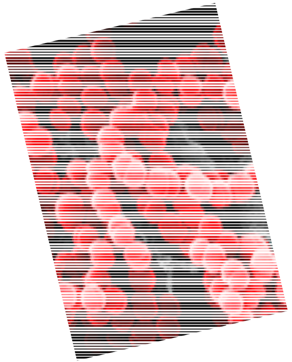
The intestinal microbiota and the link with diseases

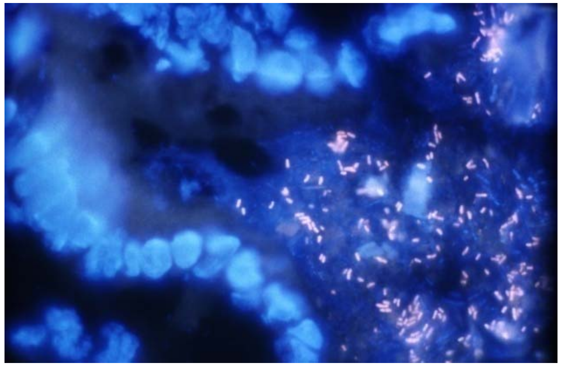
FIRST INTERNATIONAL CONFERENCE ON
CLINICAL METAGENOMICS,
October 14, 2016, Campus Biotech, Geneva, Switzerland

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

The human intestinal microbiota is a neglected organ…

- ✓ 100 trillion microorganisms ; 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

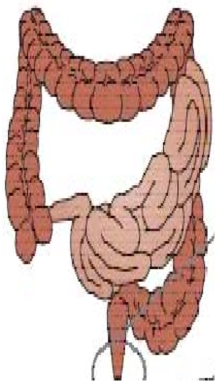




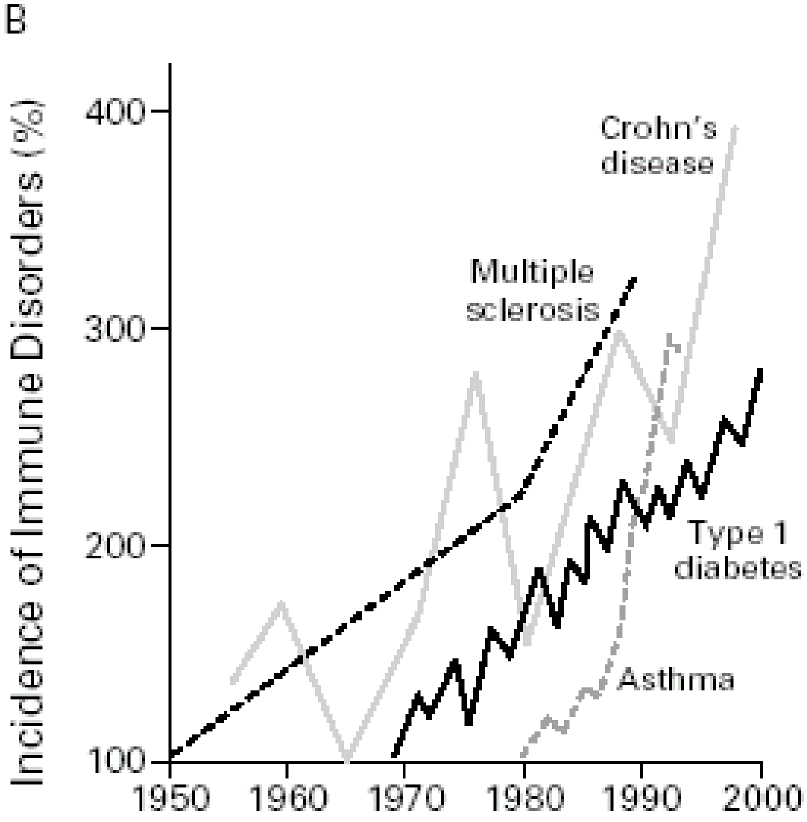
…with a major role in health & disease !

Chronic diseases potentially impacted by the gut microbiome

Frailty in seniors	Van Tongeren et al., 2005
Crohn’s disease	Seksik et al., 2003; Sokol et al., 2006, 2008, 2009
Ulcerative colitis	Sokol et al., 2008; Martinez et al., 2008
Rheumatoid arthritis	Vaahtovuo et al., 2008; Scher et al., 2013
Obesity	Ley et al., 2007; Kalliomäki et al., 2008
Type 2 diabetes	Cani and Delzenne, 2009
Type 1 diabetes	Dessein et al., 2009; Wen et al., 2008
Celiac disease	Nadal et al., 2007; Collado et al., 2009
Allergy	Kirjavainen et al., 2002; Björkstén, 2009
Autism	Finegold et al., 2002; Paracho et al., 2005
Colorectal cancer	Mai et al., 2007; Scanlan et al., 2008
Breast cancer	Velicer et al., 2004
HIV	Gori et al., 2008
Cirrhosis	Gunnarsdottir et al. 2003
Atherosclerosis	Wang et al. 2011
Other….	



Chronic diseases increase steadily in industrialized countries



- No PREVENTION
- No CURE

Seven of the top 10 causes of death in 2010 were chronic diseases. Two of these—heart disease and cancer—accounted for nearly 48% of deaths in the US

Bach JF, N Eng J Med 2002

Centers for Disease Control and Prevention, 2013

Prevention of chronic diseases could impact public health greatly

Prevention = Risk detection & alleviation

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

Assessment of the microbiome needed

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.

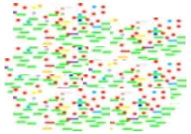
Quantitative metagenomics

- Sample collection
- Sequencing
- Reference construction
- Gene profiling
- Bioinformatics & statistics analyses

Stool sample

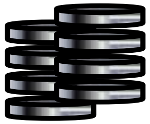
Total DNA

NGS

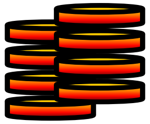


20 million sequences

Mapping to gene catalog



Known genomes

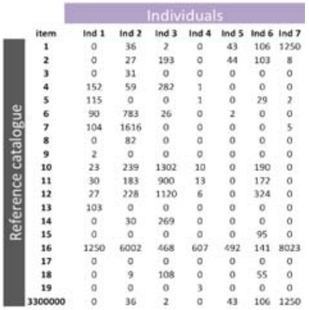


Reference Gene Catalog

Gene counts

individuals

genes

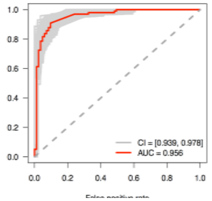


	Individuals						
item	Ind 1	Ind 2	Ind 3	Ind 4	Ind 5	Ind 6	Ind 7
1	0	36	2	0	43	106	1250
2	0	27	193	0	44	103	8
3	0	31	0	0	0	0	0
4	152	59	282	1	0	0	0
5	115	0	0	1	0	29	2
6	90	783	26	0	2	0	0
7	104	1616	0	0	0	0	5
8	0	82	0	0	0	0	0
9	2	0	0	0	0	0	0
10	23	239	1302	10	0	190	0
11	30	183	900	13	0	172	0
12	27	228	1120	6	0	324	0
13	103	0	0	0	0	0	0
14	0	30	269	0	0	0	0
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

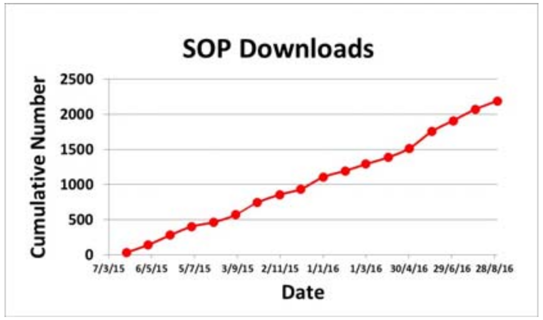
Preprocessing / normalization and dimension reduction

Relate to human data

Identify relevant microbial players



Build and test prediction models

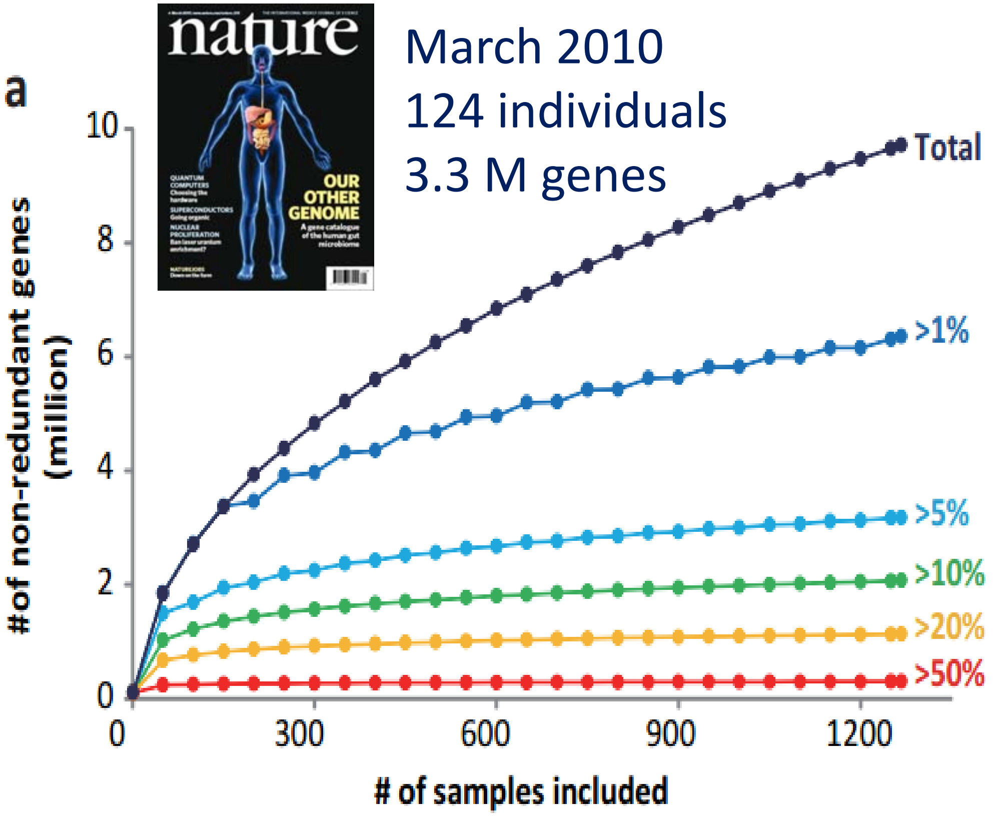


The prime way to characterize a microbiome

Standardization is critical

http://www.microbiome-standards.org/#SOPS

An integrated 9.9 M genes reference catalog



March 2010
124 individuals
3.3 M genes

Rare genes are increasing
- Transient species ?
- Strain differences ?
Pan-metagenome

