



metagenopolis  
**mgps.eu**

# Gut microbial richness in health and disease

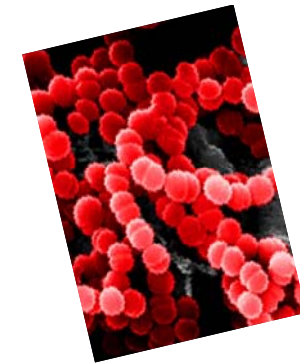
Personalized medicine initiative Bern, March 31, 2014

S. Dusko Ehrlich,

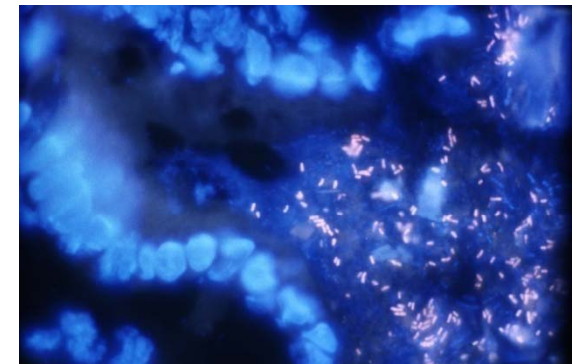
MetaGenoPolis, INRA Jouy en Josas; King's College, London, UK

# 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

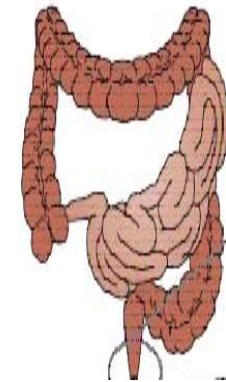


...with a major role in health & disease !



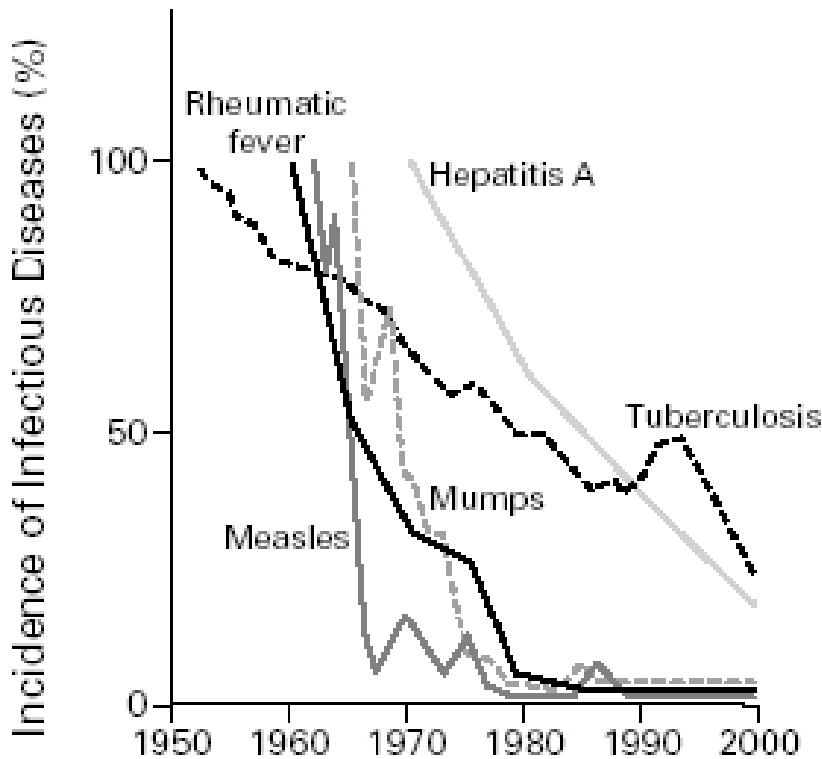
# Les maladies chroniques potentiellement impactées par le microbiote intestinal

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 Coéliquaue	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...	

