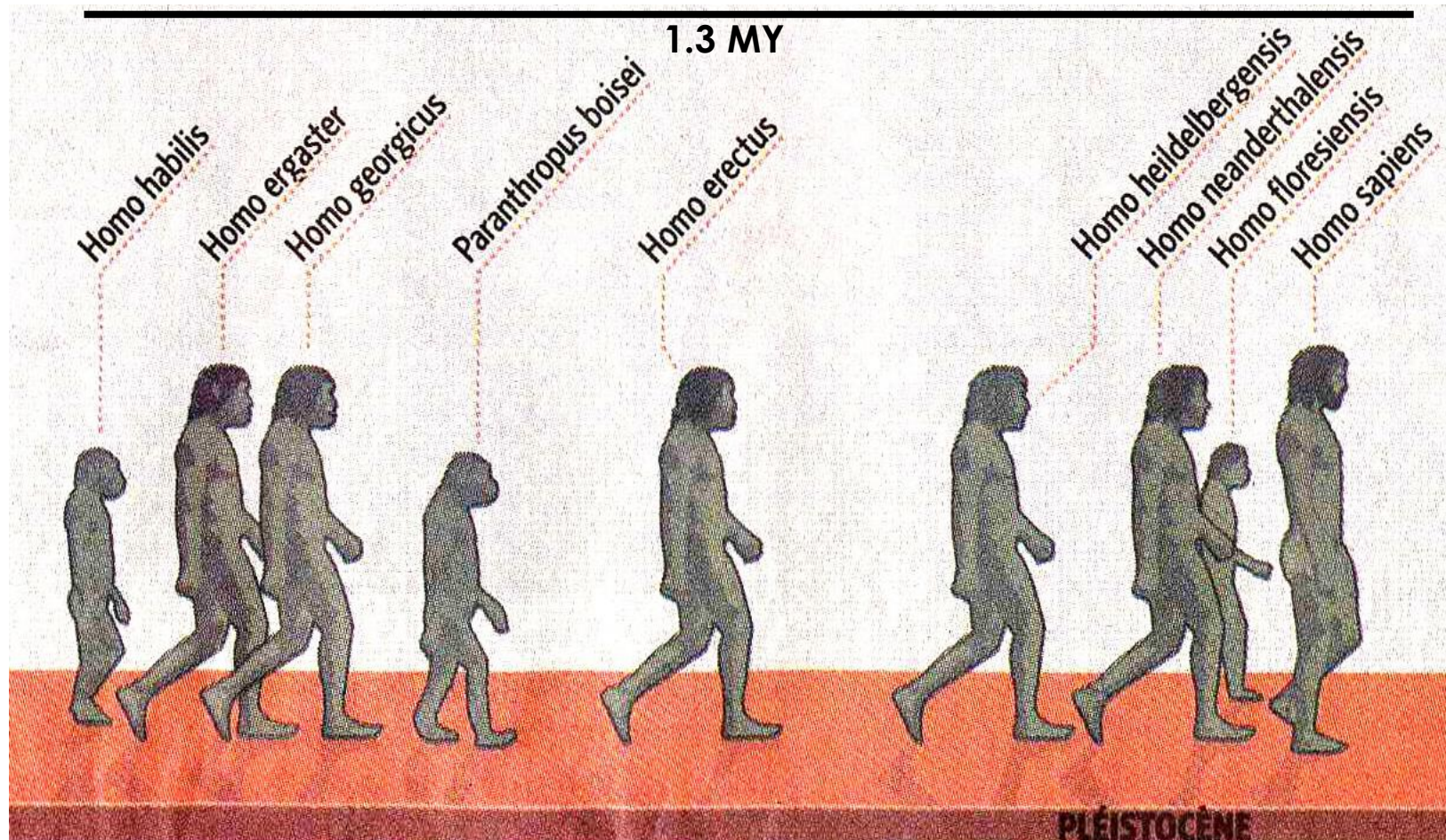
D'HOMO SYMBIOTICUS À HOMO DYSBIOTICUS

INCIDENCE OF CHRONIC DISEASES STEADILY INCREASED FOR THE PAST 60 YEARS

No prevention ;
No cure !
for diseases of
man-microbes symbiosis



UN BOULEVERSEMENT DANS LA SYMBIOSE DEPUIS QUELQUES GÉNÉRATIONS



100'000 to 130'000 generations with fiber-rich diet
(>60% of energy from fruits, veg, roots, nuts,..)

2 to 3 gen. with <10% fiber
diet

courtesy of Walter Wahly & Joël Doré

FIBRE ALIMENTAIRE ET SANTÉ



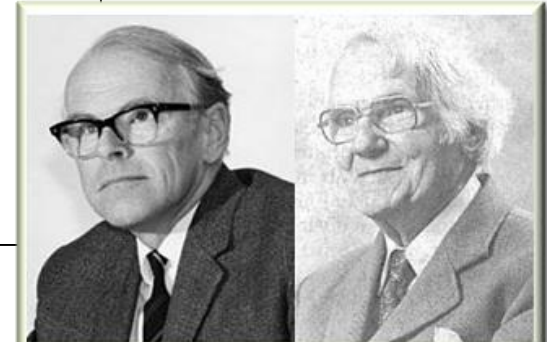
Surgeon Captain
Thomas Latimer Cleave

Molec. Aspects Med. Vol. 9, pp. 7-15, 1987
Printed in Great Britain. All rights reserved.