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

Individuals from MetaHIT, Chinese and HMP studies, n=1267
Sequenced reference gut genomes

Li et al. Nature Biotech, 2014

Improving microbiome description

Towards a common gut gene catalog

- Rheumatoid arthritis catalog
- Liver cirrhosis catalog
- Vegan catalog
- ….

Comparability of studies requires a common catalog – we should cooperate to make it!

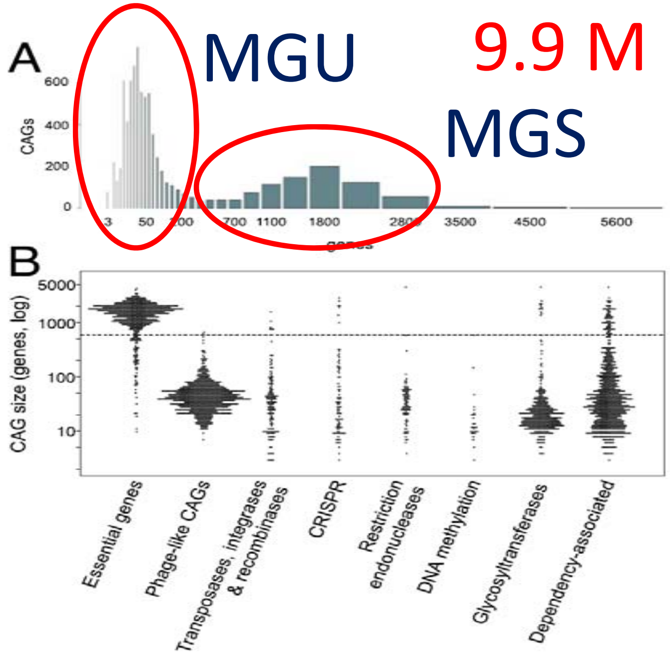
Gene catalog clustered in MetaGenomic Units by co-abundance binning

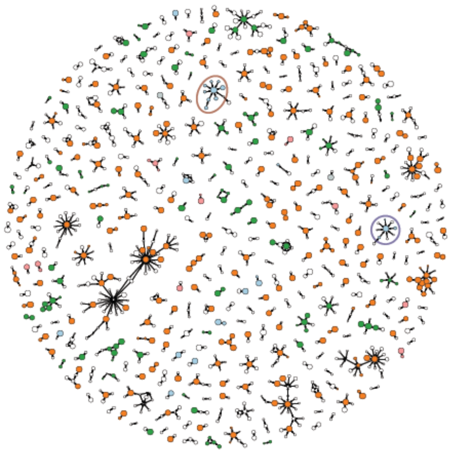
741 large MGU (>700 Genes) correspond to bacterial species (MetaGenomic Species; 85% previously unknown)

238 high quality genomes reconstructed

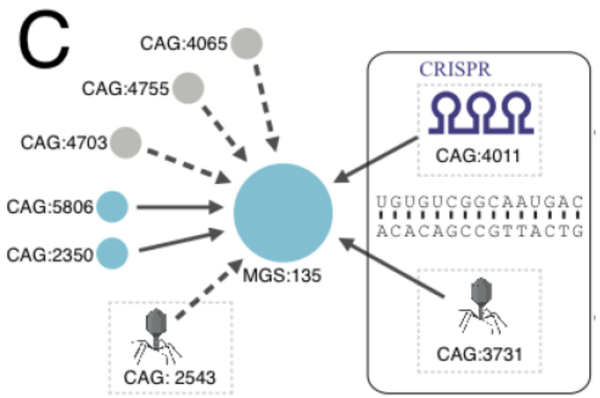
6640 small MGU: phages, plasmids, virulence islands, CRISPR..

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



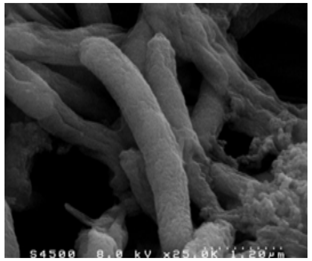


Interaction network

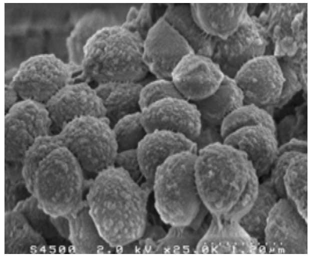


Nielsen, et al. Nature Biotech, 2014

Microbiome assessment



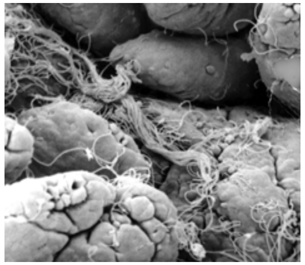
Faecalibacterium prausnitzii



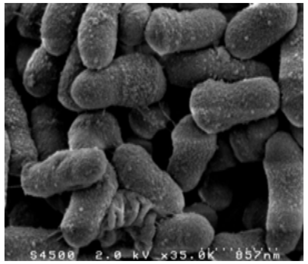
Ruminococcus spp

Photos UEPSD

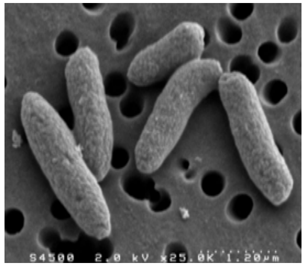
Clostridium difficile en caecum souris



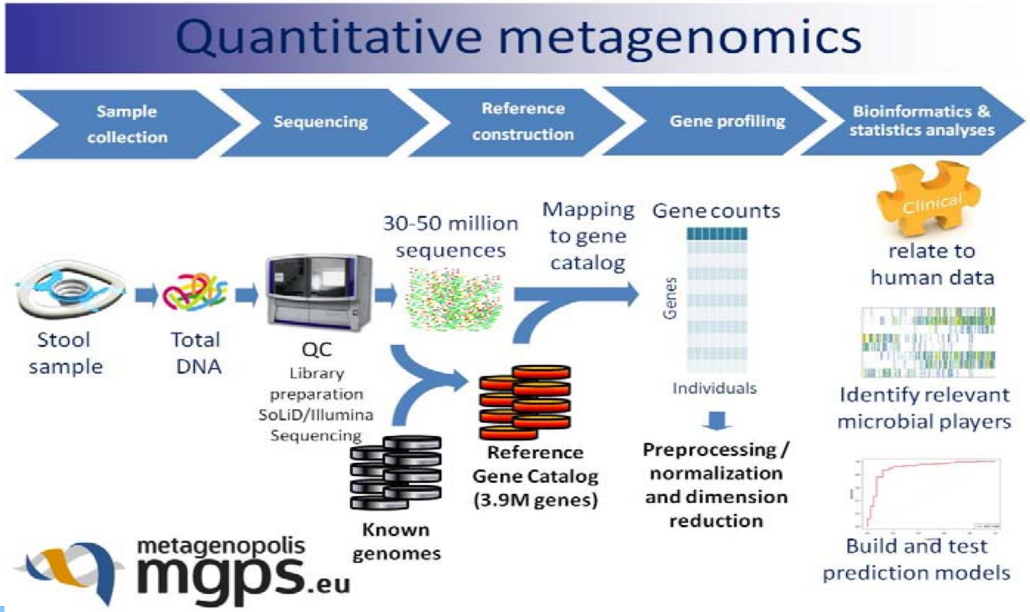
Bactéries ancrées dans une Plaque de Peyer, Intestin de souris



Bacteroides dorei



Escherichia coli



Quantitative metagenomics

Sample collection → Sequencing → Reference construction → Gene profiling → Bioinformatics & statistics analyses

Stool sample

Total DNA

QC
Library preparation
SoLiD/Illumina Sequencing

30-50 million sequences

Known genomes

Reference Gene Catalog (3.9M genes)