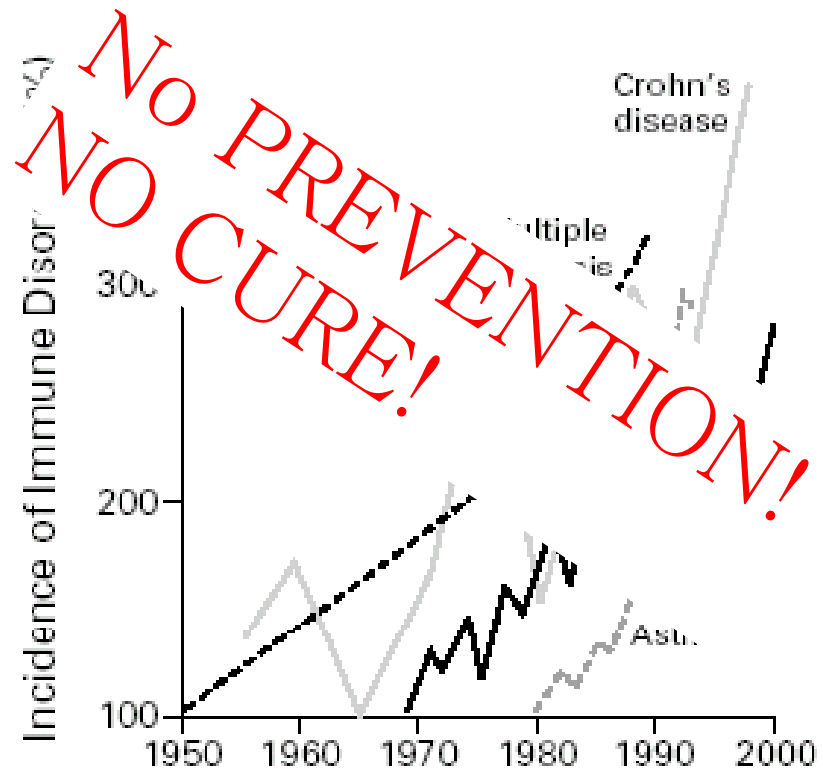


# Chronic diseases increase steadily in industrialized countries

A



B



Bach JF, N Eng J Med 2002

# Prevention = Risk detection + Risk alleviation

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

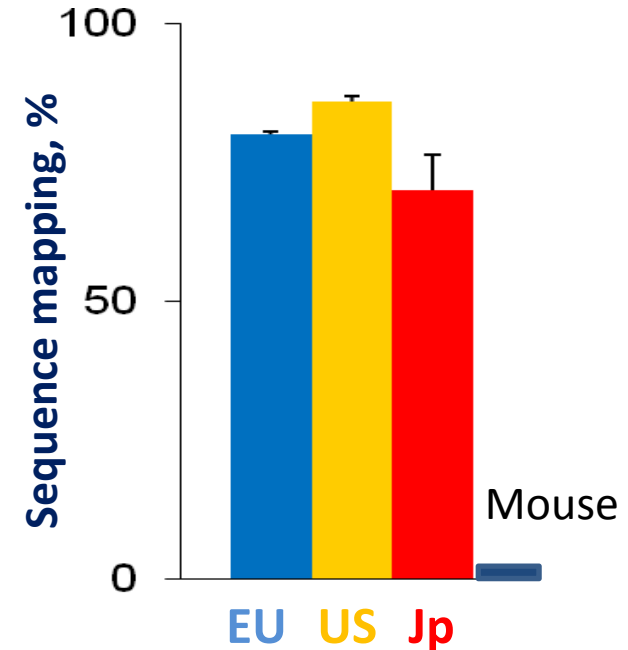
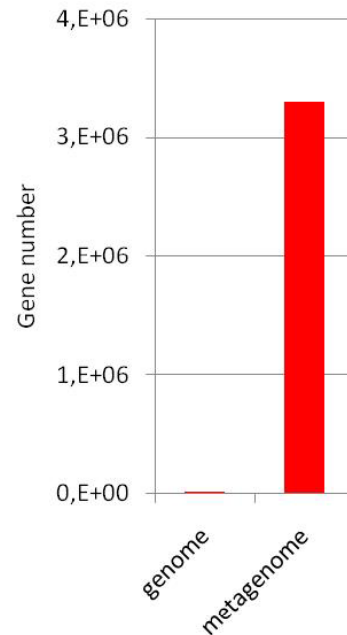
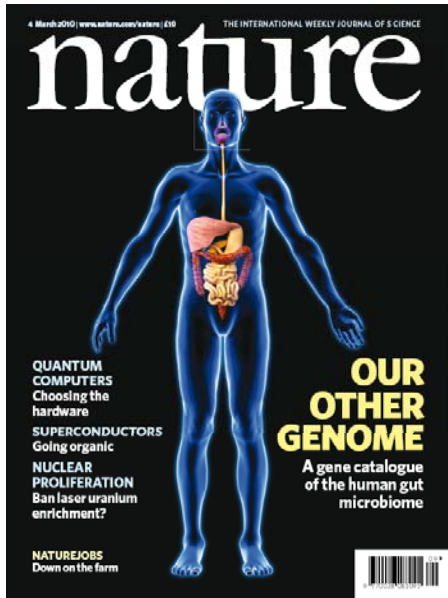
# How to assess the state of the neglected organ in each & every individual?

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 – 10 years after the genome

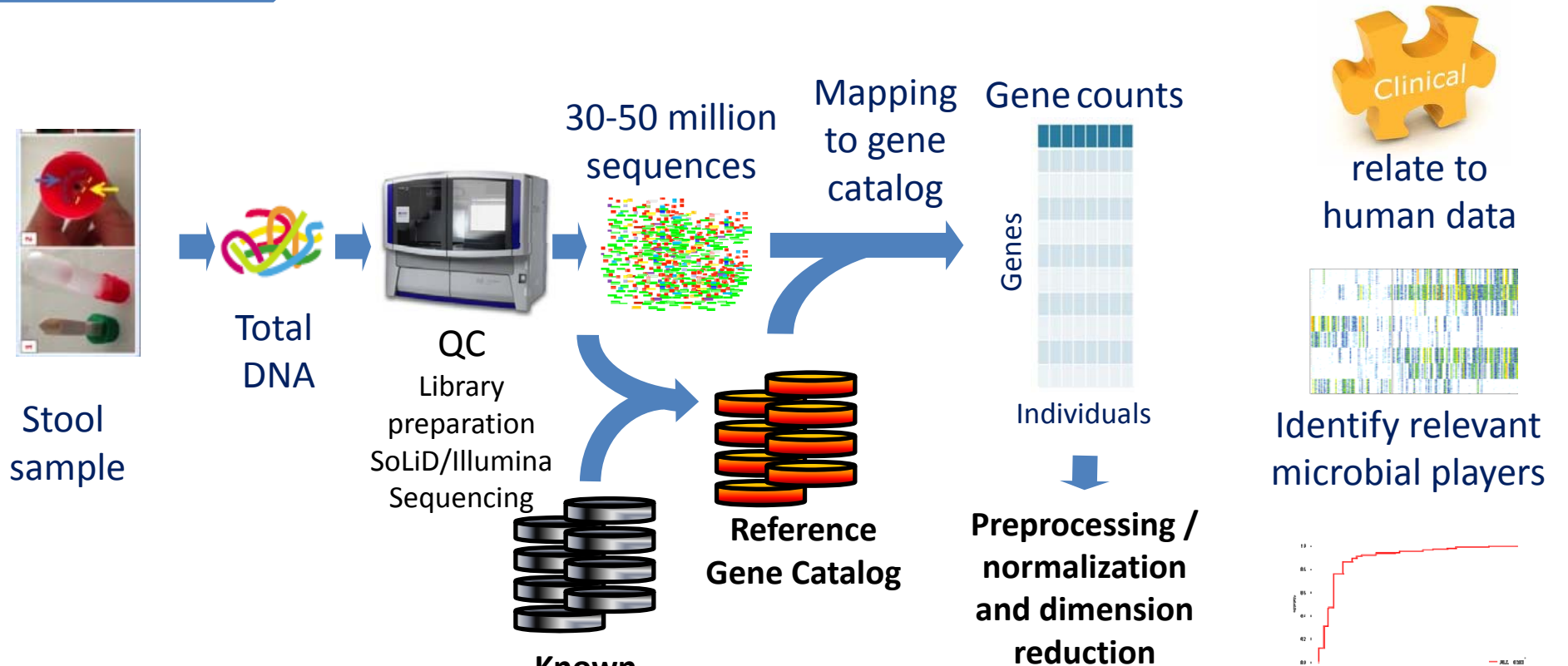
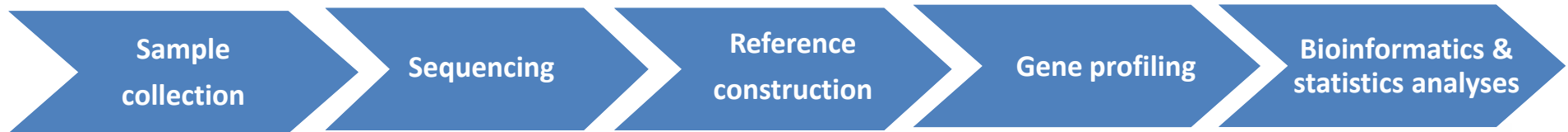
3.3 million bacterial gene catalog : 150-fold human genome

