



metagenopolis
mgps.eu

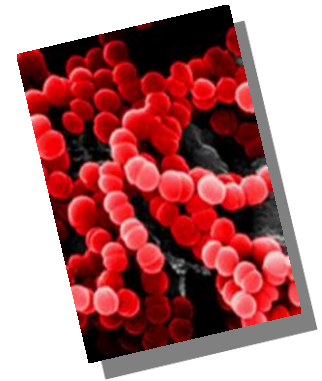
The healthy microbiome – prospects for diagnostics and intervention

3rd Innovation Academy Symposium, June 27, 2014
Apothecaries Hall, London UK

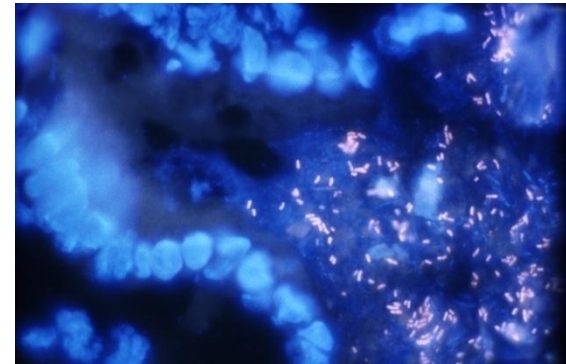
S. Dusko Ehrlich, MetaGenoPolis, INRA, Jouy en Josas; King's
College London, UK

The human intestinal microbiota is a neglected organ...

- ✓ 100 trillion microorganisms ; 10-fold more cells than the human body; up to 2 kg of mass!
- ✓ Interface between food and epithelium
- ✓ In contact with the 1st pool of immune cells and the 2nd pool of neural cells of the body



...with a major role in health & disease !



Les maladies chroniques potentiellement impactées par le microbiote intestinal

Fragilité des seniors

Van Tongeren et al., 2005

Crohn

Seksik et al., 2003; Sokol et al., 2006, 2008, 2009

Rectocolite

Sokol et al., 2008; Martinez et al., 2008

Pauchite

Lim et al., 2009, Kühbacher et al., 2006

Obésité

Ley et al., 2007; Kalliomäki et al., 2008

Type-2 diabète

Cani and Delzenne, 2009

Type-1 diabète

Dessein et al., 2009; Wen et al., 2008

Maladie Coeliaque

Nadal et al., 2007; Collado et al., 2009

Allergie

Kirjavainen et al., 2002; Björkstén, 2009

Autisme

Finegold et al., 2002; Paracho et al., 2005

Cancer colorectal

Mai et al., 2007; Scanlan et al., 2008

Cancer du sein

Velicer et al., 2004

HIV

Gori et al., 2008

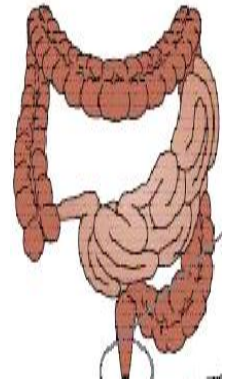
Cirrhose

Gunnarsdottir et al. 2003

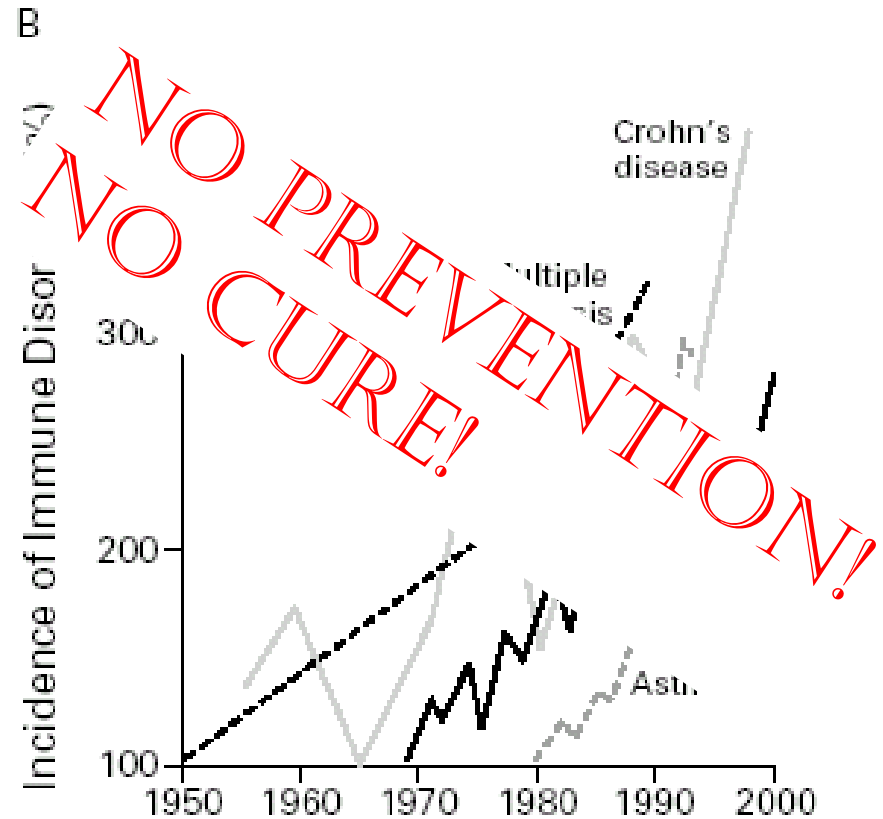
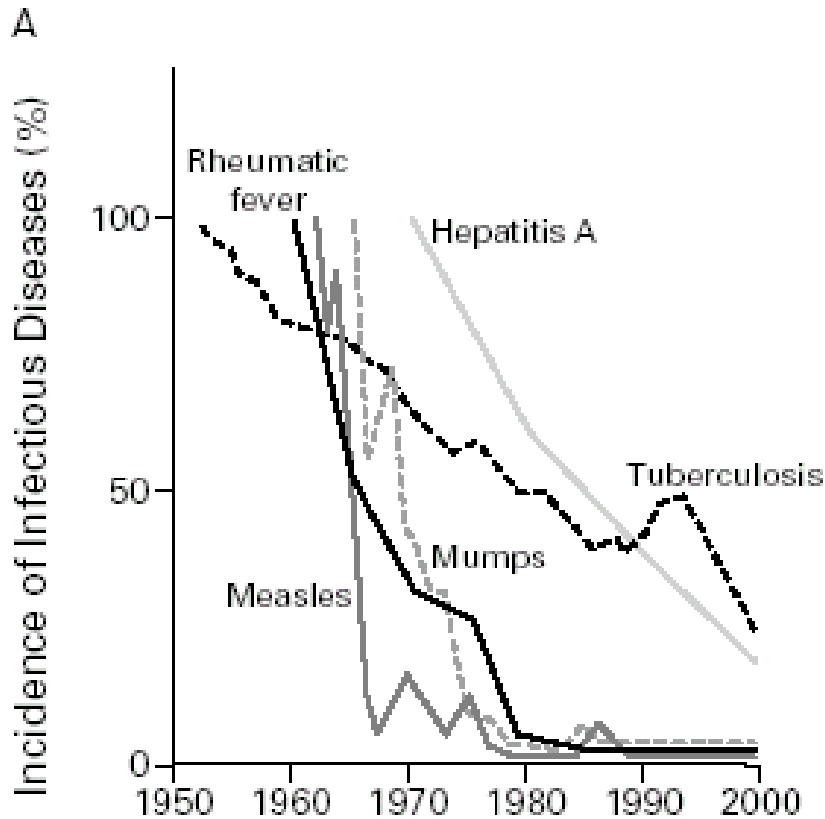
Cardiovasculaire

Wang et al. 2011

Autres....



Chronic diseases increase steadily in industrialized countries



Bach JF, N Eng J Med 2002

Prevention = Risk prediction+ Risk alleviation

- ✓ Can the neglected organ inform on a risk of chronic diseases?
- ✓ Can it be a target for intervention?

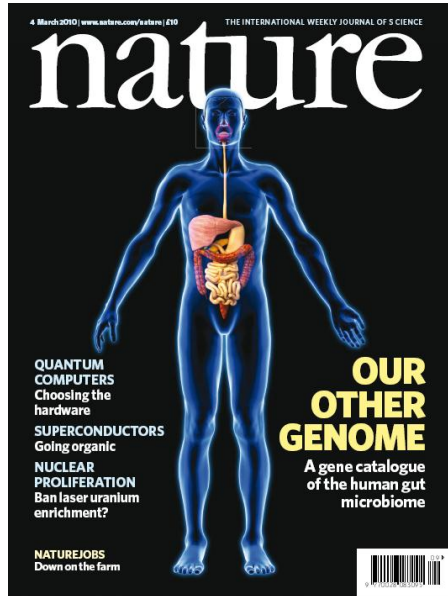
How to assess the state of the neglected organ in each & every individual?