0098-2997/87 \$0.00 + .50
Copyright © 1986 Pergamon Journals Ltd.

THE DEVELOPMENT OF THE CONCEPT OF DIETARY FIBRE

H. C. Trowell*‡ and D. P. Burkitt†‡



Denis Burkitt

Hugh Trowell

Health benefits and practical aspects of high-fiber diets^{1,2}

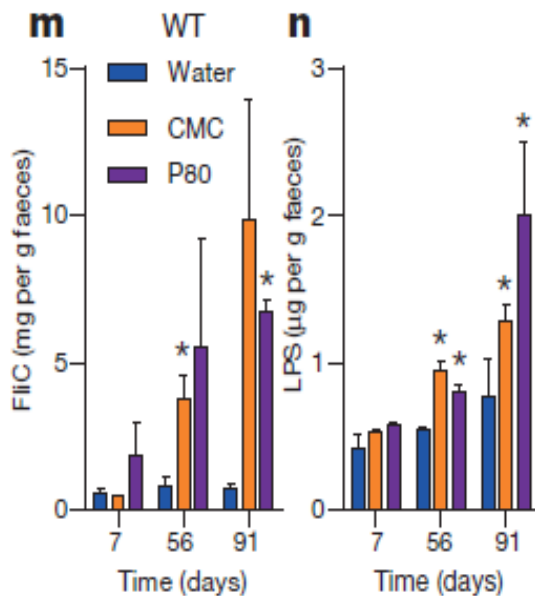
James W Anderson, Belinda M Smith, and Nancy J Gustafson

Over the past 20 y dietary fiber has emerged as a leading dietary factor in the prevention and treatment of chronic diseases. High fiber intakes are associated with lower serum cholesterol concentrations, lower risk of coronary heart disease, reduced blood pressure, enhanced weight control, better glycemic control, reduced risk of certain forms of cancer, and improved gastrointestinal function. 1994.

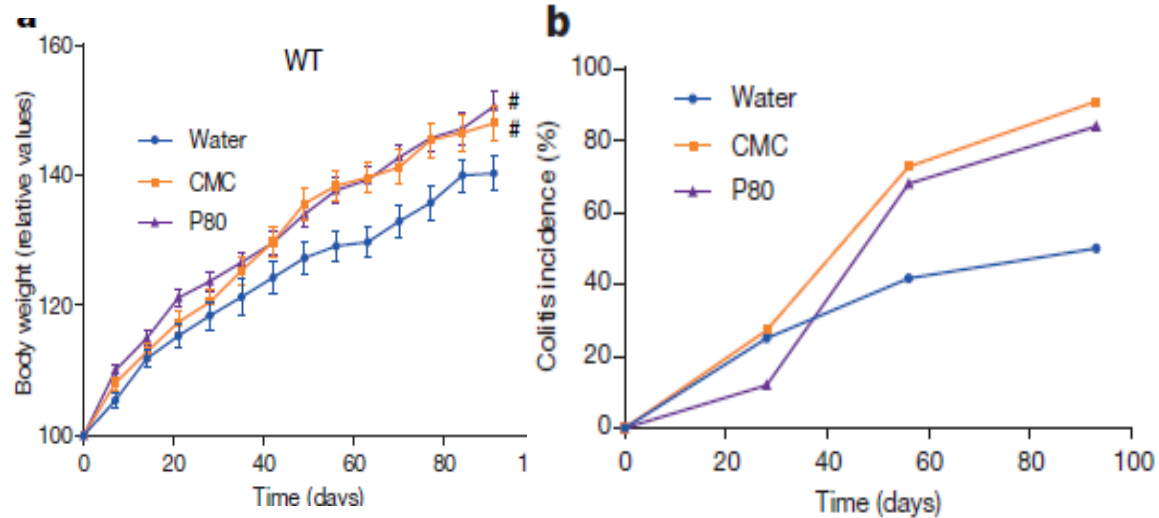
'NATURAL' HISTORY OF THE GENUS HOMO : WE CHANGED ENVIRONMENTAL EXPOSURE TO XENOBIOTICS

with potentially major impact on man-microbes symbiosis

Direct impact of dietary emulsifiers on intestinal microbiota and thereby on permeability, immunity and symbiosis ;
Loss of protection against metabolic and inflammatory risk



Chassaing..Gewirtz **Nature** 2015



Maurice, Haiser, Turnbaugh **Cell** 2013

Other xenobiotics would include pollutants, drugs, antibiotics ...

'NATURAL' HISTORY OF THE GENUS HOMO : WE CHANGED PERINATAL MANAGEMENT AND ENVIRONMENT

with potentially major impact on man-microbes symbiosis



Altering mother-to-child vertical transfer of the microbiota, *Through several generations* via:

- Duration of gestation (preterm births)
- Mode of delivery
(cesarean section >30% in Europe ; >80% in different places in the world)
- Hygiene of neonatal environment
- Exposure to antibiotics in mothers, neonates and infants
- Early life food and feeding mode (formula milk ; weaning diet)
- Maternal microbiomes

Extended hygiene hypothesis (Bach NEJM 2002)

“disappearing microbiota hypothesis” (Blaser EMBO-Report 2006)

& ... Missing Microbes (Blaser 2014)

PERTURBATION OF INTESTINAL MICROBIOTA AS A POSSIBLE CHRONIC DISEASE FACTOR

Dysbiosis :