Mapping to gene catalog

Gene counts

Preprocessing / normalization and dimension reduction

relate to human data

Identify relevant microbial players

Build and test prediction models

A Powerful Microscope to Scan the neglected organ

Diagnostics

Diagnostics of liver cirrhosis by gut metagenomic species

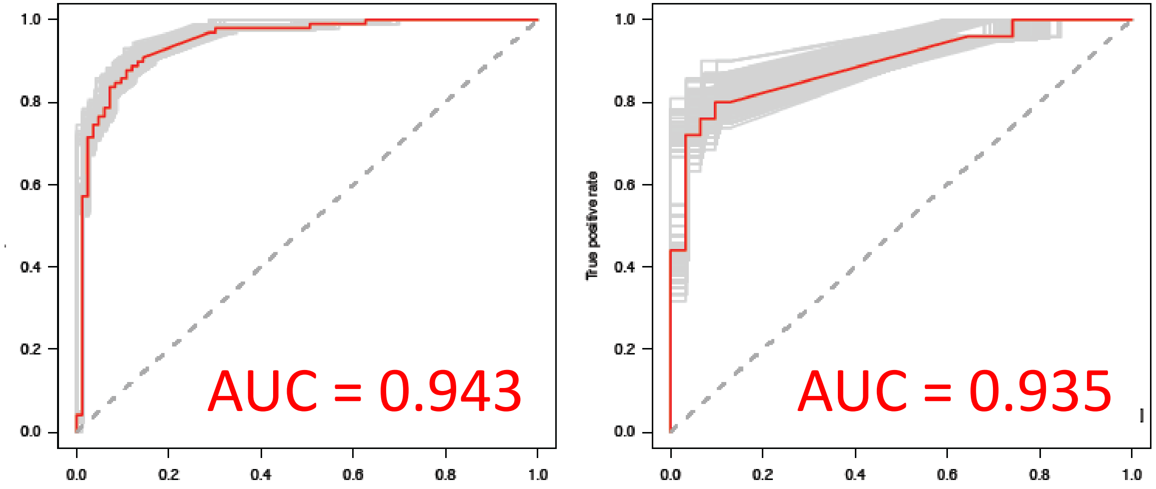
123 patients

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, doi: 10.1038/nature13568.

Zhejiang University, Hangzhou, China & MGP, Jouy en Josas, France

Diagnostics of liver cirrhosis by gut metagenomic species

Accurate diagnostics irrespective of etiology & the disease status:

Viral & alcoholic, compensated & de-compensated (ascites w/wo encephalopathy) patients are diagnosed with 95% accuracy

No effect of medication:

Patients taking antivirals, beta blockers, proton pump inhibitors and those that do not are diagnosed with 95 % accuracy

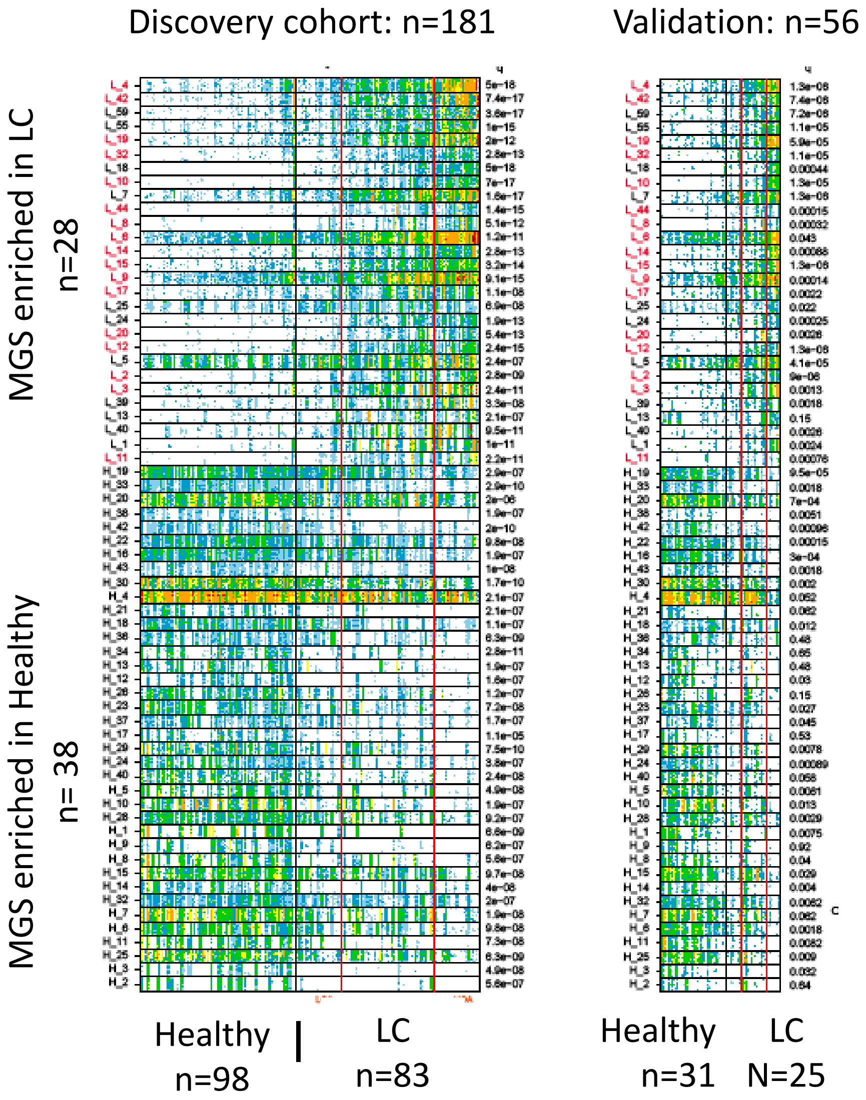
Diagnostics by a non-invasive method – stool analysis

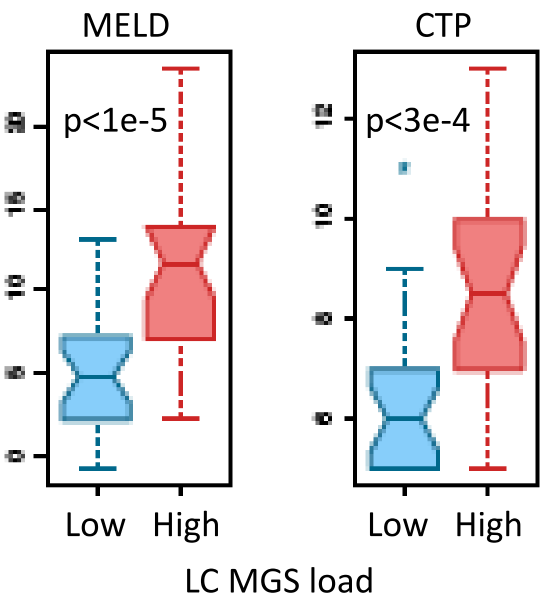
Qin et al.(2015) Nature 525, E2-3. doi:10.1038/nature14852

Zhejiang University, Hangzhou, China & MGP, Jouy en Josas, France

Patient monitoring

Microbiome informs on the state of disease





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

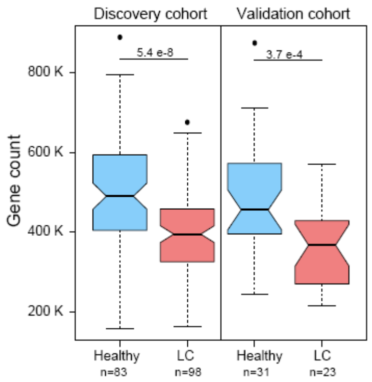
less 🟦🟦🟩🟨🟥 more

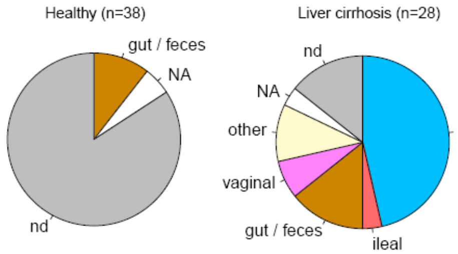
Qin N. et al. Nature 2014, doi: 10.1038/nature13568.

Zhejiang University, Hangzhou, China & MGP, Jouy en Josas, France

Massive microbiome changes in cirrhosis

Low gene richness (p<10 e-10)





Oral species

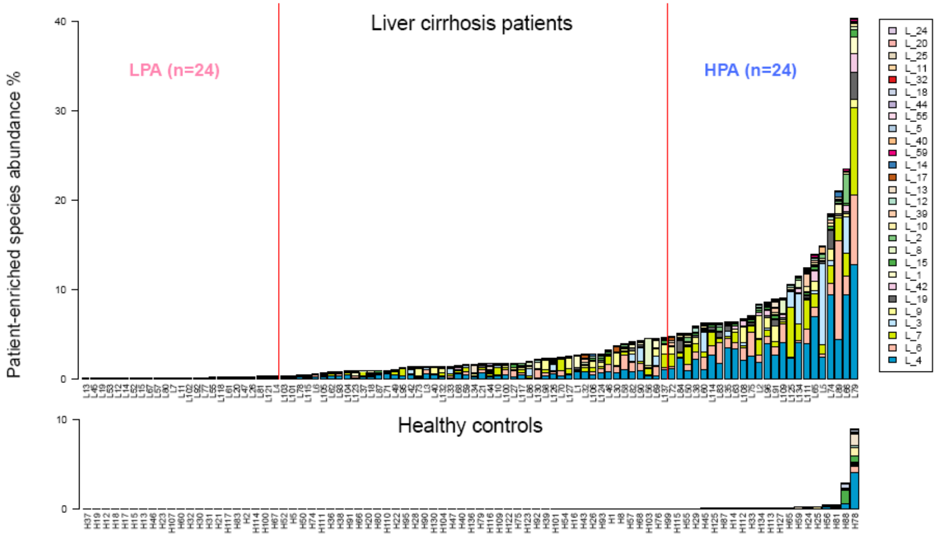
The invaders - targets for intervention?