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...

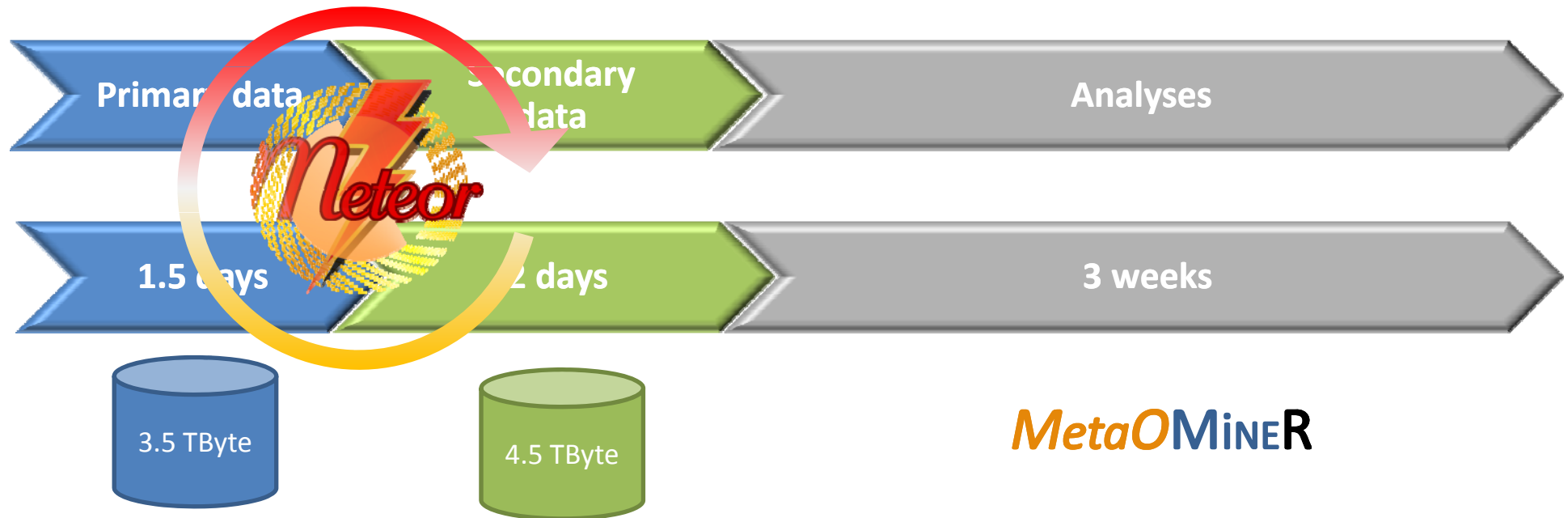
# Quantitative metagenomics





# MetaQuant – bioinformatics

Treatment & storage: 200 samples (a routine study size; 50M reads – 3.3 M gene catalogue)



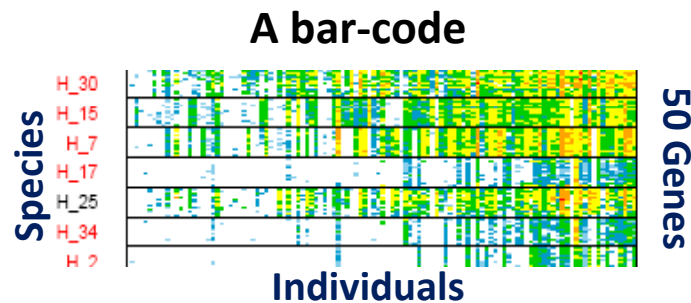
(cluster HPC Windows 2008RC2 16 nœuds/192  
cœurs avec ordonnanceur ProActive)

*Petabyte later this year*

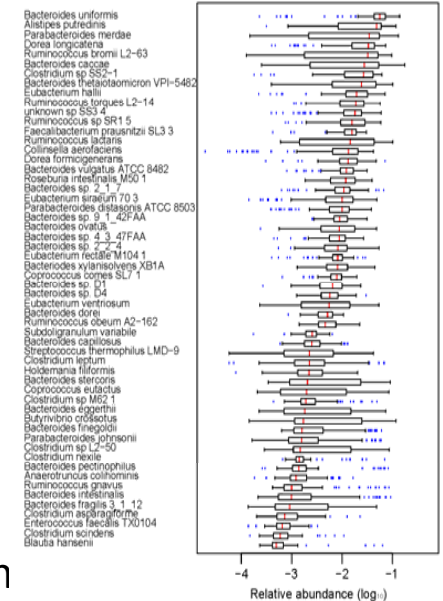
APP number : IDDN.FR.001.420008.000.R.P.2013.000.30000

# Clustering genes into MetaGenomic Units (MGU)

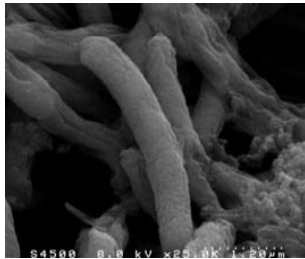
All genes of a species have the same frequency in an individual  
Abundance of a species varies greatly between individuals  
Genes that co-vary in abundance belong to the same species