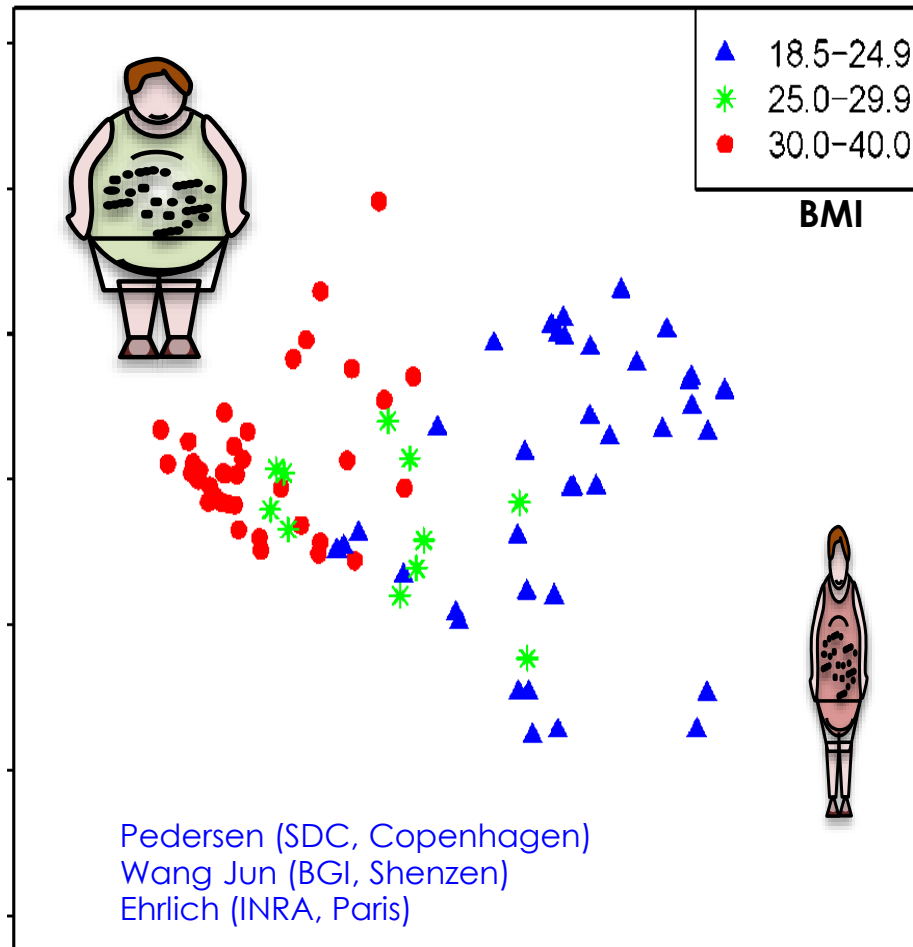
- ✓ Loss of keystone species
- ✓ Loss of richness
- ✓ Increased pathobionts
- ✓ Metabolic shift
- ✓ Broken microbiota-host cross-talk



Pathologies	References
Crohn's disease	Qin, Nature 2010 <i>Gevers, Cell Host Microbe 2014</i>
Ucerative colitis	Qin, Nature 2010 <i>Lepage , Gastroenterology, 2011</i>
Celiac disease	<i>D'Argenio, Am J Gastroenterol 2016</i>
Irritable bowel syndrome	<i>Saulnier, Gastroenterology 2011</i> <i>Rajilic-Stojanovic, Gastroenterology 2011</i>
Colorectal cancer	Zeller, Mol Syst Biol 2014 <i>Sobhani PLoS one 2011</i>
Obesity	Le Chatelier, Nature 2013 <i>Ley, Nature 2006</i>
Type 1 diabetes	Kostic, Cell Host Microbes 2015 <i>Murri, BMC medicine 2012</i>
Type 2 diabetes	Forslund, Nature 2015
Seniors frailty	<i>Claesson Nature 2012</i>
GVHD	<i>Taur, Blood, 2014</i>
Allergy	<i>Abrahamsson, J Allergy Clin Immunol 2012</i>
Liver pathologies	Qin, Nature 2014
Cardiovascular diseases	Karlsson Nat Commun 2012 Projet MetaCardis
Autism, Depression	<i>Finegold, Anaerobe 2010</i> <i>Naseribafrouei Neurogastroenterol Motil 2014</i>

Human clinical studies :
Shotgun sequencing
 16S sequencing

METAGENOMIC SIGNATURES OF DYSBIOSIS IN OBESITY



Bacterial genes and genomes specific of the microbiome of patients

INTESTINAL MICROBIOTA AND OBESITY IN HUMAN

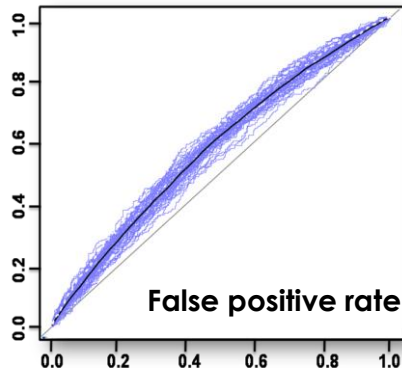
Metagenomic species show a good discrimination power between obese and lean, in contrast to human genome