NH_3 ↗

Mn^{2+} ↗

GABA ↗

Possible role in encephalopathy



Invasion of the gut by bacterial species rare in health: up to 40% of abundance!

"patients with higher MELD scores presented poorer dental health than those with lower scores". Helenius-Hietala et al. Transplant International 25, 158-165 (2012)

Qin N. et al. Nature 2014, doi: 10.1038/nature13568.

Zhejiang University, Hangzhou, China & MGP, Jouy en Josas, France

metagenopolis mgps.eu

QJM AN INTERNATIONAL JOURNAL OF MEDICINE

Current concepts in the assessment and treatment of Hepatic Encephalopathy

W.J. Cash, P. McConville , E. McDermott, P.A. McCormick, M.E. Callender, N.I. McDougall, 2009. DOI: *http://dx.doi.org/10.1093/qjmed/hcp152*

Pathophysiology – impacted by the microbiome?

✓The ammonia theory
✓GABA/benzodiazepine receptor complex theory
✓Manganese theory

Treatments – impact the microbiome?

✓Oral laxatives
✓Enemas
✓Antibiotics

Novel treatments to improve the microbiome more permanently - FMT?

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”

Advent of less healthy/toxic microbiome may be triggered by many factors

Loss of barrier scenario in liver cirrhosis

➢Trigger:

- Virus infection, alcohol, obesity, autoimmunity…

➢Barrier fall:

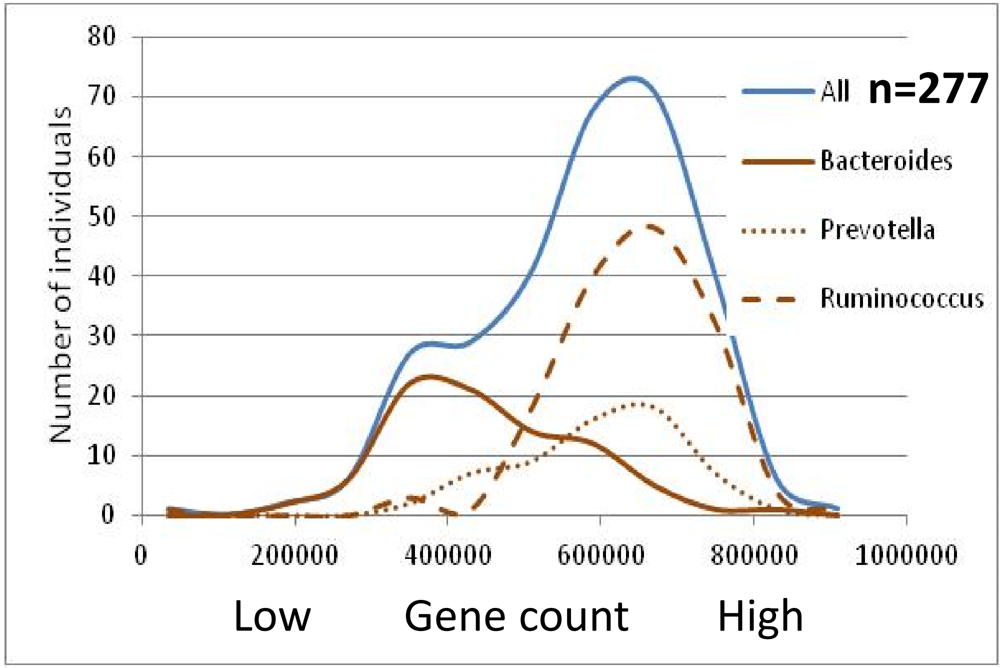
- Impaired bile production

➢Result:

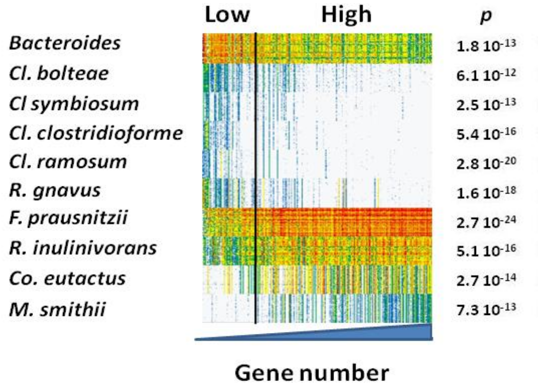
- Invasion of the gut by oral bacteria and food-borne pathogens that impact ammoniac, manganese and GABA metabolism and contribute to hepatic encephalopathy

Risk detection / prediction

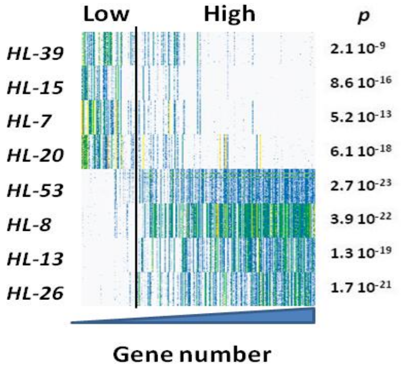
High and low gene count people



Low gene count individuals **(1/4)** 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**

Le Chatelier et al. Nature 2013, doi: 10.1038/nature12506.

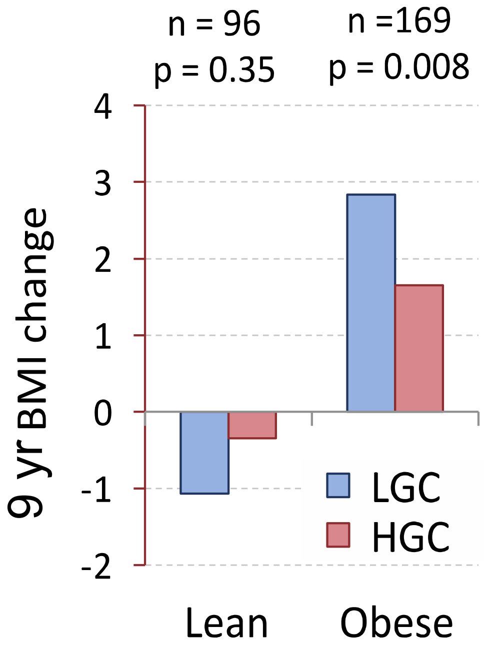
Low bacterial richness – a risk phenotype?

	LGC	**HGC**	***p***	***q***
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

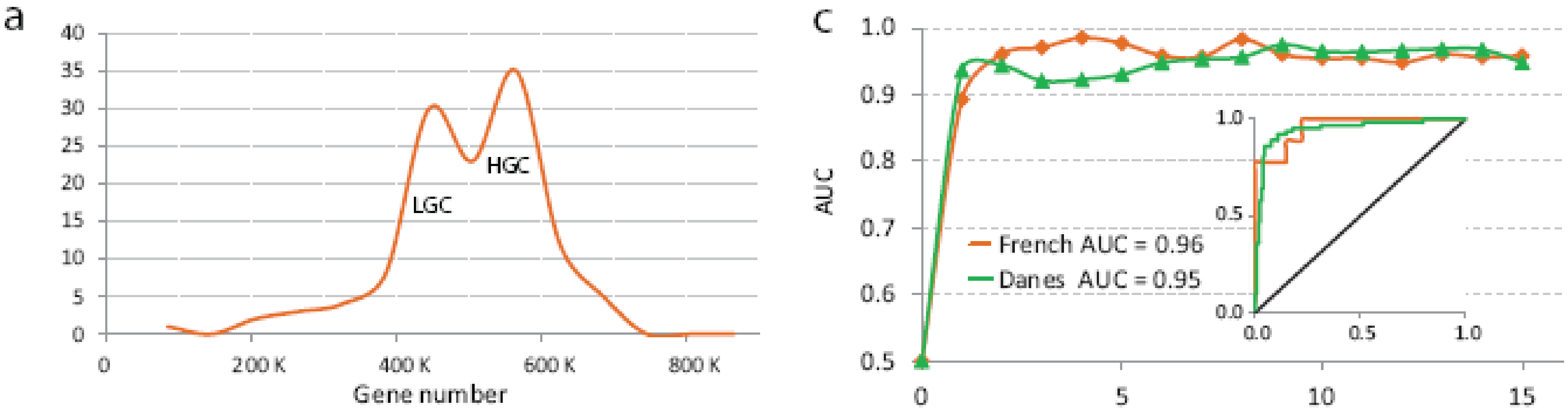
higher risk for type 2 diabetes, cardio-vascular & hepatic complications

Microbiome-poor obese Danes gain more weight



Le Chatelier et al. Nature 2013, doi: 10.1038/nature12506.