The MetaHIT approach
(the EC large human
microbiome project)

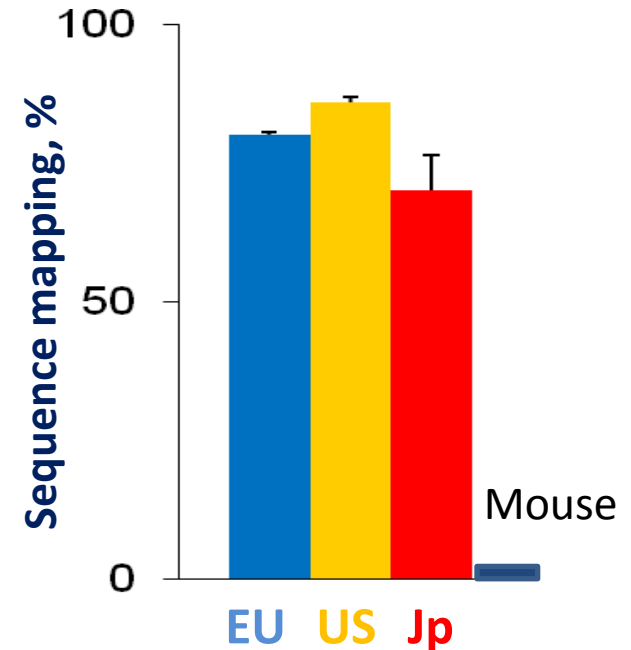
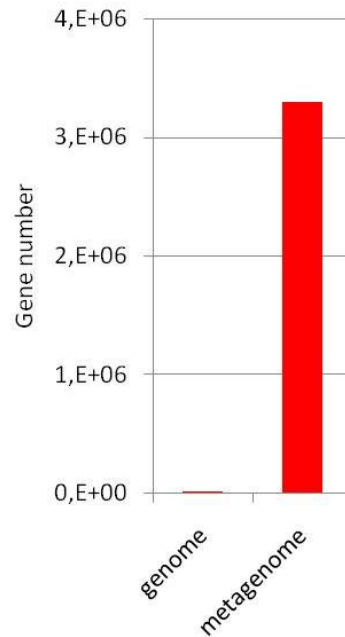


- ✓ Construct a reference gene catalog of the gut microbes – **the other human genome.**
- ✓ Develop a quantitative metagenomic pipeline for gene profiling – **the other genome of an individual.**

The reference gene catalog



March 2010



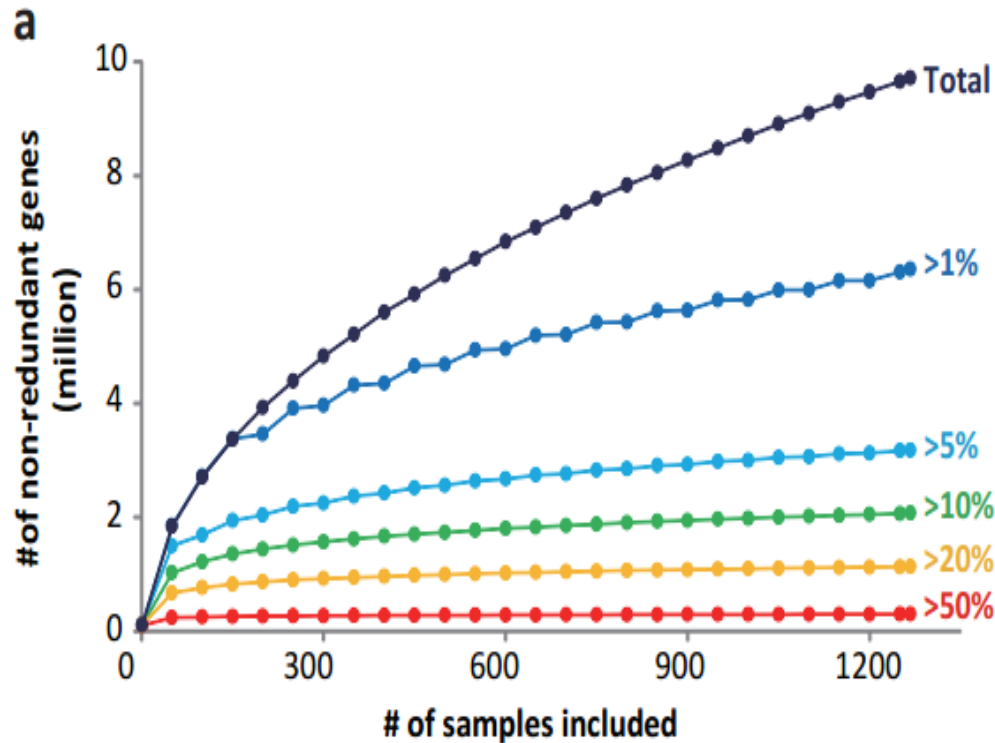
3.3 million bacterial gene catalog : 150-fold human genome

85% of abundant gut genes from a cohort of 124 European individuals

Represents well individuals from US & Japan

Catalog improvements: gene definition, more individuals, different populations...

A 10 M gut genes reference catalog

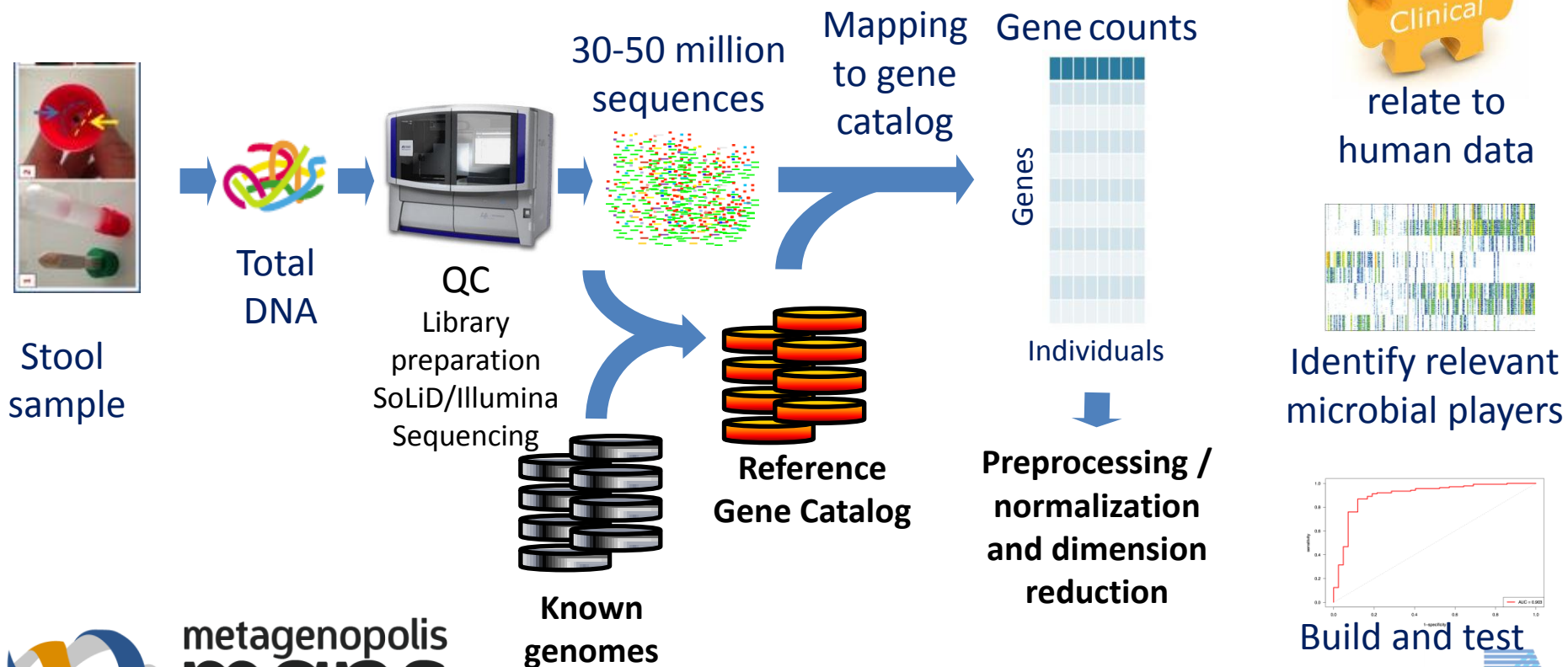
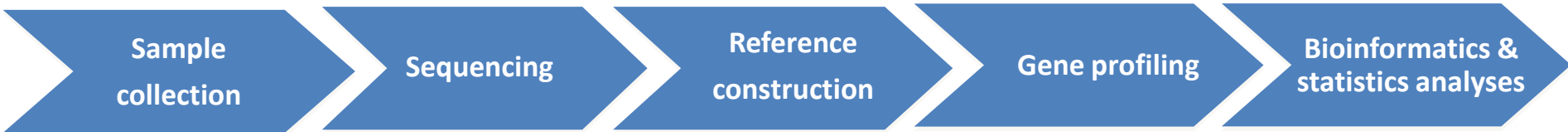