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

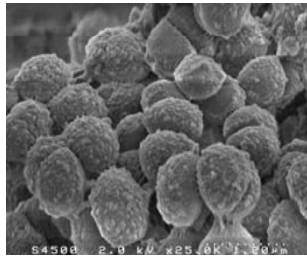


# Microbiome assessment

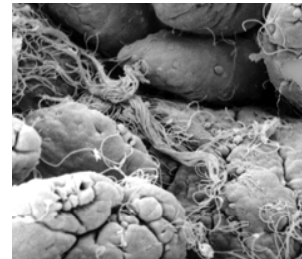


*Faecalibacterium prausnitzii*

Photos UEPSD



*Clostridium difficile*  
en caecum souris



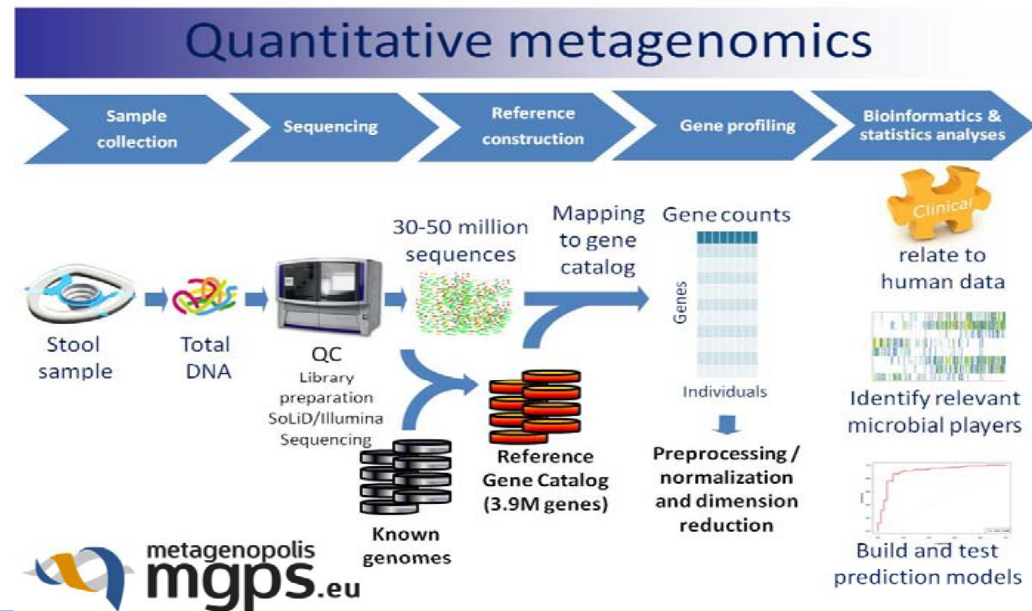
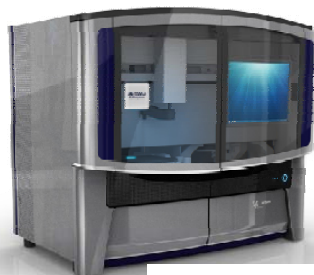
Bactéries ancrées dans  
une Plaque de Peyer,  
Intestin de souris



*Bacteroides dorei*



*Escherichia coli*



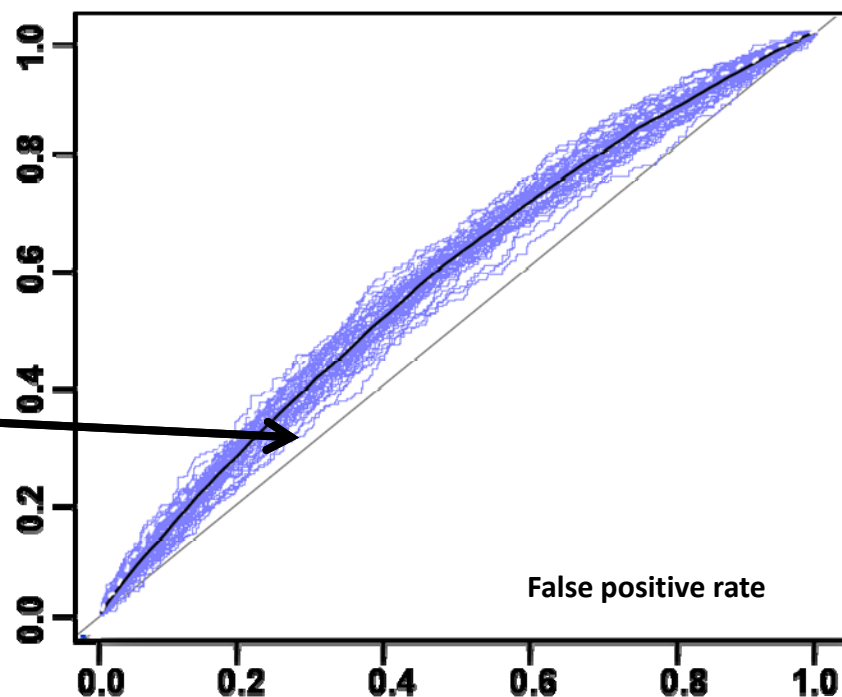
**A Powerful Microscope to Scan the neglected organ**