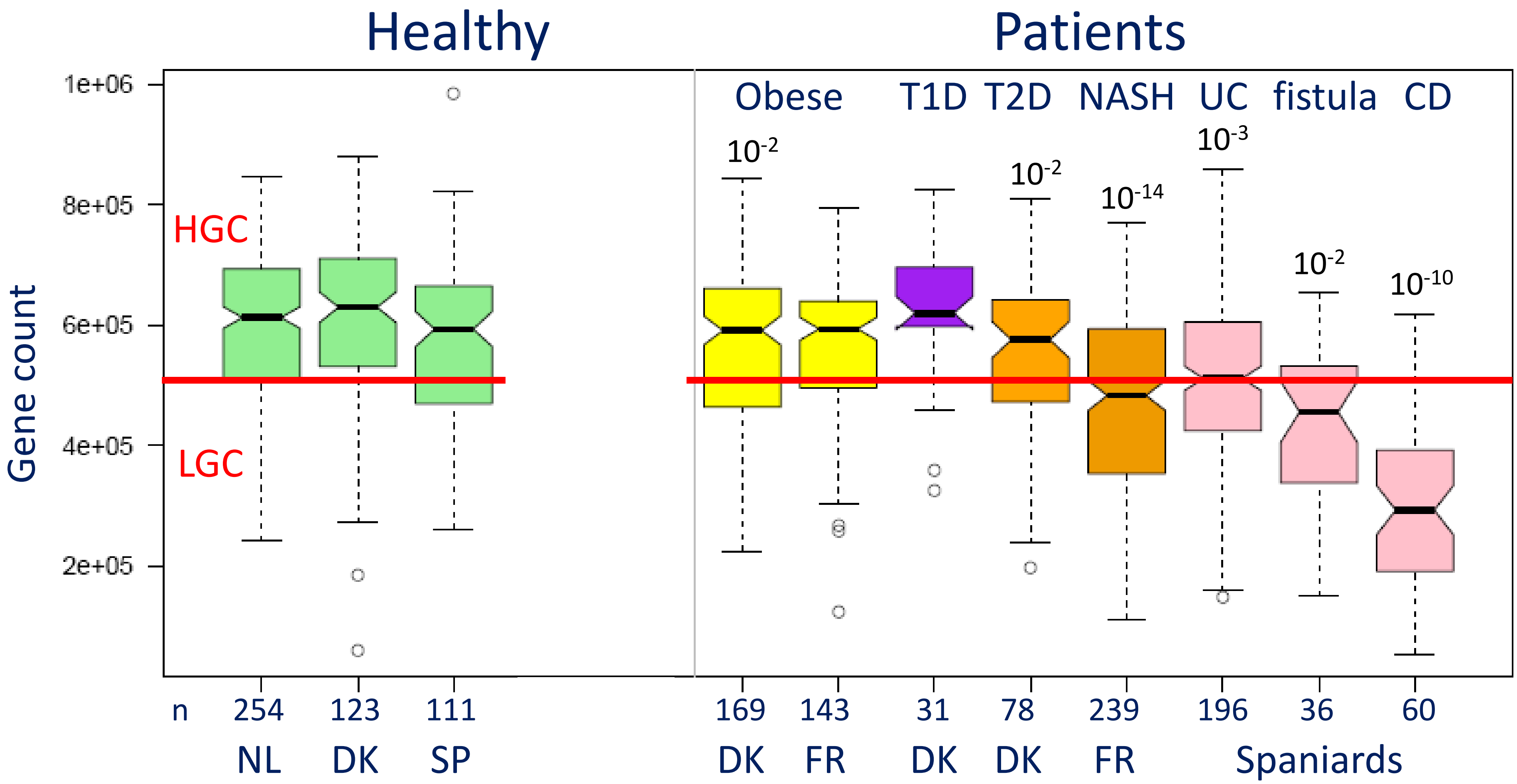
Similar microbial and metabolic/inflammatory profiles in French (n=49) and Danes (n=292)



6 MGS identify at-risk individuals that are microbe-poor with 95% accuracy

Cotillard et al. Nature 2013, doi: 10.1038/nature12480.39

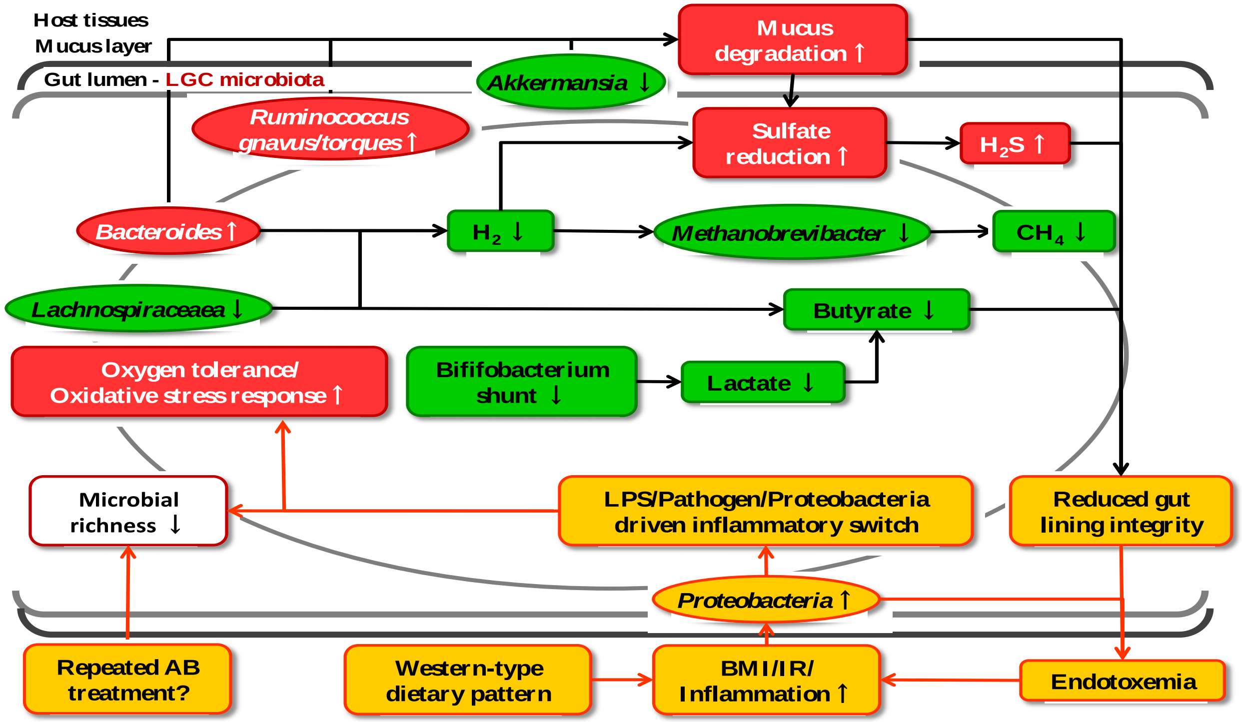
Gut gene richness in health & disease, n=1400



Atrophy of the neglected organ in some diseases

Emmanuelle Le Chatelier, Edi Prifti et al.

Microbe-poor gut microbiome is less healthy



Host tissues
Mucus layer
Gut lumen - LGC microbiota

Mucus degradation ↑

Akkermansia ↓

Ruminococcus gnavus/torques ↑

Sulfate reduction ↑

H_2S ↑

Bacteroides ↑

H_2 ↓

Methanobrevibacter ↓

CH_4 ↓

Lachnospiraceaea ↓

Butyrate ↓

Oxygen tolerance/ Oxidative stress response ↑

Bififobacterium shunt ↓

Lactate ↓

Microbial richness ↓

LPS/Pathogen/Proteobacteria driven inflammatory switch

Reduced gut lining integrity

Proteobacteria ↑

Repeated AB treatment?

Western-type dietary pattern

BMI/IR/ Inflammation ↑

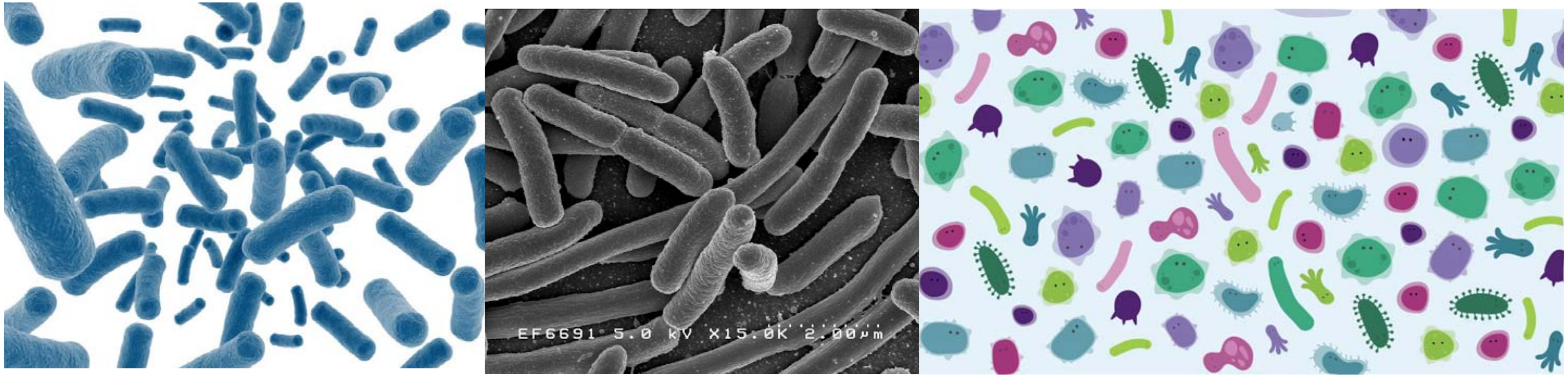
Endotoxemia

Low butyrate, high LPS, high H_2S

Le Chatelier et al. Nature 2013, doi: 10.1038/nature12506.

Microbiome richness is associated with health and well-being

It is better to be rich than poor



We need more gut bacteria!

Integration of clinical phenotypes, microbiome and metabolome data reveals microbial species important for a disease

Pedersen et al. Nature 2016, 535, 376-381.