Rare genes are increasing

Common genes are not
They may be most clinically useful for common diseases

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

Quantitative metagenomics

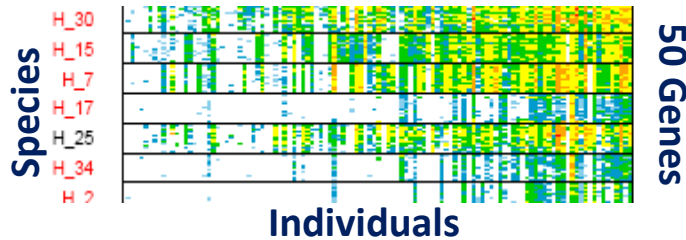


Clustering genes into **MetaGenomic Units (MGU)**

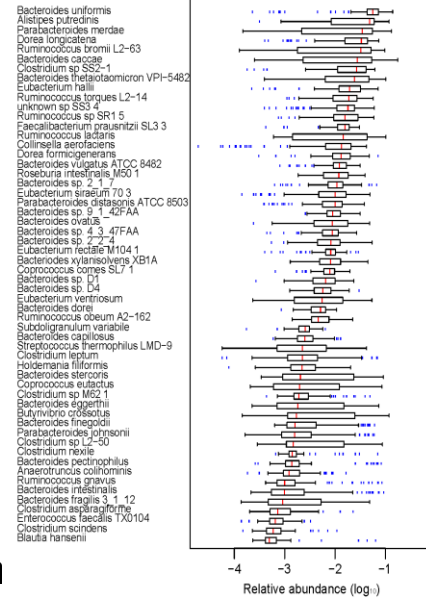
All genes of a species have the same frequency in an individual
 Abundance of a species varies greatly between individuals
Genes that co-vary in abundance belong to the same species



A bar-code

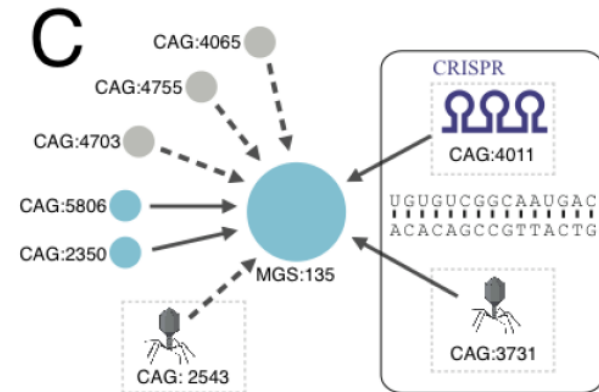
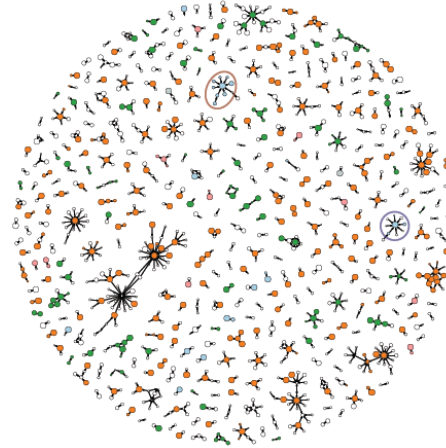
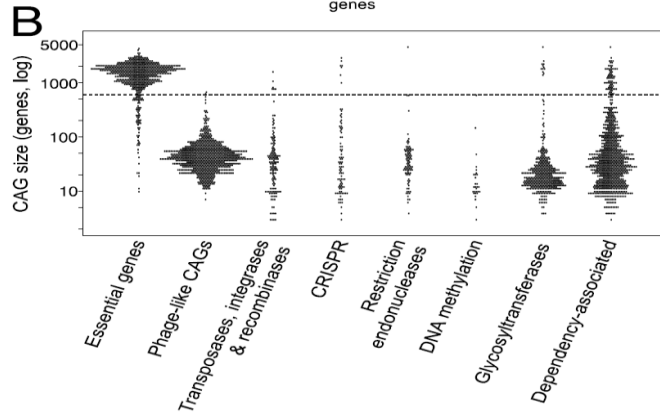
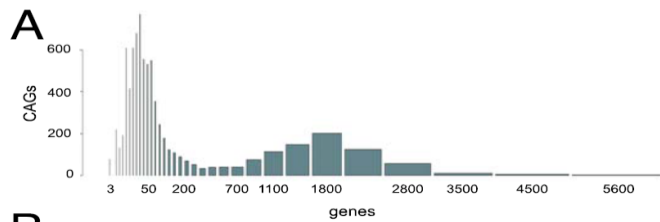


- Each column is an individual
- Each row is a gene, 50 are displayed for each species
- Colors indicate gene abundance



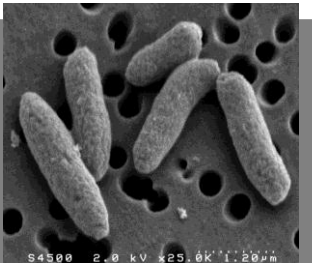
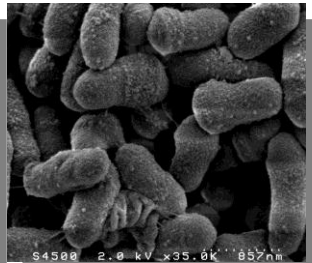
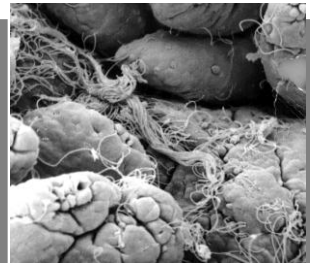
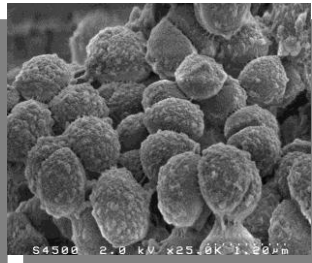
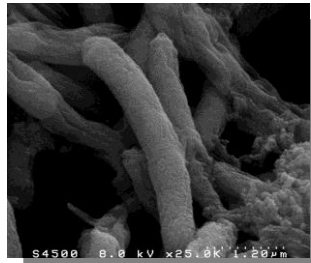
57 Common species