12 MGS
AUC = 0.84

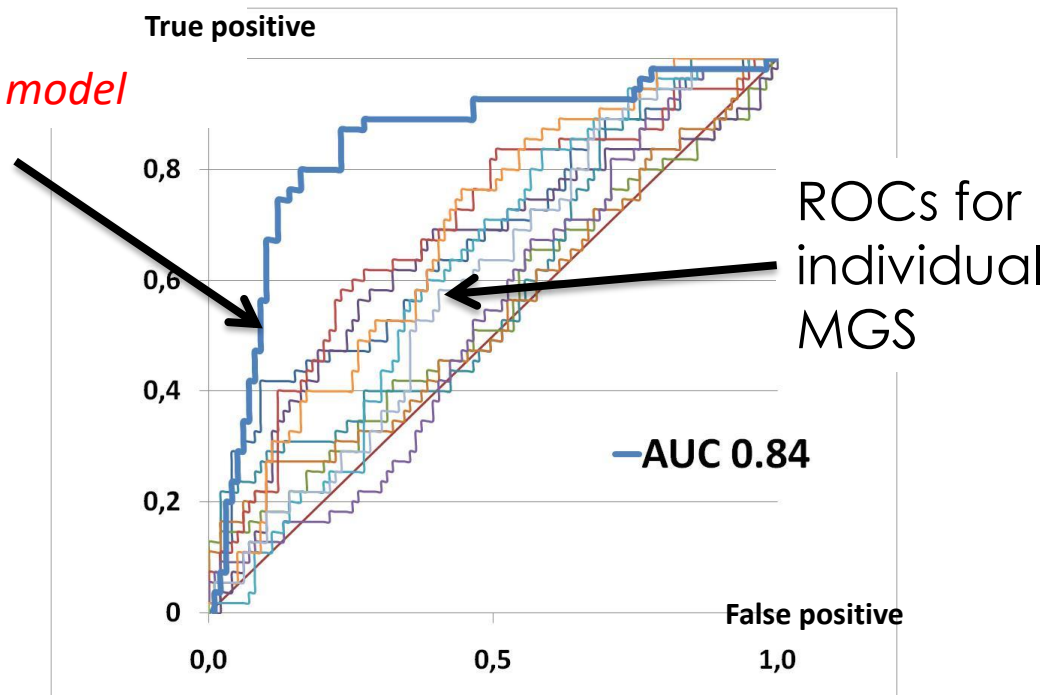
Linear additive model

32 obesity risk loci
AUC = 0.58



n = 8,120
European

n = 154 Danes



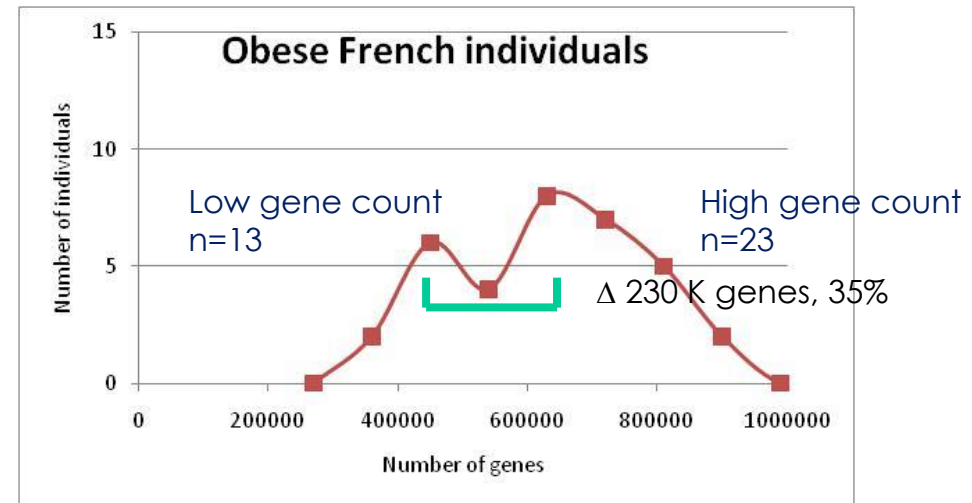
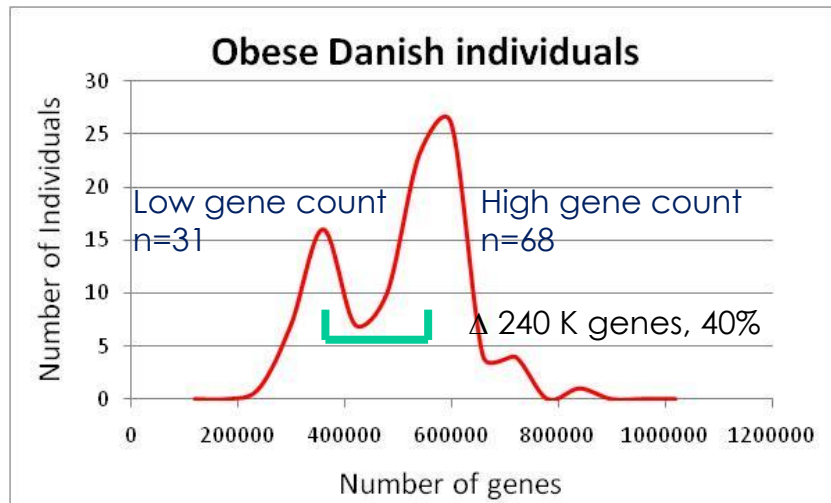
Speliotes et al. Nature Genetics 2010