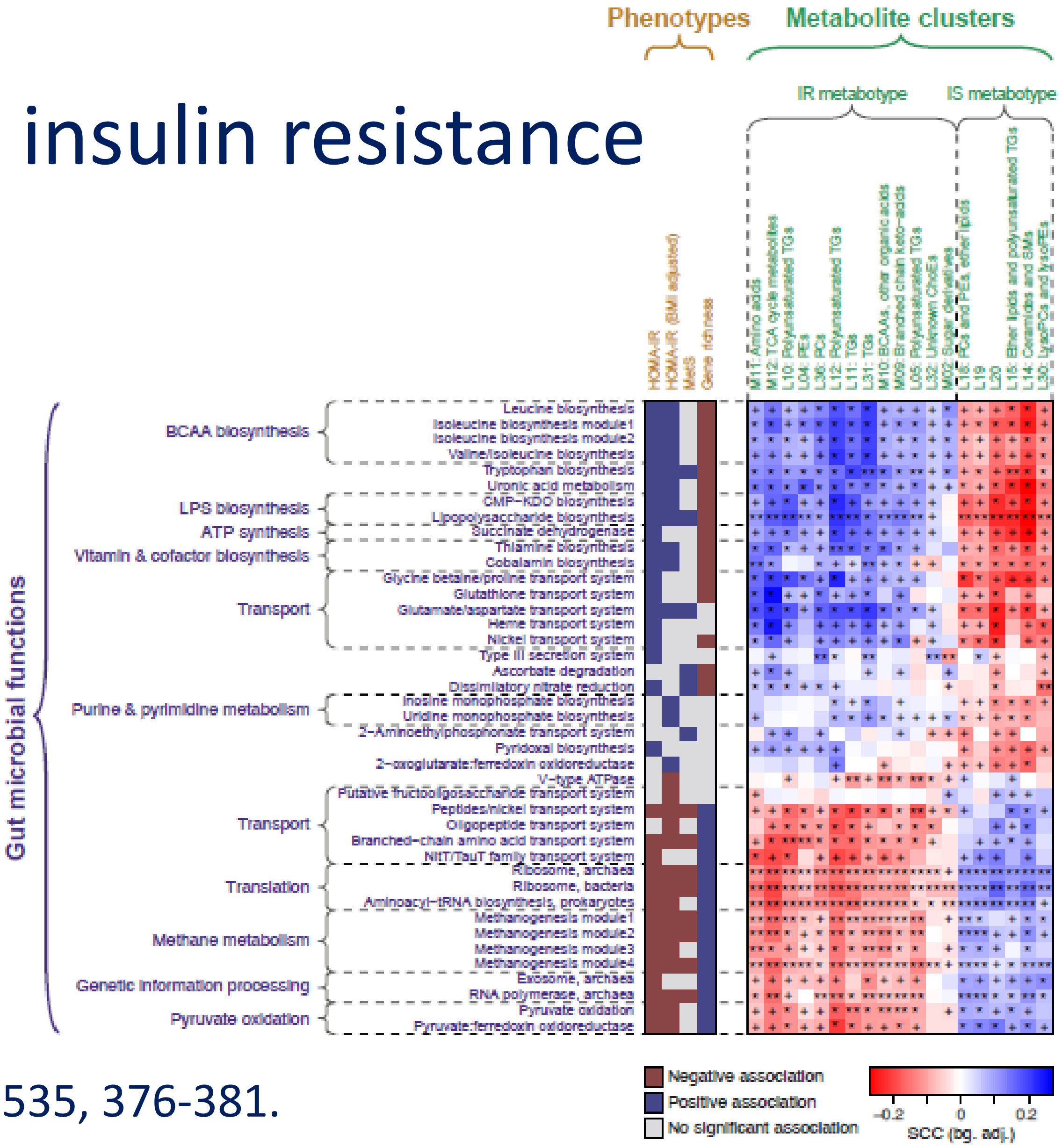
Microbiome and insulin resistance

- ➢277 non-diabetic individuals
- ➢75 T2D patients

The IR-associated metabolome was associated with the gut microbiome-encoded functions:

- ➢Higher potential for LPS and BCAA biosynthesis
- ➢Reduced potential for BCAA transport into bacterial cells

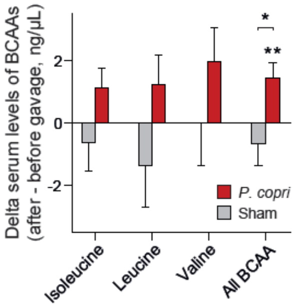
Pedersen et al. Nature 2016, 535, 376-381.



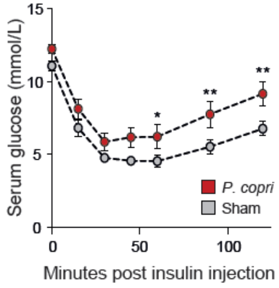
A few species drive the association

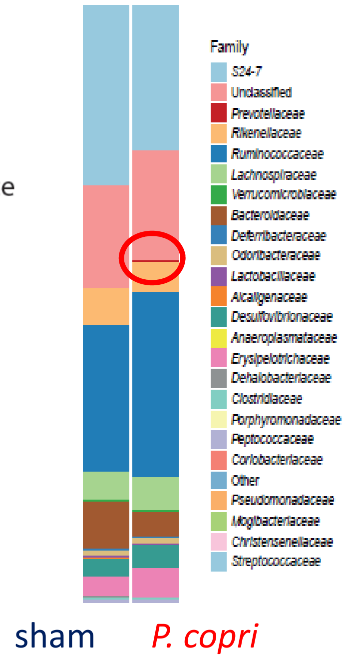
Positive correlations between microbial functions and IR are largely driven by a few species, notably *Prevotella copri* and *Bacteroides vulgatus*, suggesting that they may directly impact host metabolism. We tested this hypothesis in mice on a high-fat diet, and found that a challenge with *P. copri* led to increased circulating serum levels of BCAAs and insulin resistance.

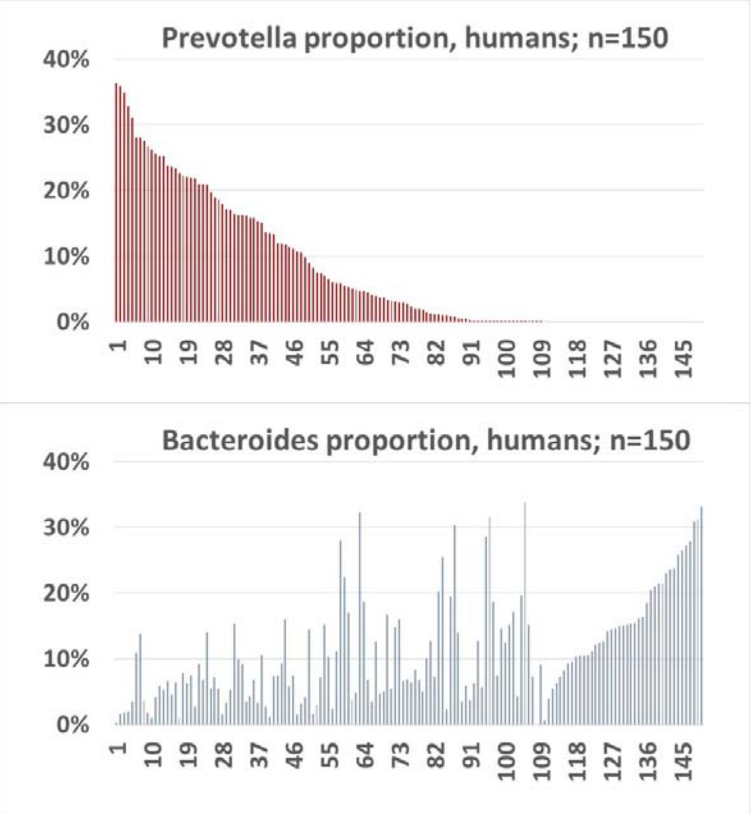
Serum BCAA levels are increased in *P. copri* compared to Sham gavaged mice



Insulin sensitivity is diminished in *P. copri* compared to Sham gavaged mice







Pedersen et al. Nature 2016, 535, 376-381.

Dysbiosis of the human gut microbiota impacts the serum metabolome and contributes to insulin resistance.

Pedersen et al. Nature 2016, 535, 376-381.