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



Simplifies analyses: 4M gut genes to 8 K MGU or 741 MGS

Microbiome assessment



Faecalibacterium prausnitzii *Ruminococcus* spp

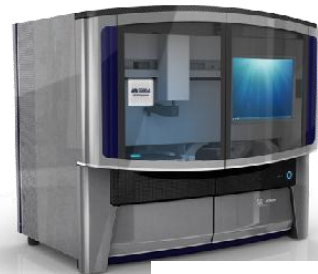
Clostridium difficile
en caecum souris

*Bactéries ancrées dans
une Plaqué de Peyer,
Intestin de souris*

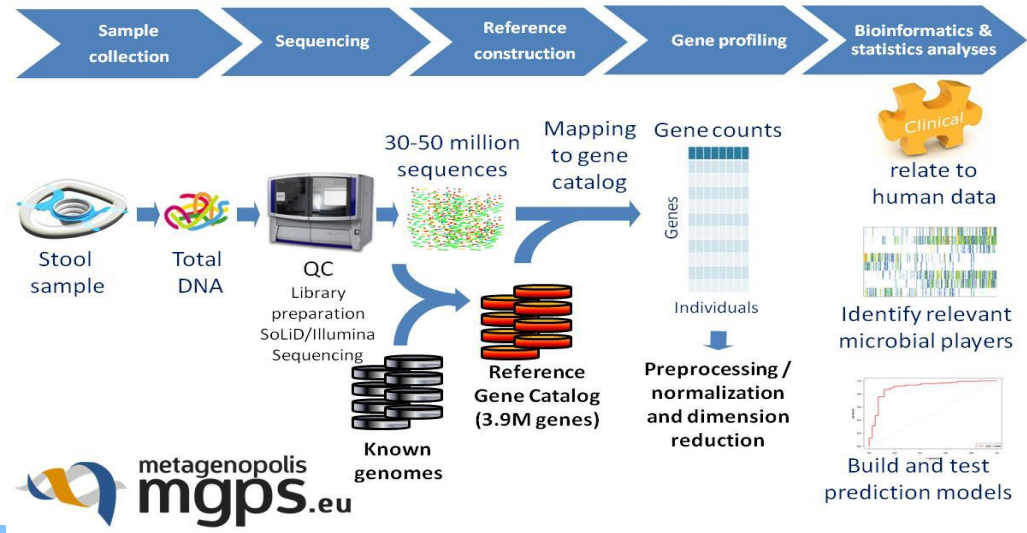
Bacteroides dorei

Escherichia coli

Photos UEPSD



Quantitative metagenomics



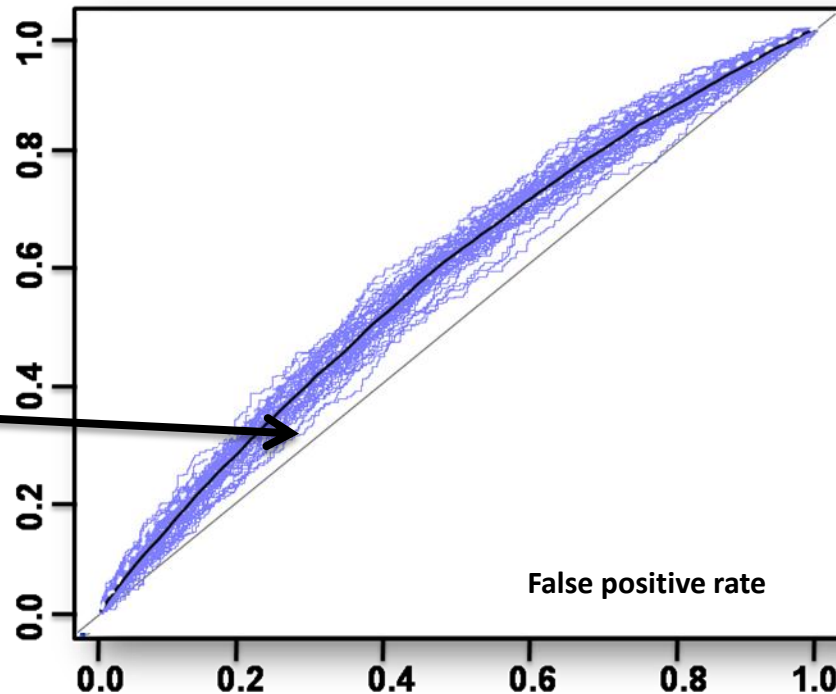
A Powerful Microscope to Scan the neglected organ

Diagnosics by gut bacterial biomarkers

Obesity: Human genome markers show a modest discriminatory value between lean and obese people

A study of a population-based sample of 6,510 middle-aged Danes

True positive rate
32 validated obesity risk loci
AUC = 0.58



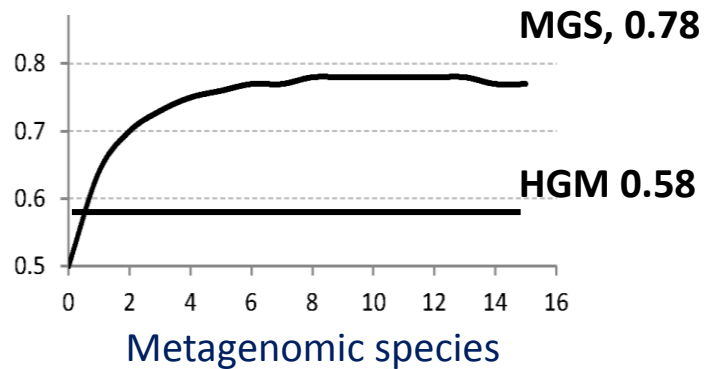
Receiver operator characteristics (ROC) discrimination analysis

Perfect: AUC = 1
Random: AUC = 0.5

AUC = area under curve

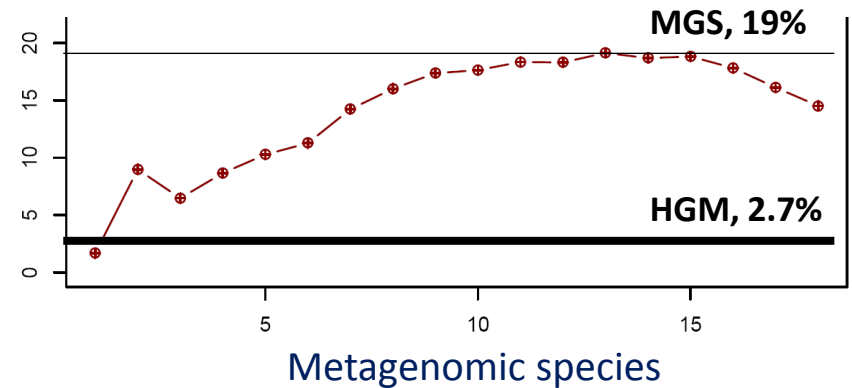
Obesity: Human **metagenome** markers work better

AUC, lean/obese



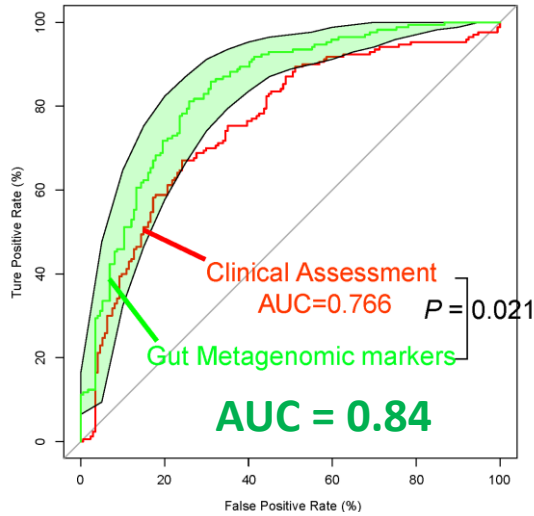
HGM= 32 human genome markers
 Speliotes et al. Nature Genetics 2010

BMI Variance explained, %

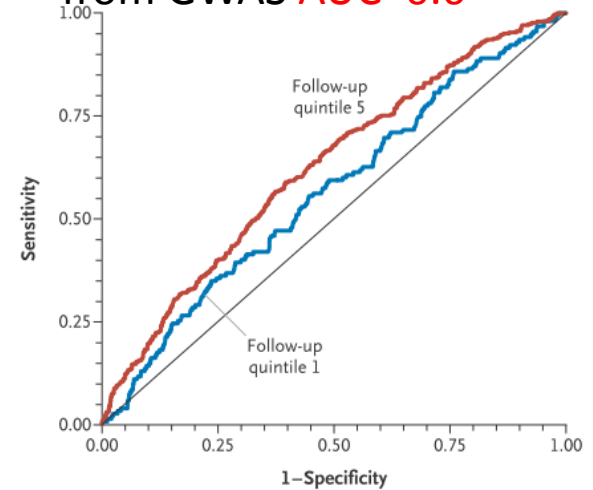


HGM= 97 human genome markers
 Ruth Loos, Genomics in Metabolism

Type 2 diabetes



Host genetic markers
from GWAS AUC=0.6



ARTICLE

doi:10.1038/nature11450

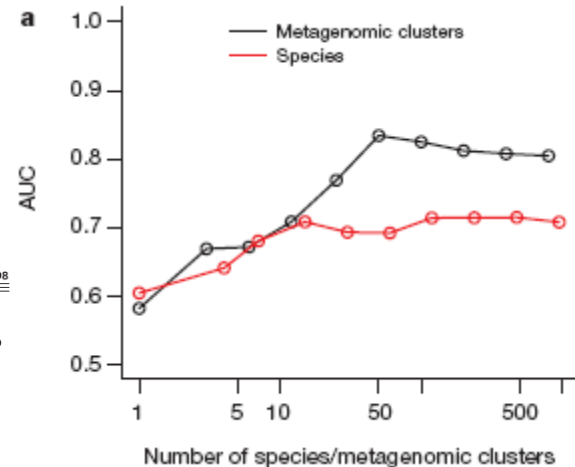
A metagenome-wide association study of gut microbiota in type 2 diabetes

LETTER

doi:10.1038/nature12198

Gut metagenome in European women with normal, impaired and diabetic glucose control

Fredrik H. Karlsson^{1*}, Valentina Tremaroli^{2*}, Intawat Nookaew¹, Göran Bergström², Carl Johan Behre², Björn Fagerberg², Jens Nielsen¹ & Fredrik Bäckhed^{2,3}