# 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

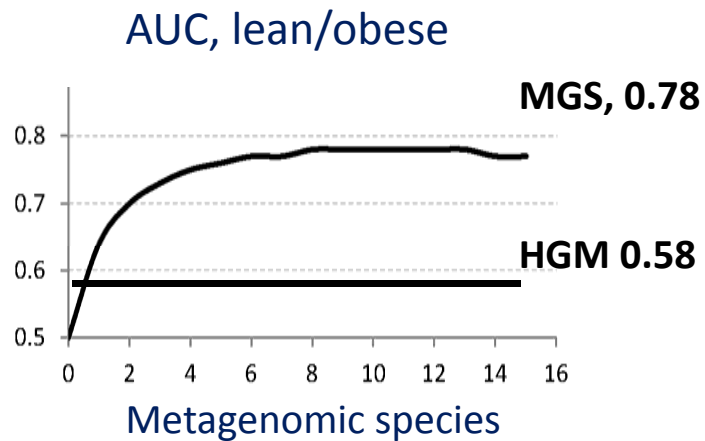


Receiver operator characteristics (ROC) discrimination analysis

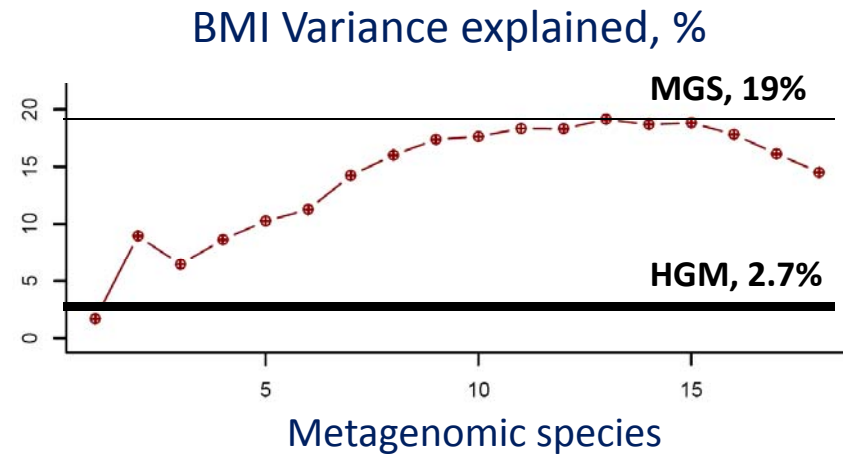
**Perfect: AUC = 1**  
**Random: AUC = 0.5**

AUC = area under curve

# Obesity: Human **metagenome** markers work better

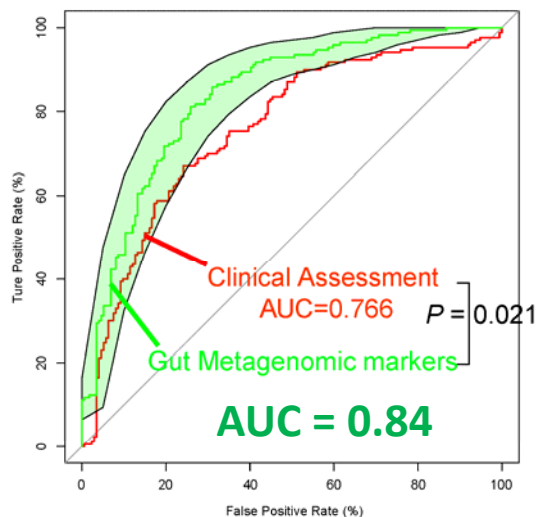


HGM= 32 human genome markers  
Speliotes et al. Nature Genetics 2010

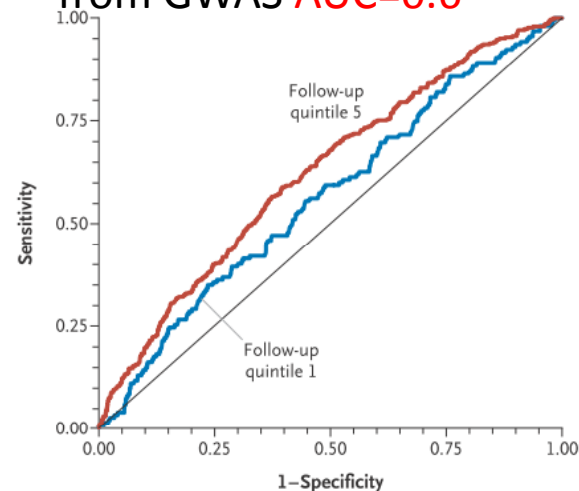


HGM= 97 human genome markers  
Ruth Loos, Genomics in Metabolism

# Type 2 diabetes



Host genetic markers  
from GWAS **AUC=0.6**



## ARTICLE

doi:10.1038/nature11450

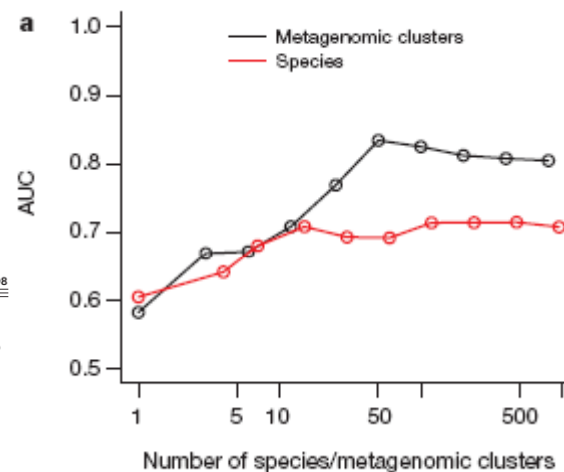
A metagenome-wide association study of gut microbiota in type 2 diabetes

## LETTER

doi:10.1038/nature12198

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

Fredrik H. Karlsson<sup>1\*</sup>, Valentina Tremaroli<sup>2\*</sup>, Intawat Nookaew<sup>1</sup>, Göran Bergström<sup>2</sup>, Carl Johan Behre<sup>2</sup>, Björn Fagerberg<sup>2</sup>, Jens Nielsen<sup>1</sup> & Fredrik Bäckhed<sup>2,3</sup>