Le chatelier et al, Nature, 2013

ARTICLE

doi:10.1038/nature12506

Richness of human gut microbiome correlates with metabolic markers

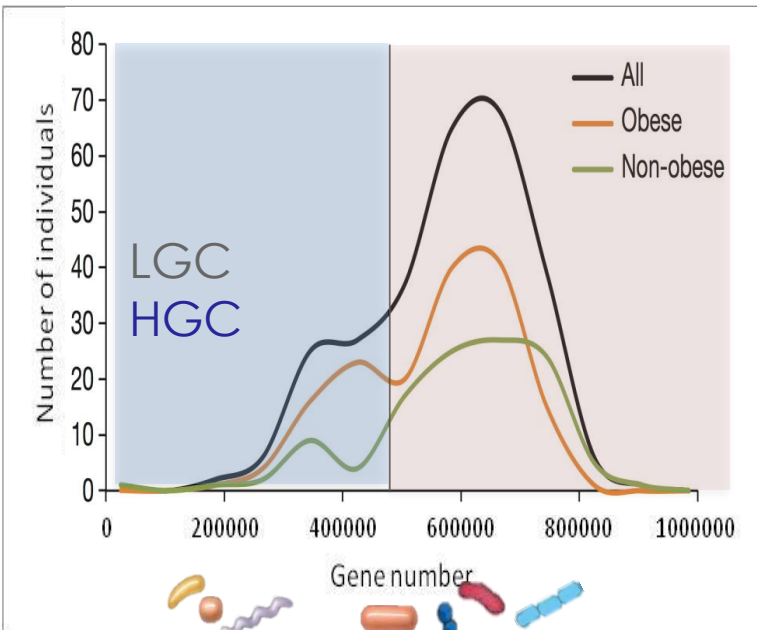


Le chatelier et al, Nature, 2013;
Cottillard et al, Nature, 2013



MICROBIOTA GENE COUNT / DIVERSITY IS A HEALTH-ASSOCIATED STRATIFIER

Low to High
gene count (French or Danes)



R. gnavus

F. prausnitzii

M. smithii

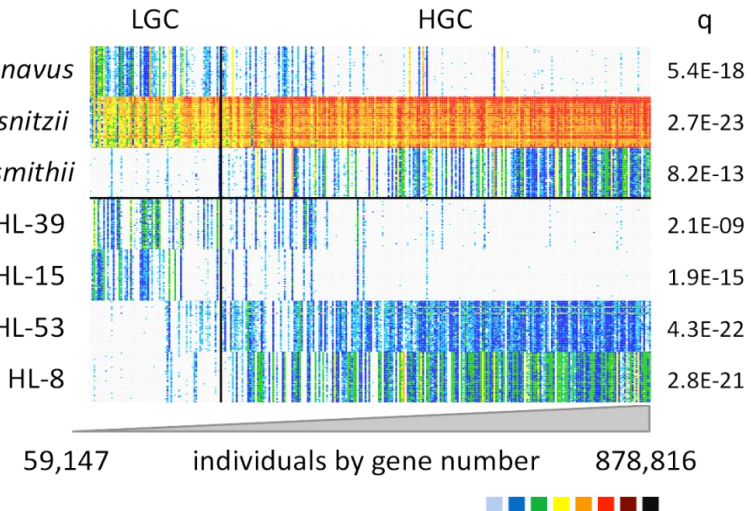
HL-39

HL-15

HL-53

HL-8

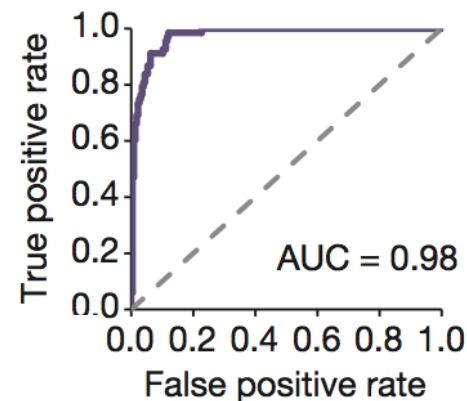
Signature species (n= 58)



Known
species
n=10

Unknown
species
n=48

ROC analysis



4 species

In overweight & obesity,
Low Gene Count (low bacterial richness)
individuals have less healthy metabolic &
inflammatory traits:

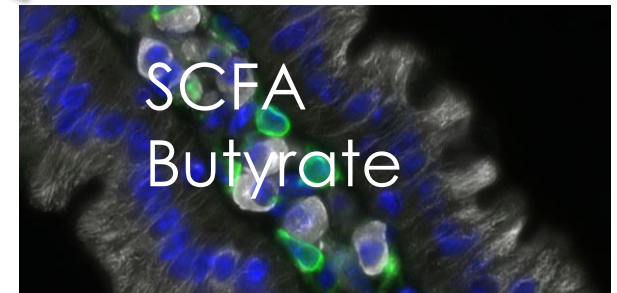
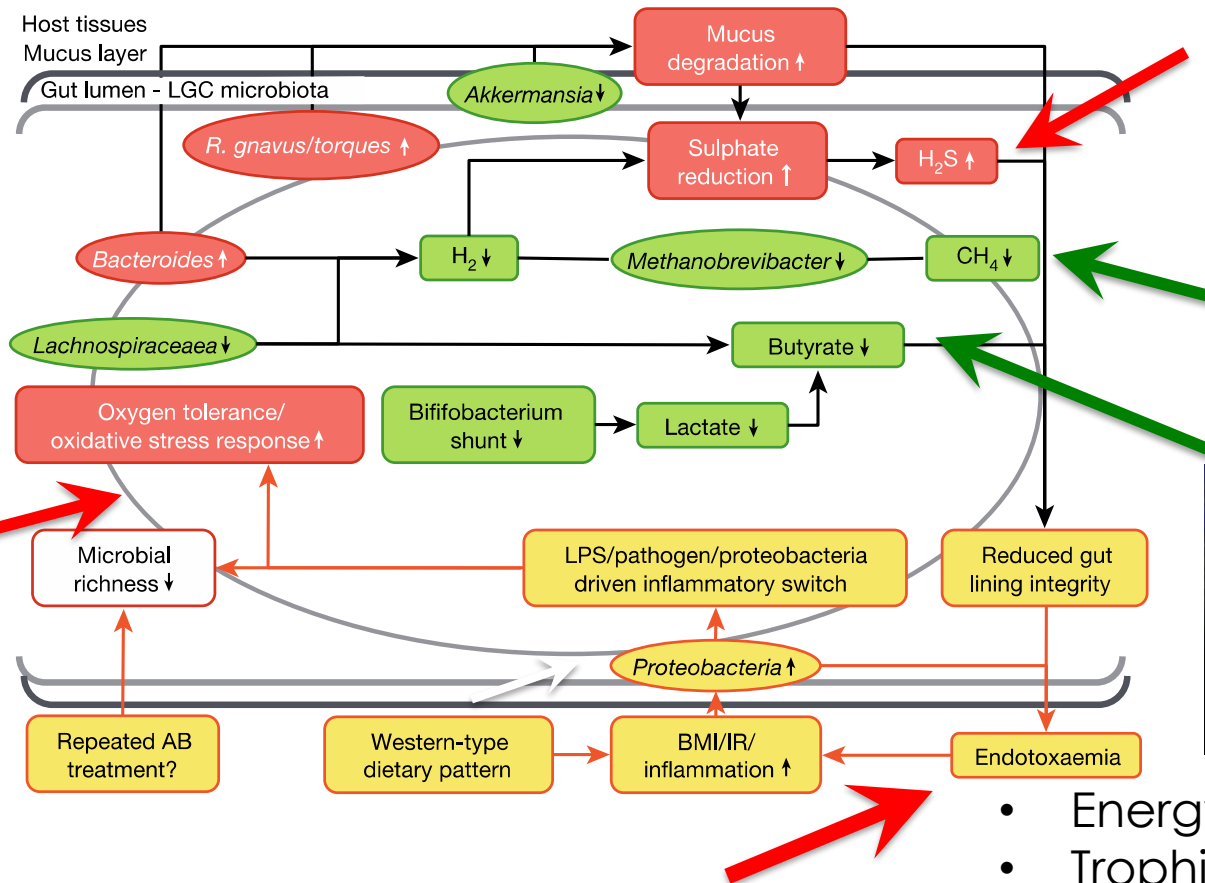
Increased adiposity, insulin resistance, dyslipidaemia,
inflammation, that predispose for type 2 diabetes,
cardio-vascular disease, cancer

Le Chatelier et al, Nature 2013



INRA
SCIENCE & IMPACT

FUNCTIONAL SHIFTS IN THE LGC MICROBIOME



- Energy source for IEC
- Trophic & barrier functions
- HDAC inhibitor (gene regulation)
- GPR agonist – PYY & GLP1
- Immunomodulatory effects

