The intestinal microbiota and the link with diseases

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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

<table>
<thead>
<tr>
<th>Chronic Disease</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frailty in seniors</td>
<td>Van Tongeren et al., 2005</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>Seksik et al., 2003; Sokol et al., 2006, 2008, 2009</td>
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<tr>
<td>Ulcerative colitis</td>
<td>Sokol et al., 2008; Martinez et al., 2008</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>Vaahтовuo et al., 2008; Scher et al., 2013</td>
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<tr>
<td>Obesity</td>
<td>Ley et al., 2007; Kalliomäki et al., 2008</td>
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<tr>
<td>Type 2 diabetes</td>
<td>Cani and Delzenne, 2009</td>
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<td>Type 1 diabetes</td>
<td>Dessein et al., 2009; Wen et al., 2008</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>Nadal et al., 2007; Collado et al., 2009</td>
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<tr>
<td>Allergy</td>
<td>Kirjavainen et al., 2002; Björkstén, 2009</td>
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<td>Autism</td>
<td>Finegold et al., 2002; Paracho et al., 2005</td>
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<tr>
<td>Colorectal cancer</td>
<td>Mai et al., 2007; Scanlan et al., 2008</td>
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<tr>
<td>Breast cancer</td>
<td>Velicer et al., 2004</td>
</tr>
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<td>HIV</td>
<td>Gori et al., 2008</td>
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<tr>
<td>Cirrhosis</td>
<td>Gunnarsdottir et al. 2003</td>
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<tr>
<td>Atherosclerosis</td>
<td>Wang et al. 2011</td>
</tr>
<tr>
<td>Other…</td>
<td></td>
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</table>
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 ➔ Gene counts

Known genomes ➔ Reference Gene Catalog ➔ Preprocessing / normalization and dimension reduction ➔ Identify relevant microbial players ➔ Relate to human data ➔ Build and test prediction models

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

The prime way to characterize a microbiome
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..

Microbiome assessment

- Faecalibacterium prausnitzii
- Ruminococcus spp
- Clostridium difficile
- Bacteroides dorei
- Escherichia coli

Quantitative metagenomics

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

7 MGS accurately diagnose liver cirrhosis

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

Zhejiang University, Hangzhou, China & MGP, Jouy en Josas, France
Patient monitoring
Microbiome informs on the state of disease

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

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

Discovery cohort: n=181
Validation: n=56

MGS enriched in LC
n=28

MGS enriched in Healthy
n=38

Healthy
n=98 | LC
n=83

Healthy
n=31 | LC
N=25

MELD
CTP

p<1e-5
p<3e-4

LC MGS load
Low High
Low High
Massive microbiome changes in cirrhosis

Low gene richness (p<10 e-10)

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

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

Zhejiang University, Hangzhou, China & MGP, Jouy en Josas, France
Current concepts in the assessment and treatment of Hepatic Encephalopathy


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


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

**Increased adiposity, insulin resistance, dyslipidaemia, inflammation higher risk for type 2 diabetes, cardio-vascular & hepatic complications**

<table>
<thead>
<tr>
<th></th>
<th>LGC</th>
<th>HGC</th>
<th>p</th>
<th>q</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (men/women)</td>
<td>68 (23/45)</td>
<td>224 (113/111)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age Yrs</td>
<td>56 ± 7.5</td>
<td>57 ± 7.3</td>
<td>0.86</td>
<td>0.89</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32 (29 - 34)</td>
<td>30 (23 - 33)</td>
<td><strong>0.035</strong></td>
<td>0.059</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>95 (75 - 100)</td>
<td>86 (71 - 100)</td>
<td><strong>0.019</strong></td>
<td>0.037</td>
</tr>
<tr>
<td>Fat %</td>
<td>37 (29 - 42)</td>
<td>31 (25 - 39)</td>
<td><strong>0.0069</strong></td>
<td>0.022</td>
</tr>
<tr>
<td>S-Insulin (pmol/l)</td>
<td>50 (35 - 91)</td>
<td>44 (26 - 66)</td>
<td><strong>0.0095</strong></td>
<td>0.023</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.9 (1.2 - 3.3)</td>
<td>1.6 (0.9 - 2.6)</td>
<td><strong>0.012</strong></td>
<td>0.027</td>
</tr>
<tr>
<td>p-Triglycerides mmol/l</td>
<td>1.32(0.97 – 1.76)</td>
<td>1.15 (0.82 – 1.57)</td>
<td><strong>0.0014</strong></td>
<td>0.013</td>
</tr>
<tr>
<td>P-Free fatty acids (mmol/l)</td>
<td>0.55 (0.39 - 0.70)</td>
<td>0.48 (0.35 - 0.60)</td>
<td><strong>0.014</strong></td>
<td>0.029</td>
</tr>
<tr>
<td>S-Leptin (µ/l)</td>
<td>17.0 (6.7 – 32.6)</td>
<td>8.3 (3.4 – 26.4)</td>
<td><strong>0.0036</strong></td>
<td>0.019</td>
</tr>
<tr>
<td>S-Adiponectin (mg/l)</td>
<td>7.5 (5.5 – 12.9)</td>
<td>9.6 (6.7 – 13.7)</td>
<td><strong>0.006</strong></td>
<td>0.022</td>
</tr>
<tr>
<td>B-leucocytes (10⁹/l)</td>
<td>6.4 (5.2 - 7.8)</td>
<td>5.6 (4.8 - 6.9)</td>
<td><strong>0.0021</strong></td>
<td>0.014</td>
</tr>
<tr>
<td>B-Lymphocytes (10⁹/l)</td>
<td>2.1 (1.6 - 2.3)</td>
<td>1.8 (1.5 - 2.1)</td>
<td><strong>0.00082</strong></td>
<td>0.012</td>
</tr>
<tr>
<td>P-CRP (mg/l)</td>
<td>2.3 (1.1 - 5.7)</td>
<td>1.4 (0.6 - 2.7)</td>
<td><strong>0.00088</strong></td>
<td>0.012</td>
</tr>
<tr>
<td>S-FIAF (µg/l)</td>
<td>88 (72 - 120)</td>
<td>78 (60 - 100)</td>
<td><strong>0.0047</strong></td>
<td>0.021</td>
</tr>
</tbody>
</table>
Microbiome-poor obese Danes gain more weight

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

Healthy

Patients

Atrophy of the neglected organ in some diseases

Emmanuelle Le Chatelier, Edi Prifti et al.
Microbe-poor gut microbiome is less healthy

Low butyrate, high LPS, high $\text{H}_2\text{S}$

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

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

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.

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

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.

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.

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
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
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€
Landmark human microbiome papers

► 60+ publications on quantitative & functional Metagenomics

2012 : Qin et al. Nature, Type II Diabetes
2013 : Le Chatelier et al. Nature, Richness of gut microbes and metabolic markers
2013 : Sunagawa et al. Nature Methods, Universal phylogenetic markers
2014 : Li et al. Nature Biotech, 10 millions genes reference catalog
2015 : Xiao et al. Nature Biotech, A mouse gut catalogue
2015 : Qin et al. Nature, Accurate liver cirrhosis diagnostic,

► 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

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Merci beaucoup!
And take good care of your microbiome...

www.mgps.eu