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

# 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<sup>1\*</sup>, Trine Nielsen<sup>2\*</sup>, Junjie Qin<sup>3\*</sup>, Edi Pridti<sup>1\*</sup>, Falk Hildebrand<sup>4,5</sup>, Gwen Falony<sup>4,5</sup>, Mathieu Almeida<sup>1</sup>, Manimozhiyan Arumugam<sup>2,3,6</sup>, Jean-Michel Batto<sup>1</sup>, Sean Kennedy<sup>1</sup>, Pierre Leonard<sup>1</sup>, Junhua Li<sup>3,7</sup>, Kristoffer Burgdorf<sup>2</sup>, Niels Grarup<sup>2</sup>, Torben Jørgensen<sup>8,9,10</sup>, Ivan Brandslund<sup>11,12</sup>, Henrik Florence Levenez<sup>1</sup>, Nicolas Pons<sup>1</sup>, Simon Rasmussen<sup>13</sup>, Shinichi Søren Brunak<sup>13</sup>, Karine Clément<sup>15,16,17</sup>, Joël Doré<sup>1,18</sup>, Michiel Kleer Thomas Sicheritz-Ponten<sup>13</sup>, Willem M. de Vos<sup>14,20</sup>, Jean-Daniel Zoetendal<sup>14</sup>, Peer Bork<sup>6</sup>, Jun Wang<sup>3,19,23,24,25</sup>, S. Dusko Ehrlich<sup>1</sup> & Pierre Renault<sup>18</sup>

NEWS & VIEWS RESEARCH

### Wealth management in the gut

SUNGSOON FANG & RONALD M. EVANS

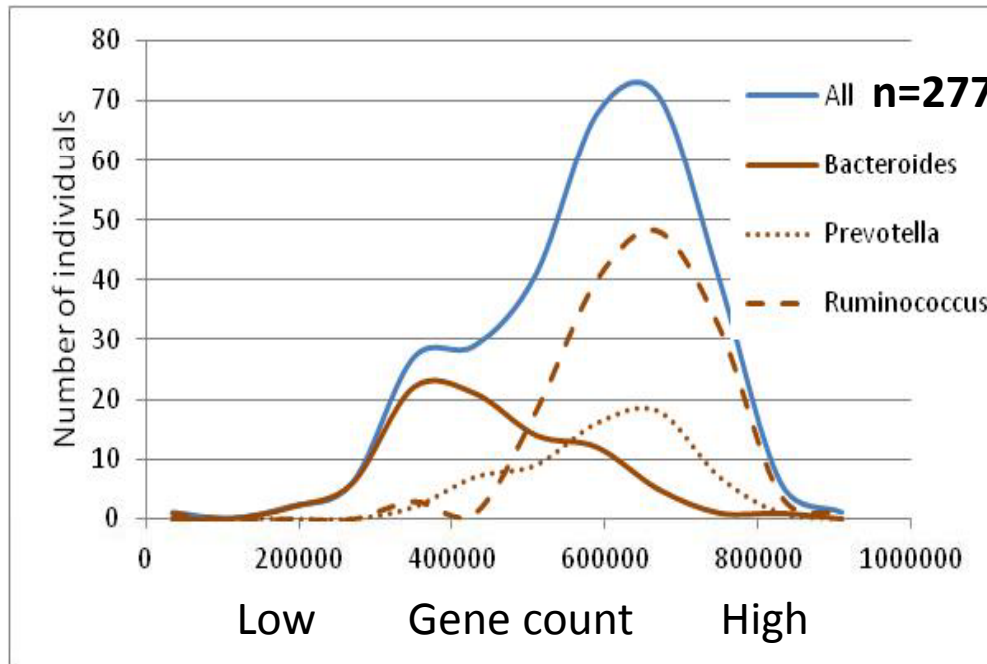
## LETTER

doi:10.1038/nature12480

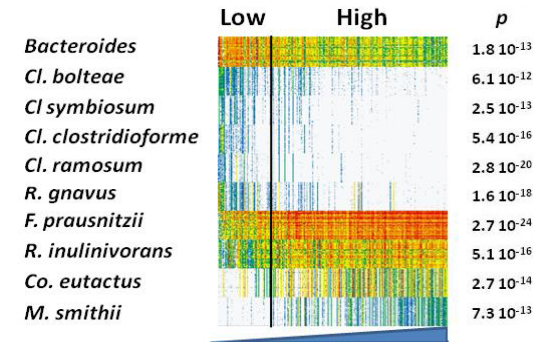
### Dietary intervention impact on gut microbial gene richness

Aurélien Cotillard<sup>1,2\*</sup>, Sean P. Kennedy<sup>3\*</sup>, Ling Chun Kong<sup>1,2,4\*</sup>, Edi Pridti<sup>1,2,3\*</sup>, Nicolas Pons<sup>3\*</sup>, Emmanuelle Le Chatelier<sup>3</sup>, Mathieu Almeida<sup>3</sup>, Benoit Quinquis<sup>3</sup>, Florence Levenez<sup>3,5</sup>, Nathalie Galleron<sup>3</sup>, Sophie Gougis<sup>4</sup>, Salwa Rizkalla<sup>1,2,4</sup>, Jean-Michel Batto<sup>3,5</sup>, Pierre Renault<sup>5</sup>, ANR MicroObes consortium†, Joël Doré<sup>3,5</sup>, Jean-Daniel Zucker<sup>1,2,6</sup>, Karine Clément<sup>1,2,4</sup> & Stanislav Dusko Ehrlich<sup>3</sup>

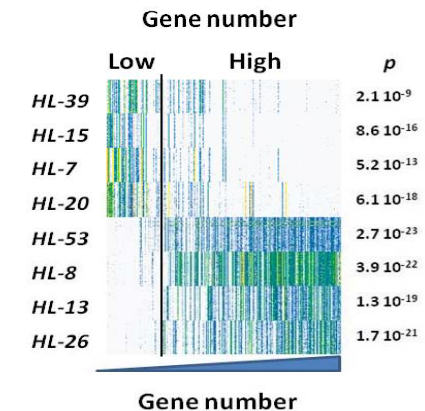
# People differ by gut bacterial gene counts and bacterial communities