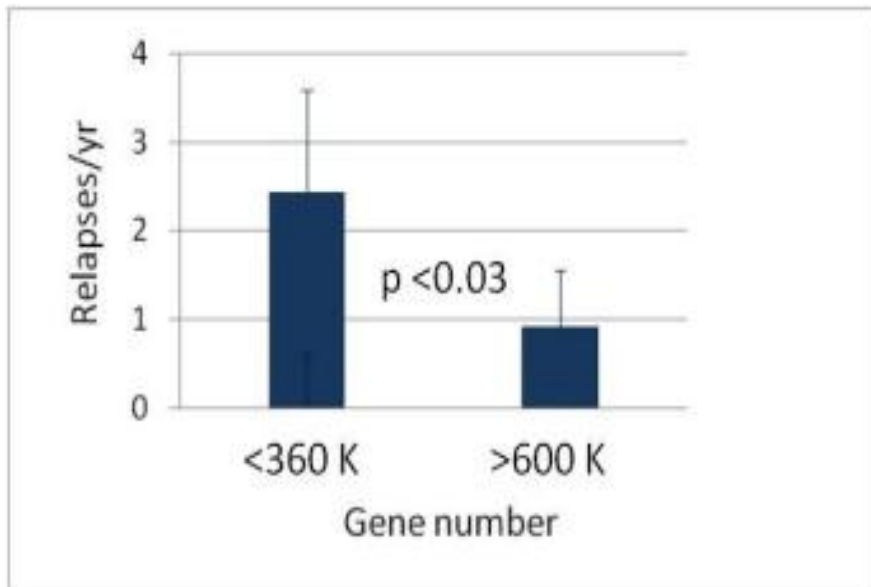
Human **metagenome** biomarkers have
better discriminatory value than
human **genome** biomarkers in several
chronic diseases

Their usefulness in many more such
diseases should be vigorously explored

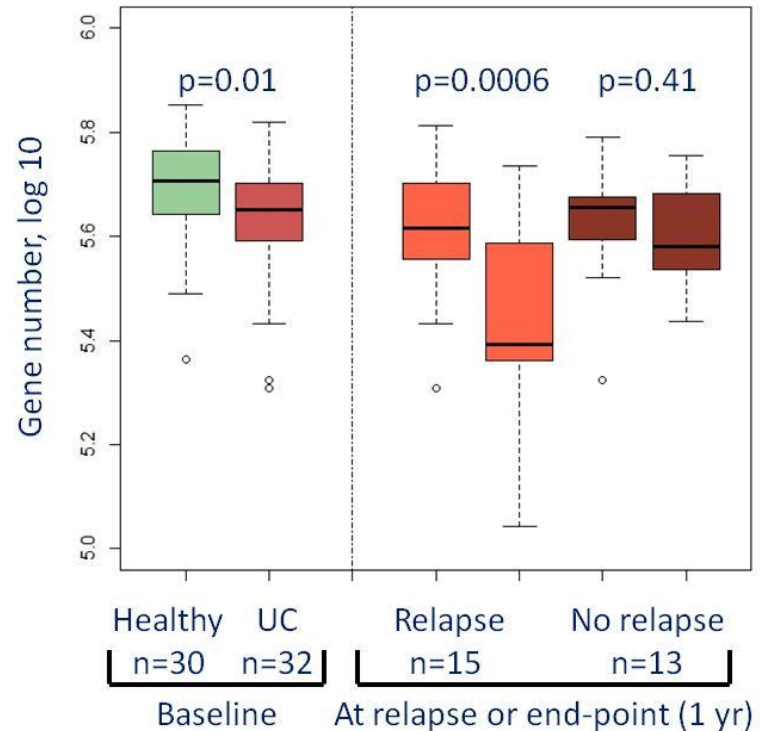
Patient monitoring by gut microbial biomarkers

Ulcerative colitis

Relapse is more frequent in low gut bacterial richness patients



Loss of richness at relapse



Risk prediction by gut microbial biomarkers

High and low Gene richness

ARTICLE

doi:10.1038/nature12506

Richness of human gut microbiome correlates with metabolic markers

Emmanuelle Le Chatelier^{1*}, Trine Nielsen^{2*}, Junjie Qin^{3*}, Edi Prifti^{1*}, Falk Hildebrand^{4,5}, Gwen Falony^{4,5}, Mathieu Almeida¹, Manimozhiyan Arumugam^{2,3,6}, Jean-Michel Batto¹, Sean Kennedy¹, Pierre Leonard¹, Junhua Li^{3,7}, Kristoffer Burgdorf², Niels Grarup², Torben Jørgensen^{8,9,10}, Ivan Brandslund^{11,12}, Henrik Florence Levenez¹, Nicolas Pons¹, Simon Rasmussen¹³, Shinichi Søren Brunak¹³, Karine Clément^{15,16,17}, Joël Doré^{1,18}, Michiel Kleer Thomas Sicheritz-Ponten¹³, Willem M. de Vos^{14,20}, Jean-Daniel Zucker consortium†, Peer Bork⁶, Jun Wang^{3,19,23,24,25}, S. Dusko Ehrlich¹ & Pierre Renault¹⁸

NEWS & VIEWS

RESEARCH

Wealth management in the gut

SUNGSOON FANG & RONALD M. EVANS

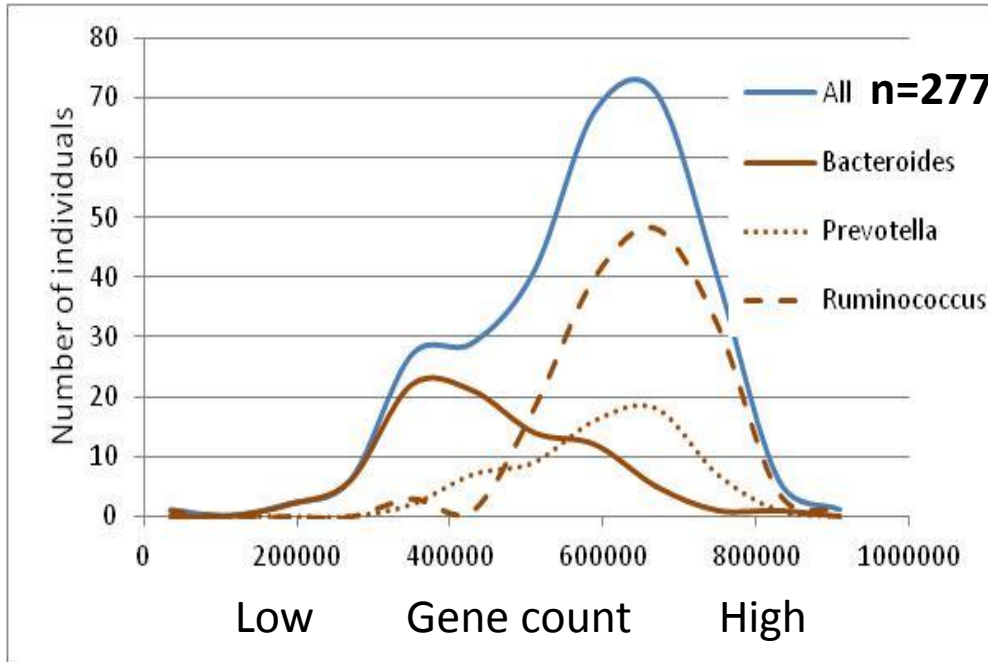
LETTER

doi:10.1038/nature12480

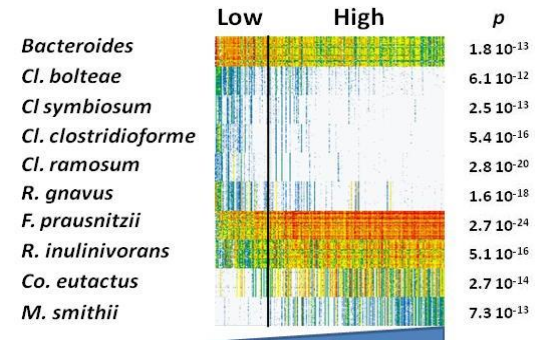
Dietary intervention impact on gut microbial gene richness

Aurélien Cotillard^{1,2*}, Sean P. Kennedy^{3*}, Ling Chun Kong^{1,2,4*}, Edi Prifti^{1,2,3*}, Nicolas Pons^{3*}, Emmanuelle Le Chatelier³, Mathieu Almeida³, Benoit Quinquis³, Florence Levenez^{3,5}, Nathalie Galleron³, Sophie Gougis⁴, Salwa Rizkalla^{1,2,4}, Jean-Michel Batto^{3,5}, Pierre Renault⁵, ANR MicroObes consortium†, Joël Doré^{3,5}, Jean-Daniel Zucker^{1,2,6}, Karine Clément^{1,2,4} & Stanislav Dusko Ehrlich³

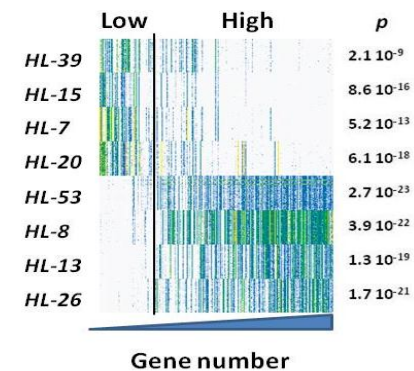
People differ by gut bacterial gene counts and bacterial communities