Effect of drugs – the case of type 2 diabetes

Forslund et al. 2015, Nature, 528:262-6. doi: 10.1038/nature15766

The drug profile in Danish patients with type 2 diabetes

Blood glucose lowering

- 77 % metformin
- 13 % sulfonylurea
- 19 % insulin
- 15 % dipeptidyl peptidase-4 (DPP4) inhibitors or glucagon-like peptide-1

Blood pressure lowering

- 73 %

Blood lipid lowering

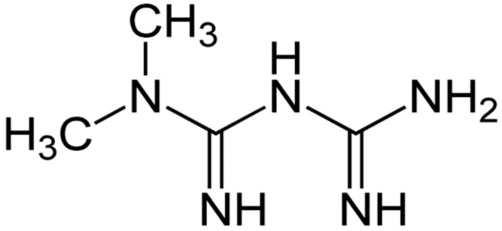
- 75 %

Blood platelet anti-aggregation

- 30%

However, therapy-attributable microbiome variability could be explained by metformin treatment status only

Forslund et al. 2015, Nature, 528:262-6. doi: 10.1038/nature15766



Metformin is the first-line drug in treatment of elevated blood glucose in type 2 diabetes

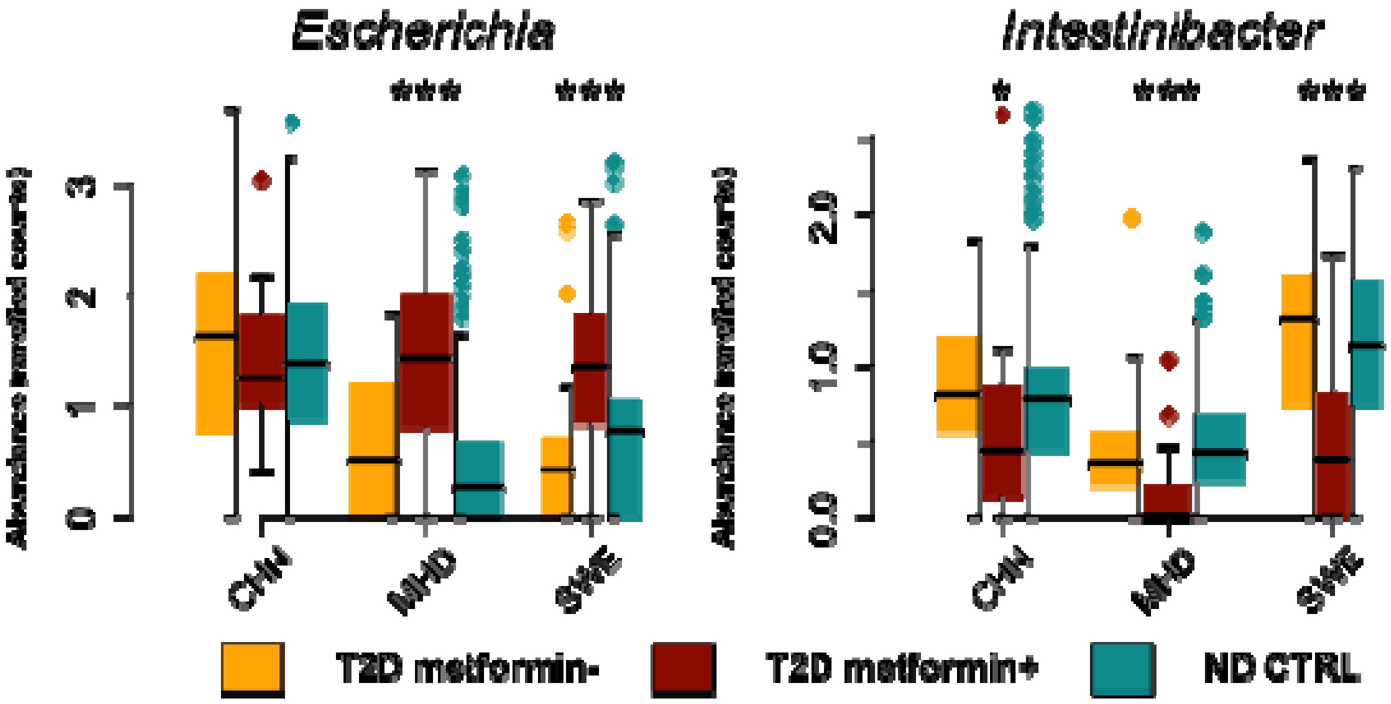
The dominant **effect** of metformin is likely an inhibition of liver gluconeogenesis
It is known for **adverse effects** including gastrointestinal pain, bloating, nausea and meteorism

Does the gut microbiome adapt to drug treatment?

The 199 type 2 diabetes patients were split into:

- **93 type 2 diabetes patients treated with metformin**
- **106 metformin-naive type 2 diabetes patients**

Metformin treatment was associated with a **reduced** ***Intestinibacter*** abundance across Danish, Chinese and Swedish samples and an **increased Escherichia** abundance in Danish and Swedish samples

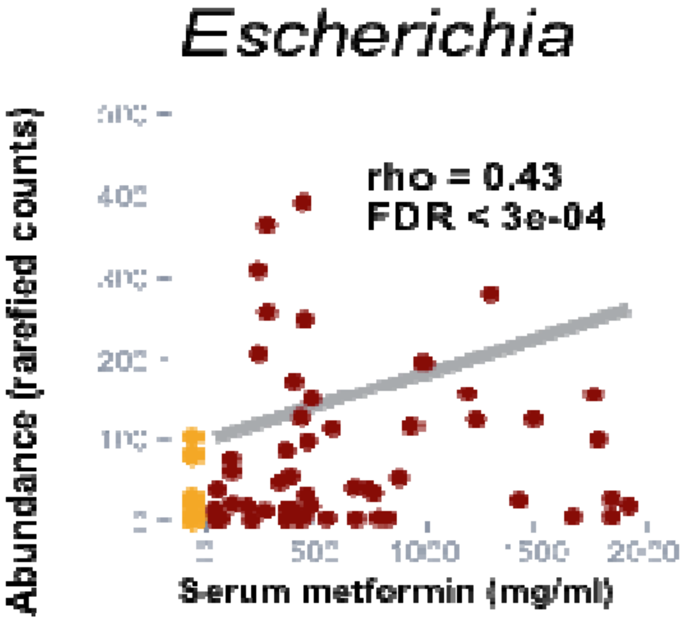


Functional annotations of *Intestinibacter bartletii* genome indicate resistance to oxidative stress and ability to degrade fucose, suggesting involvement in mucus degradation.

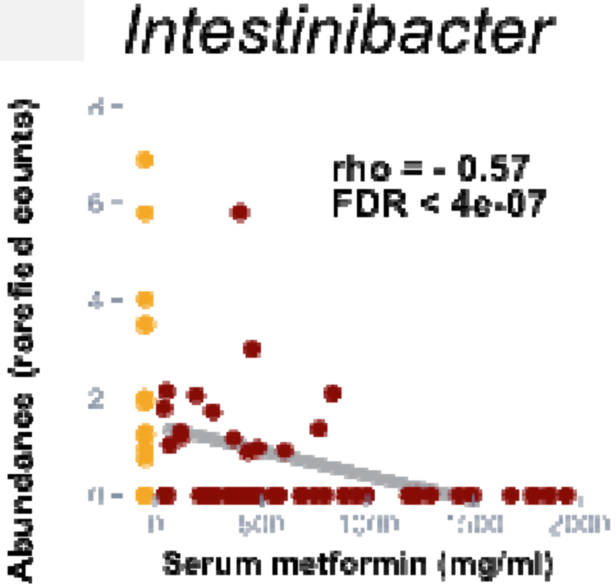
Forslund et al. 2015, Nature, 528:262-6. doi: 10.1038/nature15766

Serum metformin levels of metformin-treated T2D patients correlated positively with *Escherichia* abundance and negatively with *Intestinibacter* abundance

These metformin-induced changes might derive from taxon-specific resistance/sensitivity to the known **bacteriostatic properties of metformin**