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



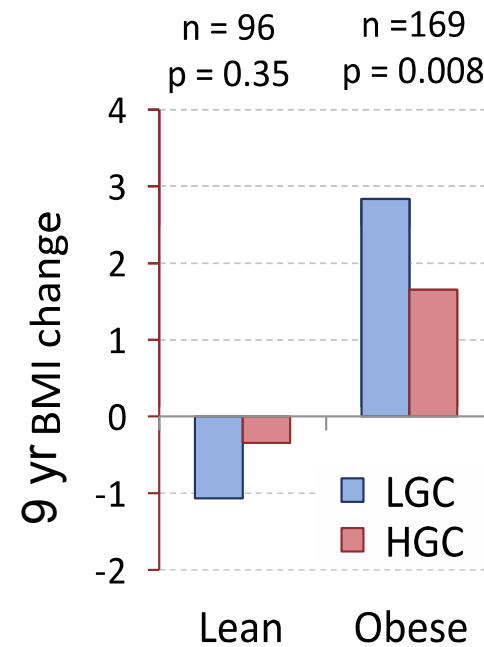
less more

# Low bacterial richness – a risk phenotype?

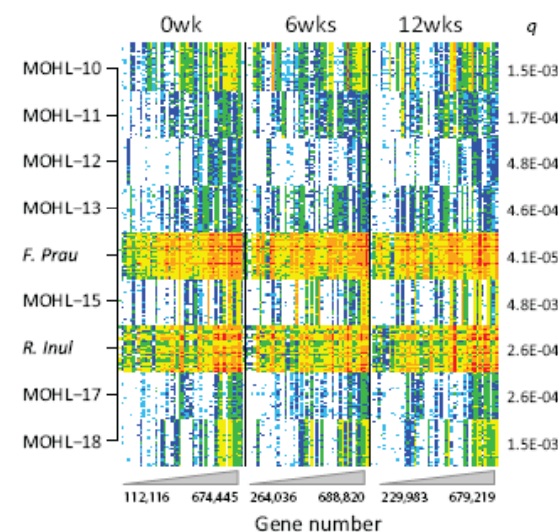
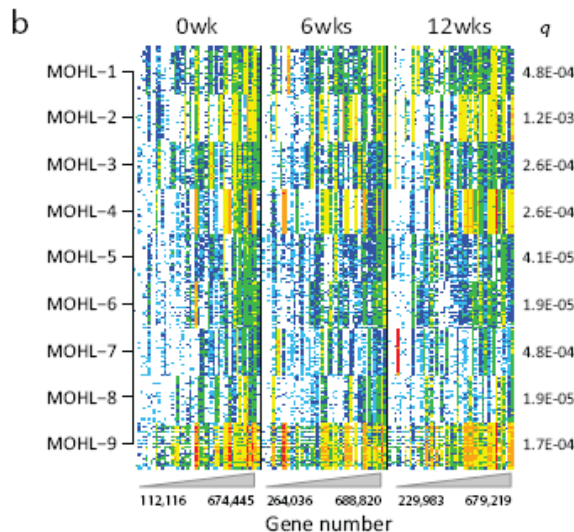
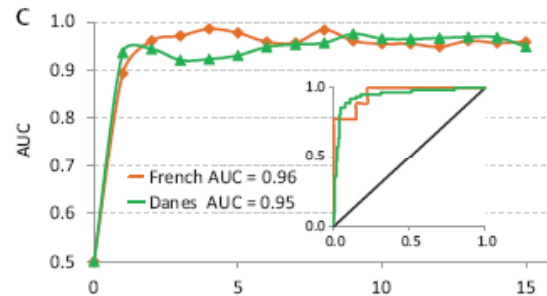
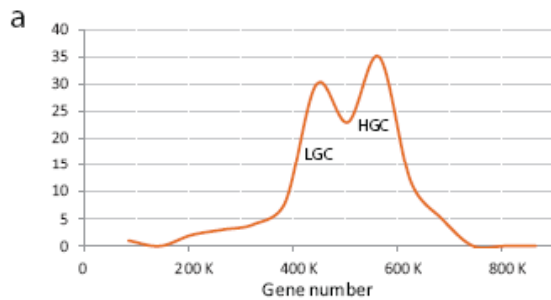
	LGC	HGC	<i>p</i>	<i>q</i>
N (men/women)	68 (23/45)	224 (113/111)		
Age Yrs	56 ± 7.5	57 ± 7.3	0.86	0.89
BMI (kg/m <sup>2</sup> )	32 (29 - 34)	30 (23 - 33)	<b>0.035</b>	0.059
Weight (kg)	95 (75 - 100)	86 (71 - 100)	<b>0.019</b>	<b>0.037</b>
Fat %	37 (29 - 42)	31 (25 - 39)	<b>0.0069</b>	<b>0.022</b>
S-Insulin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

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

# Prevention = Risk detection + 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

# Dietary intervention

controlled amount of 1200 kcal/d (35% protein, 25% lipids, and 40% carbohydrates). This diet consisted of habitually consumed foods that were supplemented daily with 4 dietary products (60–75 kcal; designed by CEPRODI-KOT Laboratory). These supplements consisted of lyophilized powder enriched in protein and soluble fiber (mainly inulin) and composed of LGI carbohydrates.

60<sup>TH</sup> ANNIVERSARY

The American Journal of  
**CLINICAL NUTRITION**



*A Publication of the American Society for Nutrition*

JANUARY 2012 • VOLUME 95 • NUMBER 1

Differential effects of macronutrient content in 2 energy-restricted diets on cardiovascular risk factors and adipose tissue cell size in moderately obese individuals: a randomized controlled trial. *SW Rizkalla et al*