Low gene count (**low bacterial richness**) individuals (**23%**) have less healthy metabolic & inflammatory traits



**Known species
n=10**



**Unknown MGS
n=58**

Each column is an individual
 Each row is a gene, 50 are displayed
 Colors reflect gene abundance

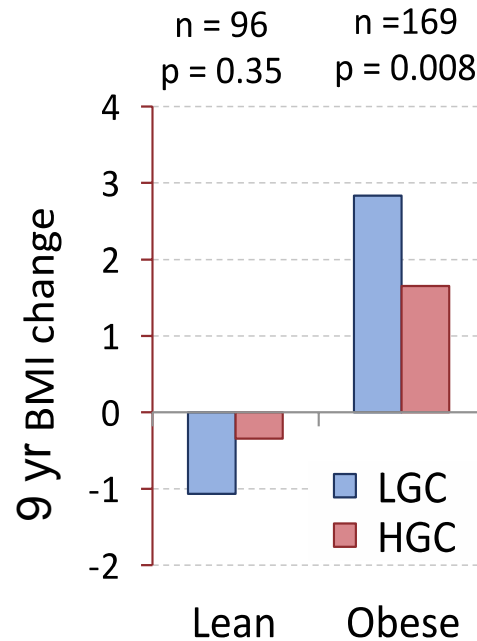


Low bacterial richness – a risk phenotype?

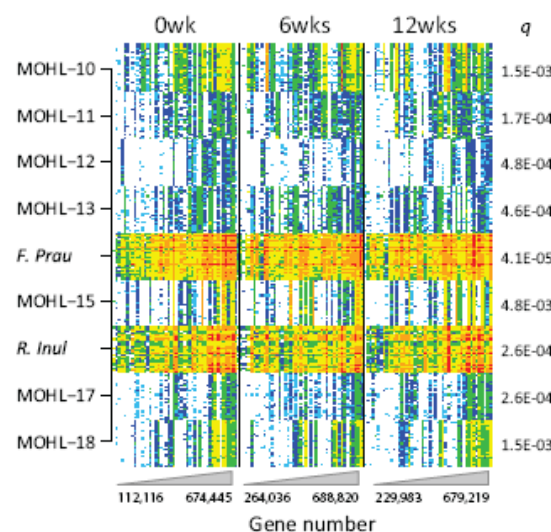
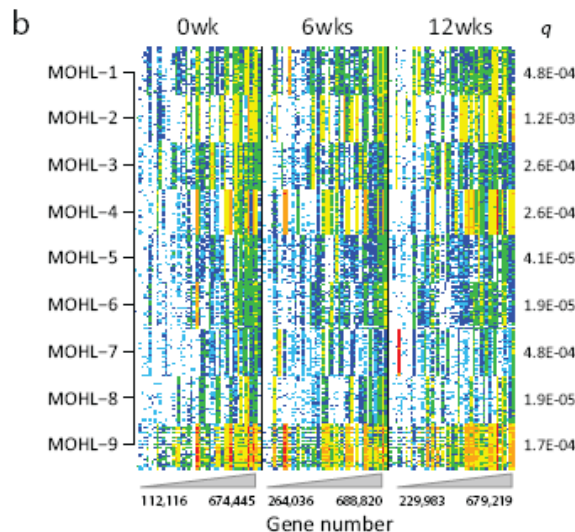
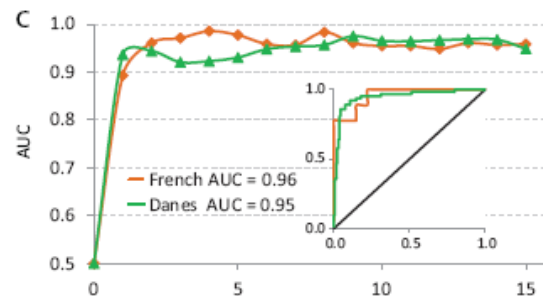
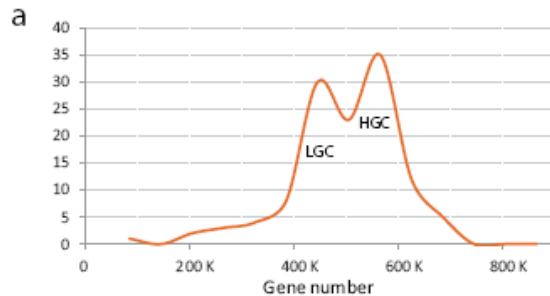
	LGC	HGC	<i>p</i>	<i>q</i>
N (men/women)	68 (23/45)	224 (113/111)		
Age Yrs	56 ± 7.5	57 ± 7.3	0.86	0.89
BMI (kg/m ²)	32 (29 - 34)	30 (23 - 33)	0.035	0.059
Weight (kg)	95 (75 - 100)	86 (71 - 100)	0.019	0.037
Fat %	37 (29 - 42)	31 (25 - 39)	0.0069	0.022
S-Insulin (pmol/l)	50 (35 - 91)	44 (26 - 66)	0.0095	0.023
HOMA-IR	1.9 (1.2 - 3.3)	1.6 (0.9 - 2.6)	0.012	0.027
p-Triglycerides mmol/l	1.32(0.97 – 1.76)	1.15 (0.82 – 1.57)	0.0014	0.013
P-Free fatty acids (mmol/l)	0.55 (0.39 - 0.70)	0.48 (0.35 - 0.60)	0.014	0.029
S-Leptin (μ/l)	17.0 (6.7 – 32.6)	8.3 (3.4 – 26.4)	0.0036	0.019
S-Adiponectin (mg/l)	7.5 (5.5 – 12.9)	9.6 (6.7 – 13.7)	0.006	0.022
B-leucocytes (10 ⁹ /l)	6.4 (5.2 - 7.8)	5.6 (4.8 - 6.9)	0.0021	0.014
B-Lymphocytes (10 ⁹ /l)	2.1 (1.6 - 2.3)	1.8 (1.5 - 2.1)	0.00082	0.012
P-CRP (mg/l)	2.3 (1.1 - 5.7)	1.4 (0.6 - 2.7)	0.00088	0.012
S-FIAF (μg/l)	88 (72 - 120)	78 (60 - 100)	0.0047	0.021