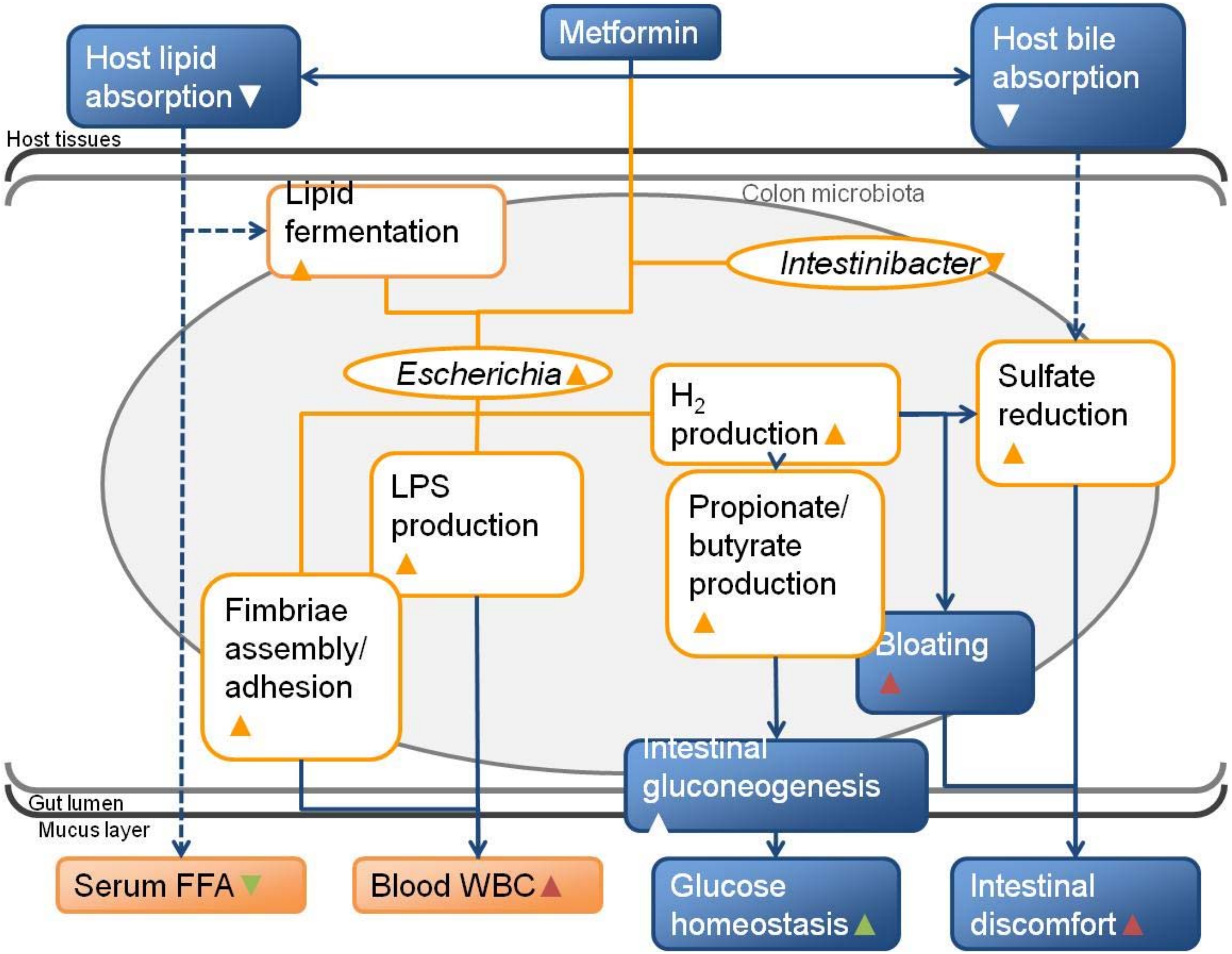
Escherichia



Intestinibacter

Forslund et al. 2015, Nature, 528:262-6. doi: 10.1038/nature15766

Mixed blessings of metformin



- improved glucose homeostasis *via* enhanced gut gluconeogenesis
- bloating and intestinal discomfort *via* increased hydrogen production and sulfate reduction

Forslund et al. 2015, Nature, 528:262-6. doi: 10.1038/nature15766

metagenopolis mgps.eu

Impact of drugs on microbiome should be considered

- when dissecting disease signatures
- when developing diagnostic/prognostic tools
- when testing for phenotype transferability in gnotobiotic mice experiments

Forslund et al. 2015, Nature, 528:262-6. doi: 10.1038/nature15766

Is microbiome alteration a cause, a consequence or a contribution to a chronic disease?

Contribution of the microbiome to the disease – two examples

- Low richness gut microbiome may be less healthy
  - Low butyrate producers (gut health)
  - Abundant pro-inflammatory species (systemic inflammation)
- Liver cirrhosis gut microbiome may be toxic
  - Ammoniac, manganese, GABA (encephalopathy)

Microbiome restoration

- ➢Diet, nutritional interventions
- ➢Molecules
  - Promoters of “good” species (prebiotics, fibers)
  - Inhibitors of “bad” species (narrow spectrum AB, bacteriocins, bacteriophages)
- ➢Microbes
  - Probiotics
  - Communities
  - Transplantation

We should strive to restore or preserve health by modulating unhealthy/toxic microbiome…

…while attempting to unravel the mechanisms which underlie its advent and its effects on our bodies

Impact of human microbiome research on public health

A tremendous potential of human microbiome

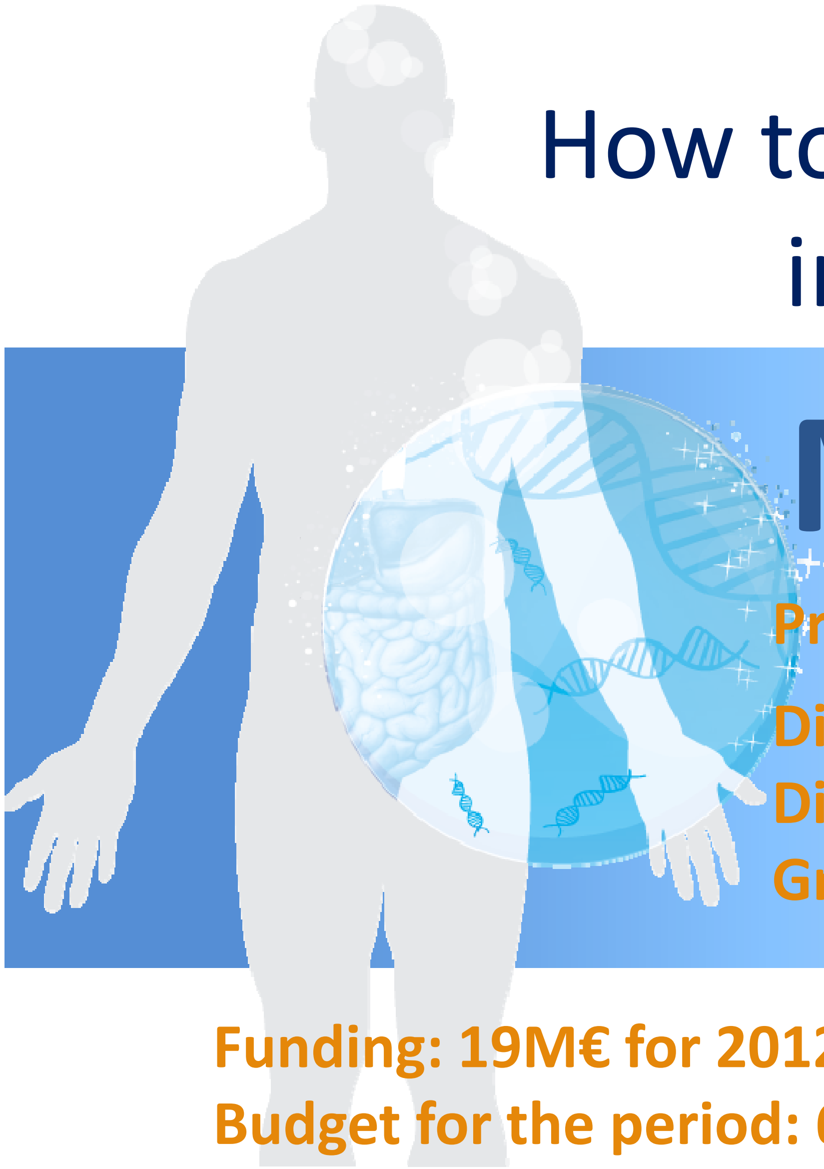
- In diagnostics
- In prognostics
- In patient monitoring
- As target for modulation to improve health

Could help us to better preserve health and better treat the disease

And thus save untold resources & human suffering

metagenopolis
mgps.eu

How to introduce microbiome into public health?



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: 60+ M€

ICAN CLINICAL STUDIES

QUANTITATIVE METAGENOMICS

METAQUANT

HIGH THROUGHPUT SEQUENCING

Composition of microbial communities

FUNCTIONAL METAGENOMICS

METAFUN

FUNCTIONAL METAGENOMICS

Bacteria / cell cross talk

SAMBO

SAMPLE BIOBANKING

Specifications, collection, aliquoting, storage & DNA extraction

IT management, developments & data interpretation

BIG DATA

INFOBIOSTAT

SOCA - ETHICS

ICAN
TOGETHER

INRA
SCIENCE & IMPACT

Université
Catholique
de Lyon

Landmark human microbiome papers

- **60+ publications on quantitative & functional Metagenomics**

2010 : Qin *et al.* Nature , The human gut reference catalogue
2011 : Arumugam et al. Nature, Enterotypes
2012 : Qin *et al*. Nature, Type II Diabetes
2013 : Cotillard *et al.* Nature, Impact of diet on gut microbiome
2013 : Le Chatelier *et al*. Nature, Richness of gut microbes and metabolic markers
2013 : Sunagawa *et al*. Nature Methods, Universal phylogenetic markers
2014 : Nielsen *et al.* Nature Biotech, Method for identifying metagenomic species
2014 : Li *et al.* Nature Biotech, 10 millions genes reference catalog
2014 : Qin *et al.* Nature, Human gut microbiome alterations in liver cirrhosis
2015 : *Xiao et al.* Nature Biotech, A mouse gut catalogue
2015 : *Qin et al.* Nature, Accurate liver cirrhosis diagnostic,
2015 : *Forslund et al.* Nature, Drug confounders in microbiome analysis
2016 : *Pedersen et al.* Nature, Microbiome & insuline resistance

- **27 patent applications; 19M € of research contracts (54% private sources) since 2012**

- Co-chair of the International Human Microbiome Consortium (2012-2014)
- Co-organizer of the International Human Microbiome Congress since 2010 (2000 participants in 2013)
- Networking with academia, clinics & industry, nationally and internationally

Acknowledgments

MetaHIT Consortium

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

INVESTISSEMENTS D'AVENIR

INRA
SCIENCE & IMPACT

metagenopolis
mgps.eu

S. Dusko EHRLICH
Nicolas PONS
Anne-Sophie ALVAREZ
Magali BERLAND
Franck GAUTHIER
Ndeye GAYE
Julienne HANA
Marie JEAMMET
Emmanuelle LE CHATELIER
Nicolas MAZIERS
Florian PLAZA ONATE
Florence THIRION
Kevin WEISZER

Florence HAIMET
S. Dusko EHRLICH
Joël DORE

Joël DORE
Florence LEVENEZ
Mickael CAMUS
Rémy PERON
Mylène ROBINET
Thierry VANDUYVENBODEN

Hervé BLOTTIERE
Véronique LEJARD
Amélie GOUDET
Aline LETUR

S. Dusko EHRLICH
Nathalie GALLERON
Benoit QUINQUIS
Mélina CORDEAU
Robin MASSEY
Laetitia PASERO

Plateforme commune

Sophie HEBERT
Marine FRAISSANGE
Anthony DOBEZ
Damien TOTY

Merci beaucoup!

And take good care of your microbiome…

www.mgps.eu