49

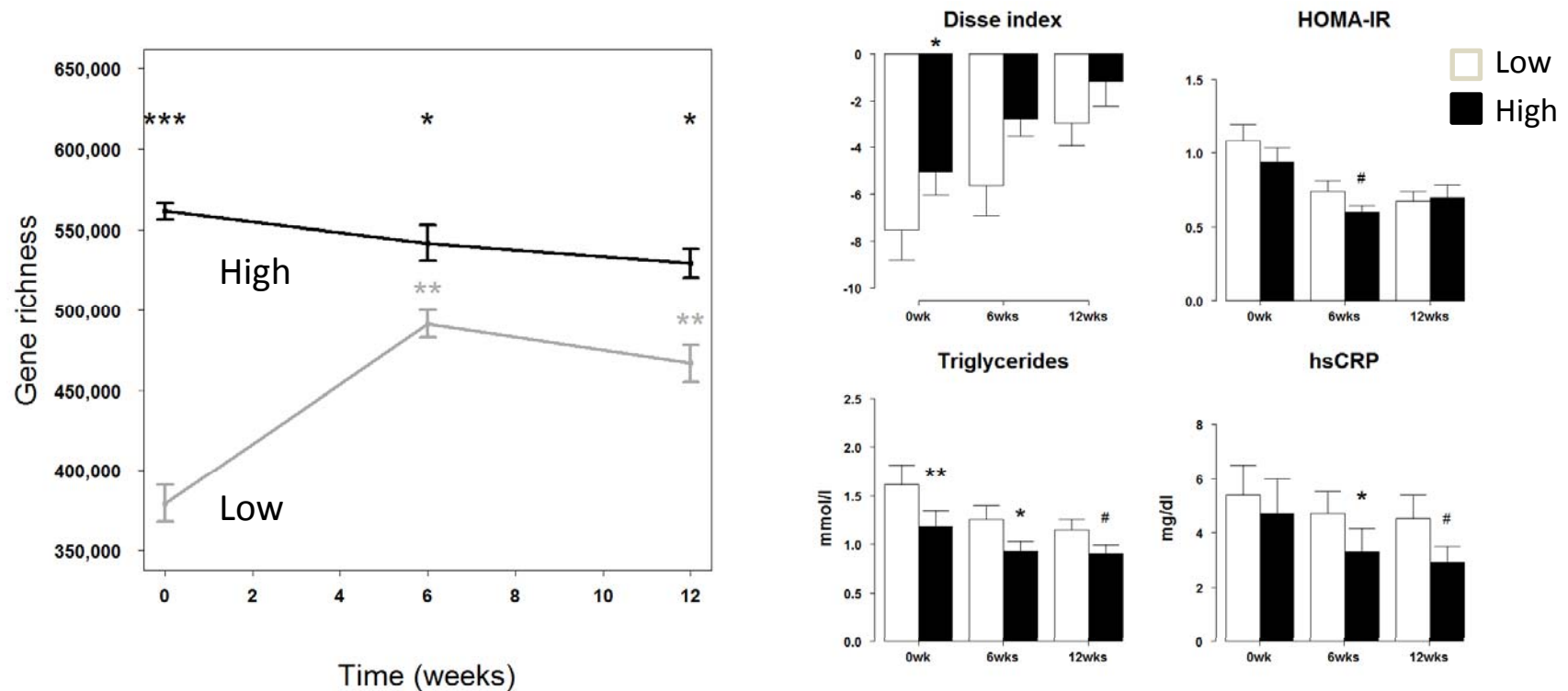
# MICRO-Obes study

## 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 (**1 of 4!!**).
- Interventions can correct it and possibly alleviate the risk.

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

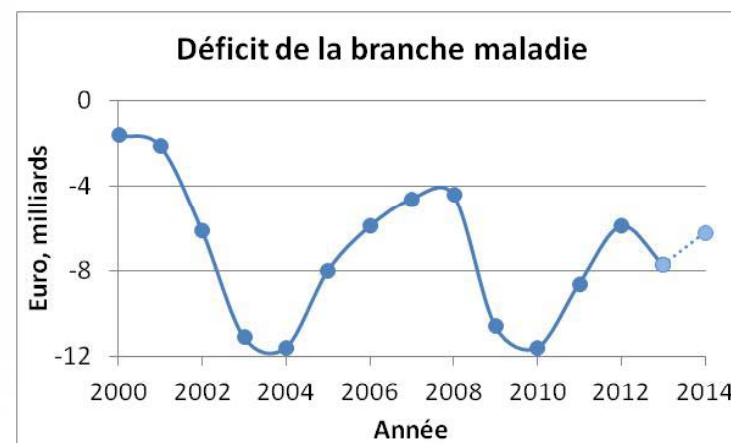
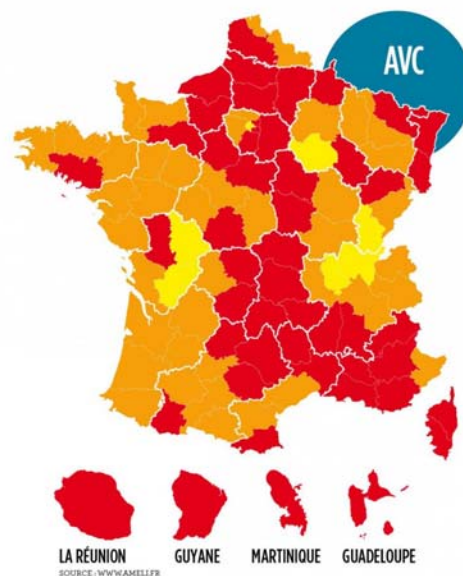
# Chronic diseases are frequent and costly

Type 2 diabetes: **4.4%**  
of French population  
**12 billion €/year** (2007)



A cerebrovascular accident:  
**every 4 minutes** in France

■ Moins de 52% ■ Entre 52,01% et 61% ■ Plus de 61,01%



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

How to meet new challenges?



# 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



**METAQUANT**  
QUANTITATIVE METAGENOMICS

Libraries, Sequences, Bioinformatics,  
Biostatistics

**SAMBO**  
SAMPLE BIOBANKING

Specifications, Collection,  
Aliquoting, Storage & Nucleic  
Acid Preparation



**METAFUN**  
FUNCTIONAL METAGENOMICS

Libraries, Screening, Mechanistics



**SOCCA** ETHICS AND SOCIETY





# IT philosophy



*free*



Google



The key to success -  
integration & mastery,  
hard & soft



**MGP follows the same  
strategy**

## > 20 publications in quantitative & functional metagenomics

2010 : Qin J et al. Nature , **The human gut reference 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  
and metabolic markers**

2013 : Sunagawa S et al. Nature Methods, **Metagenomic species  
profiling using universal phylogenetic marker genes**



## 5 patents filed last year

- ▶ 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



- ▶ Contact: [contact@mgps.eu](mailto:contact@mgps.eu)
- ▶ Site internet: [www.mgps.eu](http://www.mgps.eu)



# 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





## MGP team

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



**Merci beaucoup!**  
**Et occupez-vous bien de**  
**votre microbiote...**