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

Low richness obese Danes gain more weight

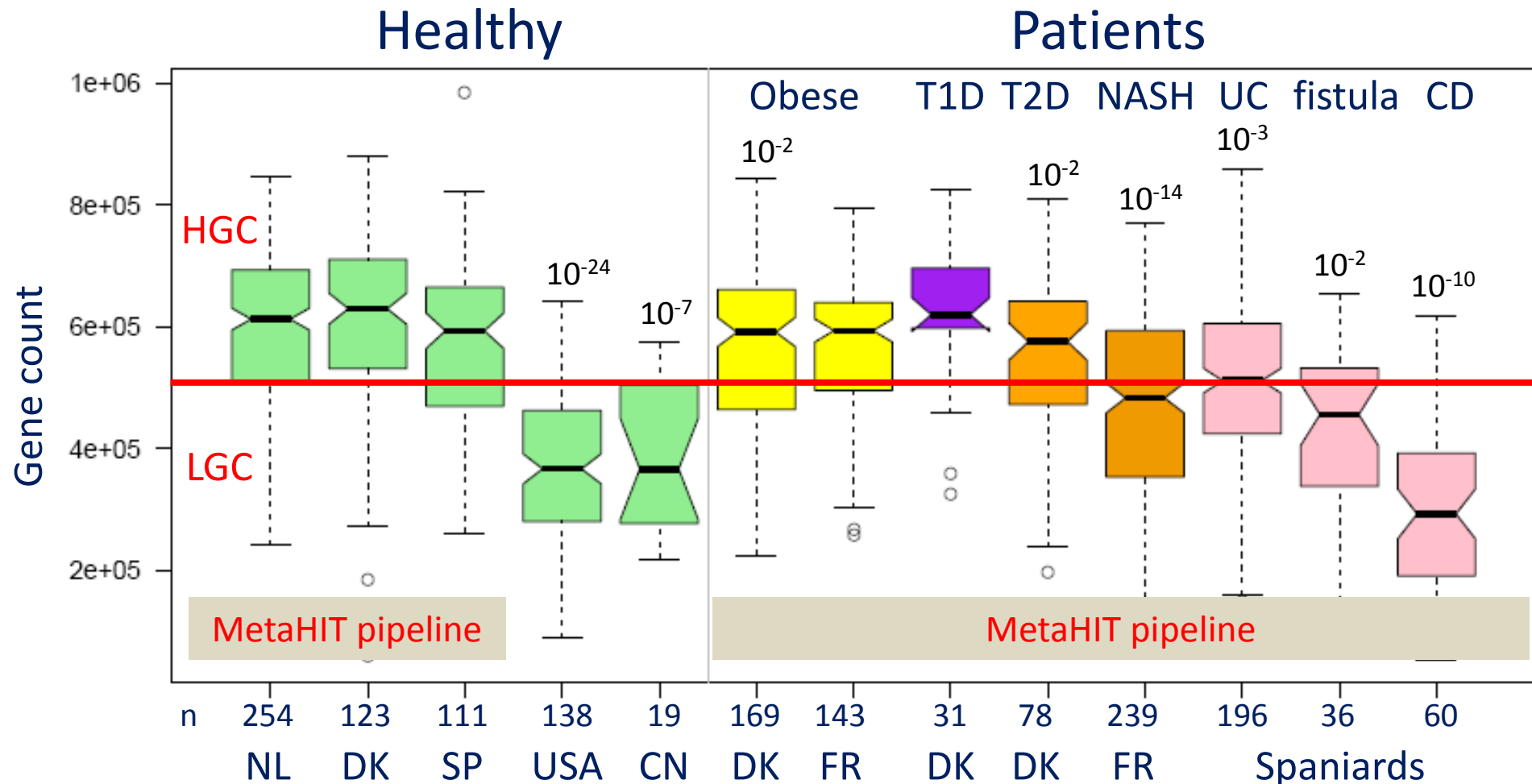


Similar microbial profiles in French (n=49) and Danes (n=292)



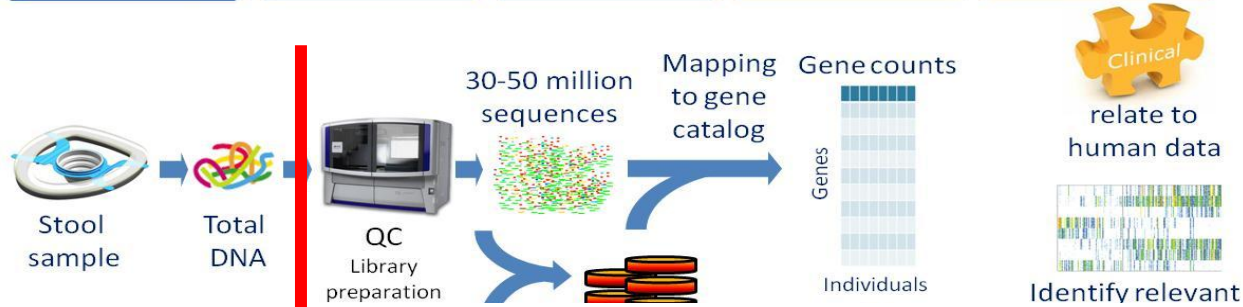
- 18 MGS highly biased in French
- 14/18 MGS also highly biased in Danes
- 6 MGS sufficient to identify at-risk people with 95% accuracy

Gut gene richness in health & disease, n=1597



Atrophy of the neglected organ in some diseases

Quantitative metagenomics



Different in MetaHIT,
US (HMP) & CN studies

Could this result in the
apparent loss of richness
in US & CN?

**Standardization in microbiome
studies is critical**

Prevention = Risk prediction + Risk alleviation

- ✓ Can the neglected organ inform on a risk of chronic diseases?
- ✓ Can it be a target for intervention?

Microbiome perturbations:
an “ecological” disease.
Not a pathogene infection!

Microbiome
modulation



Eutrophic lake

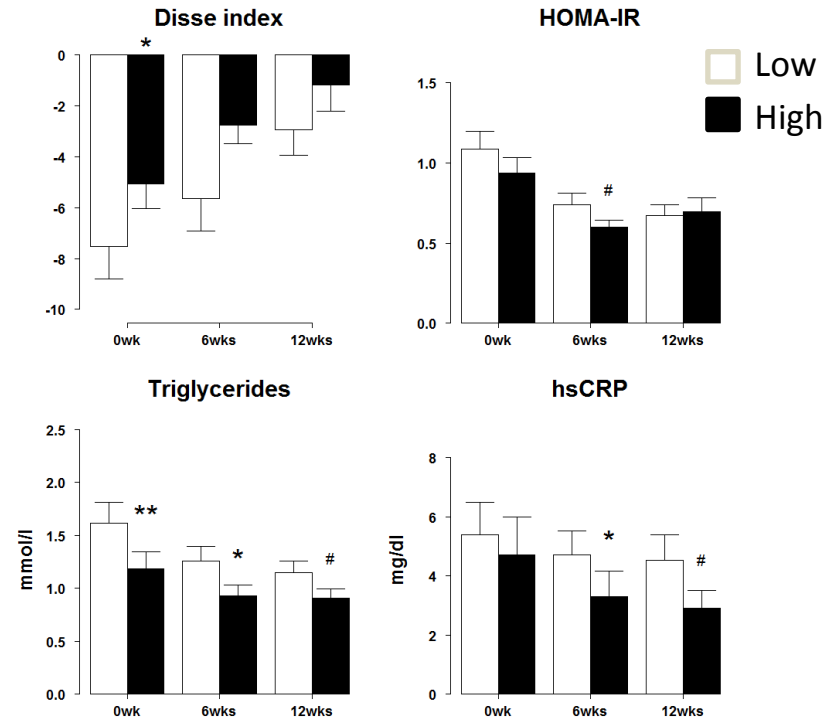
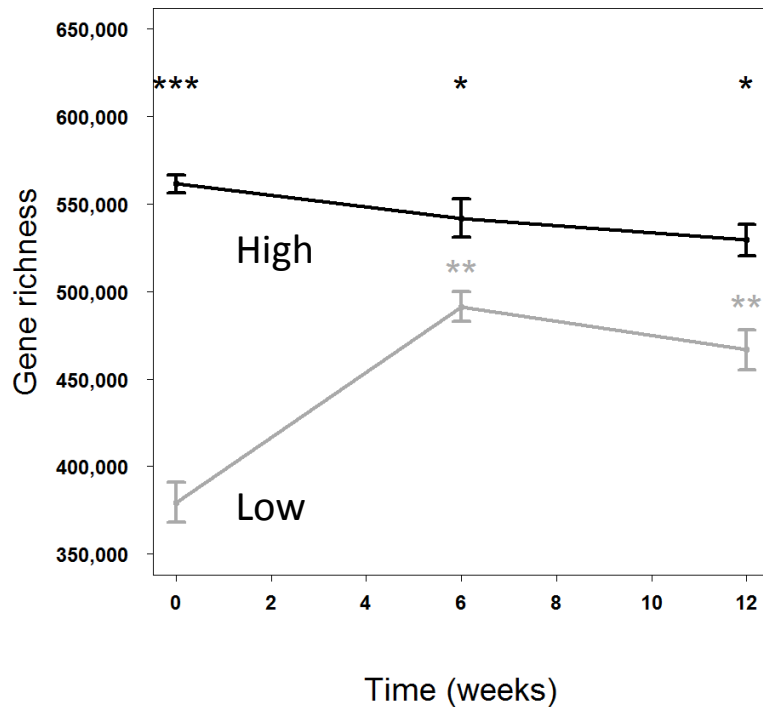
MICRO-Obes study

Nutritional intervention in France

- 49 overweight & obese individuals
- calorie-restricted diet for 6 weeks
- maintenance diet for 6 weeks

Micro-obes partners: Joël Doré, Coordinator, INRA
Karine Clément, Pitié-Salpêtrière; Denis Le Paslier, Genoscope; S.
Dusko Ehrlich, Jean-Pierre Gauchi, Philippe Bessières, INRA

Dietary intervention improves simultaneously gene richness and risk phenotypes, albeit not fully



High gene richness people respond better

Low diversity microbiome appears to be less healthy

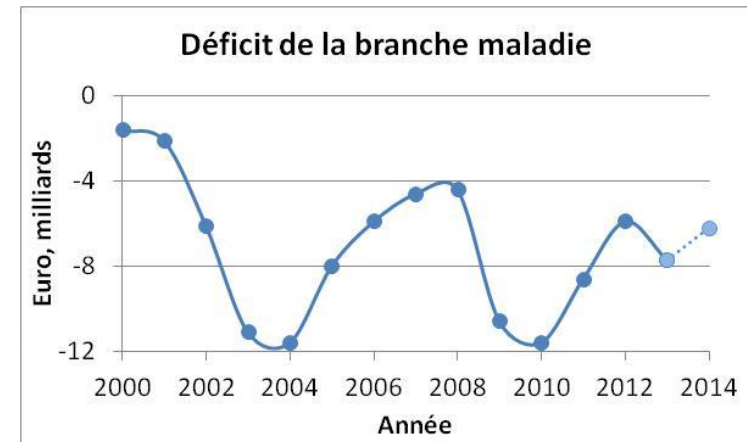
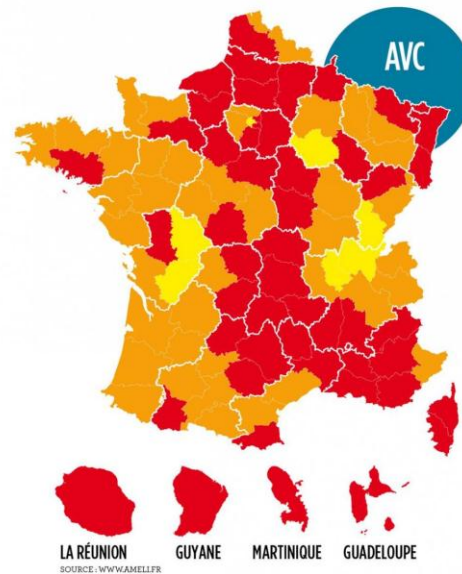
- It can be diagnosed simply, to identify individuals at risk.
- Interventions can correct it and possibly alleviate the risk.