Le chatelier et al, Nature, 2013

*Blottière et al, PNS 2003;
Segain et al, Gut, 2000*

DIAGNOSTIC OF LIVER CIRRHOSIS BY GUT METAGENOMIC SPECIES

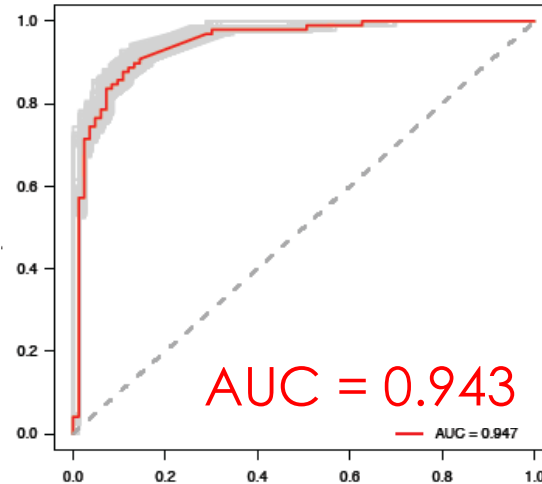
123 patients

Liver cirrhosis diagnosis

- by biopsy in 46
- by clinical symptoms or imaging in 77

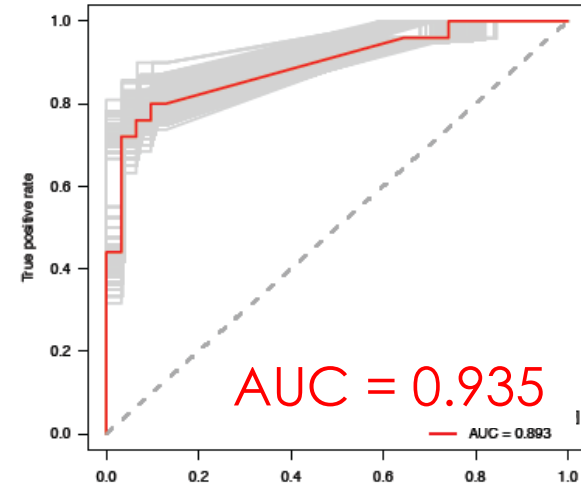
114 controls

Healthy volunteers who visited the hospital for annual physical examination



Discovery

- 98 patients
- 83 controls



Validation

- 25 patients
- 31 controls

7 MGS accurately diagnose liver cirrhosis

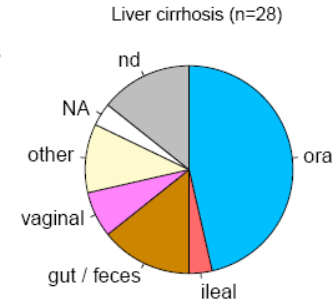
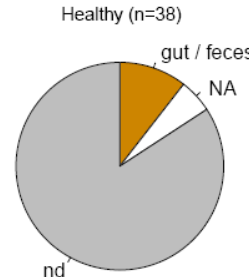
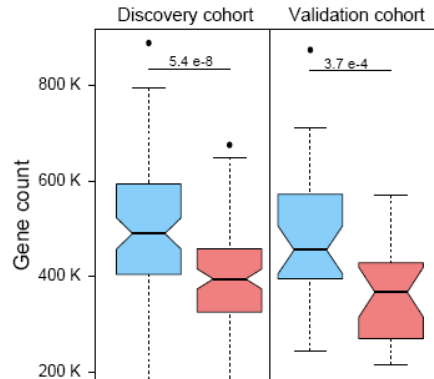
Qin N. et al. Nature 2014

Zhejiang Univ, Hangzhou, China

& MetaGenoPolis, Jouy en Josas, France

MASSIVE MICROBIOME CHANGES IN CIRRHOSIS

Low gene richness
($p < 10^{-10}$)



Known invaders
24/28 with
sequenced
genome.
Targets for
intervention?

HEPATOLOGY

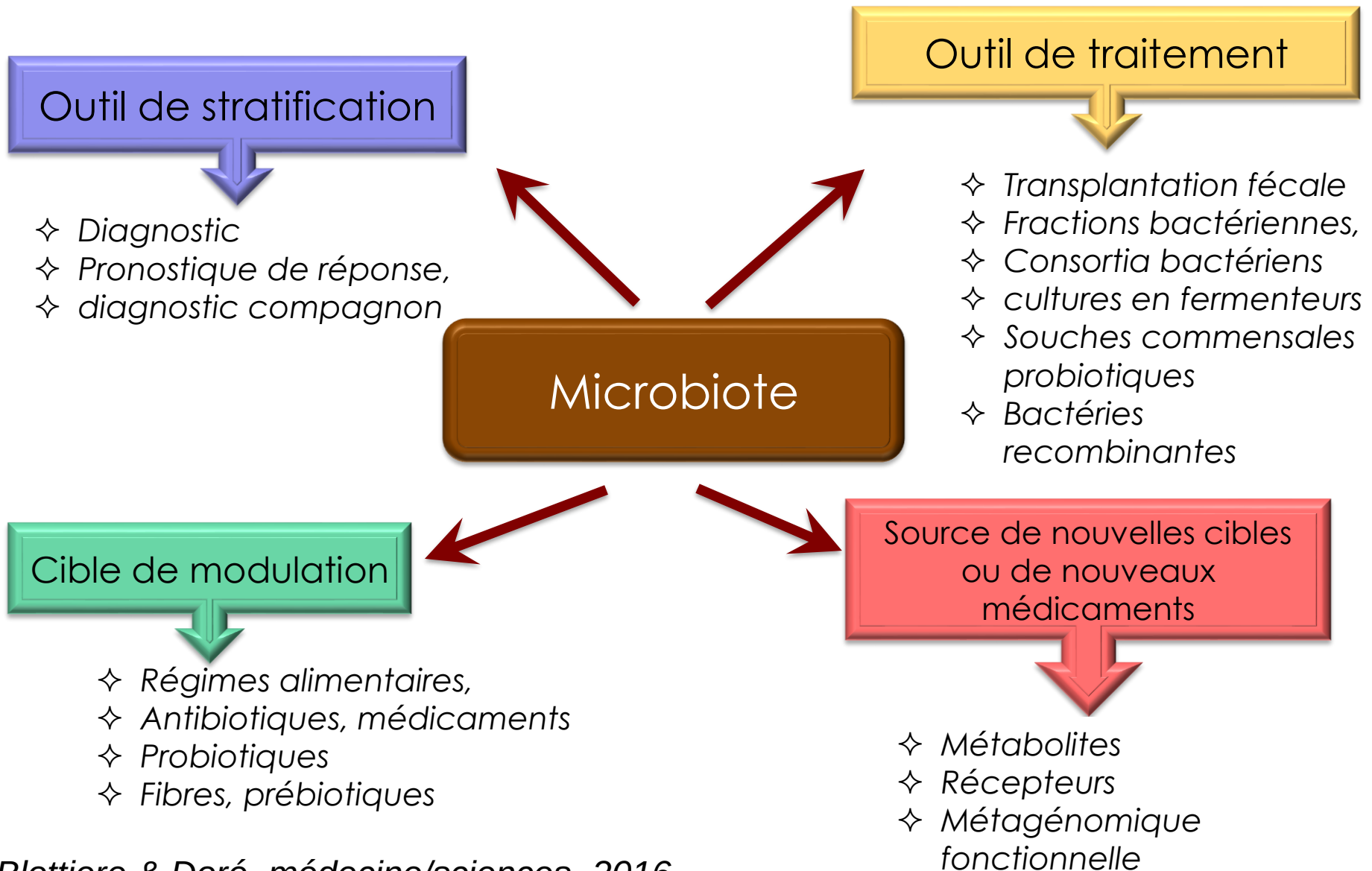
Fecal microbiota transplantation in the management of hepatic encephalopathy,