**This could have a major
impact on public health**

Chronic diseases are frequent and costly

Type 2 diabetes: **4.4%**
of French population

A cerebrovascular accident:
every 4 minutes in France



Could they be retarded by systematic analysis and treatment of microbiota (risk detection & alleviation)?

How to raise to the challenge?

MetaGenoPolis

Pre-industrial Demonstrator

Director of the INRA Unit: Florence Haimet

Director of Research: Joël Doré

Grant P.I. : S. Dusko Ehrlich

Funding: 19M€ for 2012-2019 by Investissements d'Avenir

Budget for the period: 80+ M€



Four platforms & a clinical interface

ICAN CLINICAL STUDIES: PITIE SALPÊTRIÈRE HOSPITAL



SAMBO
SAMPLE BIOBANKING

Specifications, Collection,
Aliquoting, Storage & Nucleic
Acid Preparation



METAQUANT
QUANTITATIVE METAGENOMICS
Libraries, Sequences, Bioinformatics,
Biostatistics

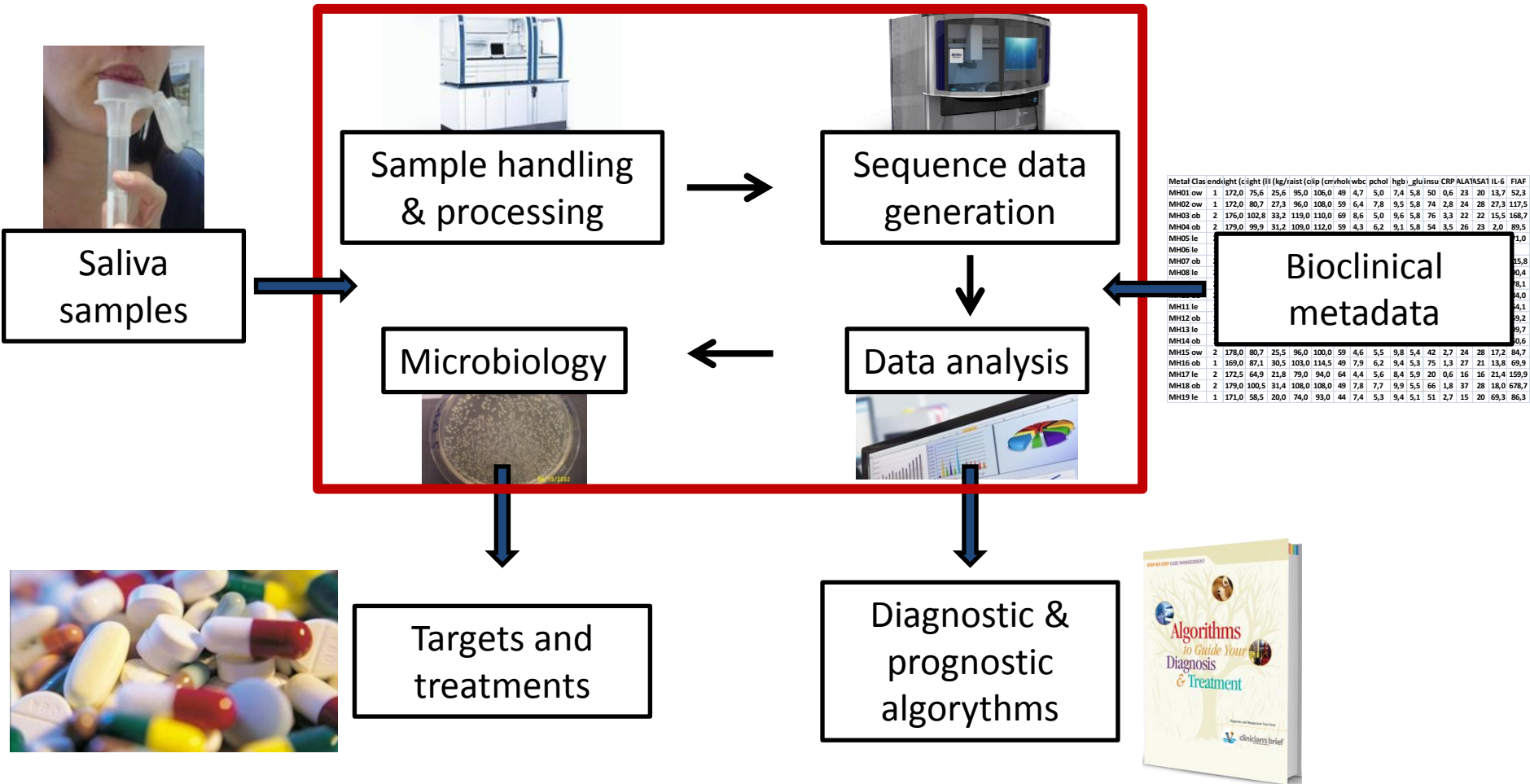


METAFUN
FUNCTIONAL METAGENOMICS
Libraries, Screening, Mechanistics

SOCCA ETHICS AND SOCIETY



KCL Center for Host Microbiome Interactions: from the mouth (CHMI) to the gut (MGP)



> 30 publications in quantitative & functional metagenomics

2010 : Qin J et al. Nature , **The human gut reference gene catalogue**

2011 : Arumugam M et al. Nature, **Enterotypes**

2012 : Qin J et al. Nature, **Type II Diabetes**

2013 : Cotillard A et al. Nature, **Impact of diet on gut microbiome**

2013 : Le Chatelier E et al. Nature, **Richness of gut microbes**

2013 : Sunagawa S et al. Nature Methods, **Universal phylogenetic markers**

2014 : Nielsen B et al. Nature Biotechnol. in press, **Catalog of metagenomic units**

2014 : Li J. et al. Nature Biotechnol. in press, **10 M gene catalog**

2014: Qin N. et al. Nature in press, **Gut microbiome in liver cirrhosis.**



5 patents filed, 1 being filed.

- ▶ Co-presidency of International Human Microbiome Consortium
- ▶ Co-organiser of all International Human Microbiome Congress since 2010 (2000 participants in 2013)
- ▶ Integration in research networks, academic, clinical & industrial; national & international

Beyond discovery : impact on the society – engaging industry



Profiling the Gut Microbiome to Manage Microbiotal Diseases

Gut bacterial richness
assessment kit by
the end of 2014



Impact of human microbiome research on public health

A tremendous potential of human microbiome

- In diagnostics
- In prognostics
- In patient monitoring
- As target for modulation to improve health

Could help us to shift focus of medicine from mainly curative today to preventive tomorrow

And thus save untold resources & human suffering

Acknowledgments

MetaHIT Consortium



Micro-Obese: K. Clement, JD. Zucker, J. Doré



MetaHIT Consortium



Jean-Michel BATTO

Hervé BLOTTIÈRE

Camille BRUNAUD

François CHEVRE

Angélique DORE

Joël DORE

Adeline DUBREUIL

Fabien DUMETZ

S. Dusko EHRlich

Marine FRAISSANGE

Nathalie GALLERON

Ndeye GAYE

Amine GHOZLANE

Florence HAIMET

Sophie HEBERT

Marie Elise JACQUET

Sean KENNEDY

Emmanuelle LE CHATELIER

Véronique LEJARD

Pierre LÉONARD

Aline LETUR

Florence LEVENEZ

Nicolas MAZIERS

Alice MOREAU

Célia PALCY

Florian PLAZA ONATE

Nicolas PONS

Edi PRIFTI

Benoit QUINQUIS

Maxime ROCHET

Etienne RUPPE

Julien TAP

Thierry VANDUYVENBODEN

Kevin WEISZER

A traditional Japanese garden featuring a pond, a stone lantern, a bamboo fence, and a thatched pavilion. The garden is surrounded by lush greenery, including large, rounded bushes and tall trees with pink cherry blossoms. The water in the pond is calm, reflecting the surrounding landscape.

Merci beaucoup!
Et prenez bien soin de
votre microbiome...

www.mgps.eu