Kao et al 2016, DOI: 10.1002/hep.28121

A case study: "the dramatic clinical improvements following serial FMT are very encouraging"



APPLICATIONS POTENTIELLES EN SANTÉ HUMAINE



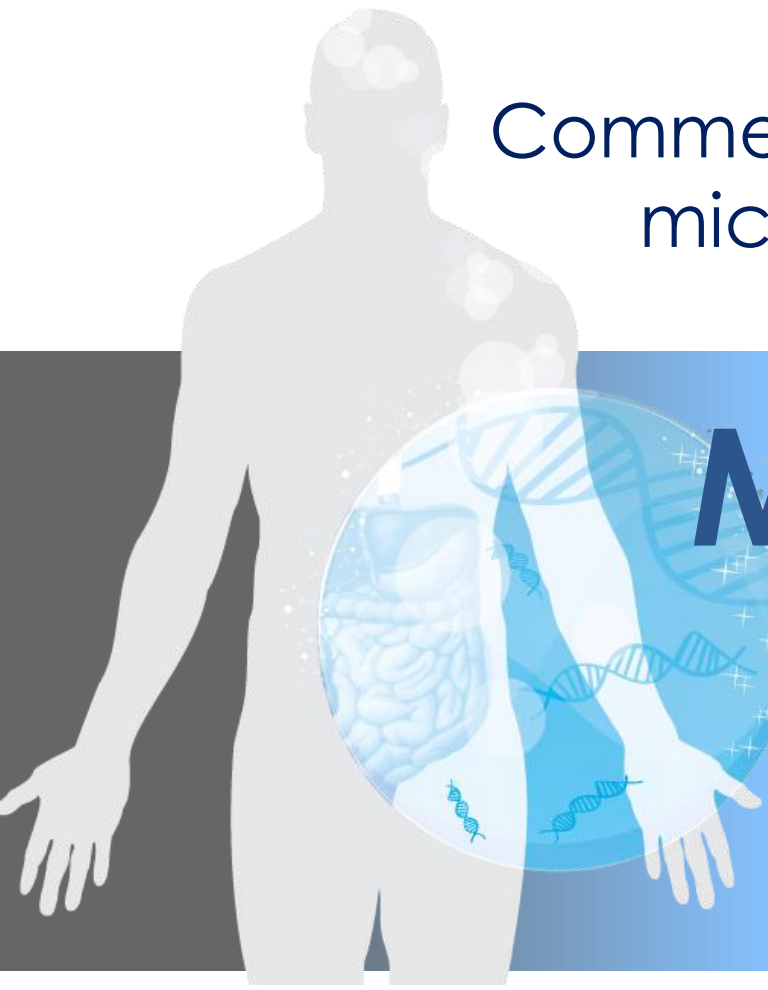
Quelques messages pour conclure

- ✓ Le microbiote intestinal humain est très complexe et diversifié : 25 fois plus de gènes microbiens que de gènes du génome humain ;
- ✓ L'équilibre de la symbiose hôte-microbiote est un garant du maintien de la santé et du bien-être ;
- ✓ L'Homme a changé à travers quelques générations à la fois l'alimentation et les modalités entourant la naissance, autant d'éléments qui impactent la mise en place de la symbiose Hôte-microbiote ;
- ✓ Des particularités du microbiote sont aujourd'hui associées aux maladies chroniques dont l'incidence ne cesse de croître, et sont prédictives de risques, d'aggravation, de réponse/non-réponse à des traitements ;
- ✓ La prise en compte du microbiote devra dans l'avenir accompagner le diagnostic, la prise en charge clinique, le suivi au cours du traitement et le développement de stratégies préventives ciblées.

Comment aller plus loin et introduire le
microbiote dans notre santé ?

MetaGenoPolis

Un ensemble de plateformes
dédiées à la métagénomique
quantitative & fonctionnelle



19M€ pour 2012-2019 des Investissements d'Avenir
Budget : > 60 M€



METAGENOPOLIS – 4 INTEGRATED PLATFORMS



*FlnE/Blotière lab,
Micalis Institute*

Joël Doré,

Nicolas Lapaque,
Catherine Juste,
Christel Maillet-Béra
Jean-Marc Lelièvre
Alexandre Jamet
Maarten van de Guchte
et al....

MetagenoPolis

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Florence Haimet
Nicolas Pons
Emmanuelle Le Chatelier
Véronique Lejard
Florence Levenez
et al....



MetaHIT consortium

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& MetaCardis Consortium



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Sven Pettersson et col. (Karolinska Institute)

Maria Rescigno (IEO, Milan)

Oluf Pederson (Novo Nordisk Foundation, Copenhagen)

Francisco Guarner (Val Hebron Hosp., Barcelone)

Mark Morisson (U of Queensland, Brisbane)

