The human gut microbiome and links to metabolic pathologies

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The supra-organism and the crowd of questions

- Which microbes?
- What are they doing?
- How does environment affect the microbiome?
- Which host genes do control the microbes?
- Which host genes respond to microbes and their products?

Microbiome

Host genome

Microbial components
Host components

Interaction

Health or Disease
Our Other Genome: *The human gut microbiome*

Discovery of 3.3 million microbial genes from distal gut of 86 Danes and 38 Spaniards

- 1,100 abundant bacterial species
- Largely unknown species
- Each individual harbors ~ 160 abundant gut bacterial species


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A human gut microbial gene catalogue established by metagenomic sequencing
The year 2014 updated reference catalog

10 mio non-redundant gut microbial genes

Rare genes are increasing

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

MetaHIT, Chinese and Human Microbiome Project studies, n=1267

Nature Biotechnology 32: 834-841, 2014)
How to make sense of the millions of microbial genes?

- About 10% of microbial genes can be mapped to known bacterial genomes.

- Since the majority of genes cannot be mapped to any known bacterial genome, bioinformatics methods based upon co-abundance were developed to enable grouping of microbial genes into **741 large MetaGenomic Units (MGU’s)** to represent yet unknown bacterial species.

- Each MGU contains >700 microbial genes.

Recent progress in ‘scanning’ the gut ecosystem

1. **Sample collection**
   - Stool sample

2. **Sequencing**
   - QC
   - Library preparation
   - Sequencing
   - 30-50 million sequences

3. **Reference construction**
   - Known genomes
   - Gene Catalog

4. **Gene profiling**
   - Mapping to gene catalog
   - Gene counts
   - Individuals

5. **Bioinformatics & statistics analyses**
   - Preprocessing / normalization and dimension reduction
   - Test of biomarkers
   - Relate to human data
   - Identify relevant microbial units

**Bioinformatics & statistics analyses**

- **Analyses**
  - Bioinformatics
  - Statistics
  - Preprocessing
  - Normalization
  - Dimension reduction

- **Identify relevant microbial units**

- **Test of biomarkers**

- **Relate to human data**
Is a rich gut flora associated with metabolic health?
Richness of human gut microbiome correlates with metabolic markers

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A bimodal distribution of gut bacterial gene abundance splitting 292 Danish people into low gene richness (LR) and high gene richness (HR) individuals.

**Low richness individuals:** 380,000 genes

**High richness individuals:** 640,000 genes

**Graph Details:**
- **LR:** 380,000 genes
- **HR:** 640,000 genes
- **480,000 genes**
Low-richness (LR) people have higher abundance of known pro-inflammatory species

- **LR** group had higher prevalence of 5 potentially pro-inflammatory bacteria including *Bacteroides* and several species of the *Clostridium* genus and *Ru. gnavus*

- **HR** group had a higher prevalence of 4 anti-inflammatory species including *F. prausnitzii*, *Ro. inulinivorans*, *C. eutactus* and the main human methanogen, *M. smithii*
Low-richness people have more body fat and are insulin resistant.
**Low-richness** individuals have elevated markers of low-grade inflammation and blood lipids.
Public health implications of low gut bacterial richness

Over 9 years low-richness obese people gain more weight (3 kg)

Nature 500; 541-546, 2013
High-richness (HR) people have increased potential for production of organic acids - including lactate, propionate and butyrate.
• One in four has on average a 40% reduction in gut microbial genes

• These people are featured by reduced gut bacterial richness and altered composition and function of the remaining gut microbiota

• Low-richness people show increased adiposity, insulin resistance and low-grade inflammation

• These individuals may be at increased risk of cardio-metabolic disorders

Nature 500; 541-546, 2013
Gut microbiome biomarker discovery

Search for bacterial signatures that discriminate low versus high richness individuals
Receiver Operator Characteristics (ROC) analysis shows that 4 gut bacterial Metagnomics Units discriminate between low richness and high richness individuals.
What is the cause(s) of the missing gut bacteria?
Known regulators of the gut flora

- Host genetics
- Antibiotics
- Diet
Dysbiosis of the gut flora – an ecological perturbation

Not a pathogene infection!

Can a narrow gut flora be restored?
Dietary intervention impact on gut microbial gene richness

Aurélie Cotillard¹,², Sean P. Kennedy³*, Ling Chun Kong¹,²,⁴*, Edi Prifti¹,²,³*, Nicolas Pons³*, Emmanuelle Le Chatelier³, Mathieu Almeida³, Benoît Quinquis³, Florence Levenez³,⁵, Nathalie Galleron³, Sophie Gougis⁴, Salwa Rizkalla¹,²,⁴, Jean–Michel Batto³,⁵, Pierre Renault⁵, ANR MicroObes consortium†, Joel Doré³,⁵, Jean–Daniel Zucker¹,²,⁶, Karine Clément¹,²,⁴ & Stanislav Dusko Ehrlich³
A low-energy, low-fat dieting improves simultaneously microbial gene richness and risk metabolic phenotypes, albeit not fully.

Nature 2013;500:585-8
A ‘narrow’ gut flora is common and appears to be less healthy. Diet intervention can partially correct it and possibly alleviate the risk. This could have a major impact on public health.
Beyond the concept of ‘richness individuals’………

Can gut bacteria discriminate between lean and obese individuals?
18 MetaGenomic Units discriminate between lean and obese individuals
Are Under the Curve: 0.96
Human gut bacteria biomarkers have tremendous discriminatory power in several chronic diseases. Their usefulness in many more such diseases should be vigorously explored.
Does the gut flora adapt to drug treatment?
Recent studies of the gut microbiome in type 2 diabetes based upon shot-gun sequencing and quantitative metagenomics

A Chinese study of 171 T2D patients and 174 control subjects  

A Swedish study of 140 elderly women with T2D, impaired glucose regulation and normal glucose tolerance  

A MetaHit study of T2D evaluating the impact of treatment on gut microbiome
The gut bacteria of Type 2 Diabetes

More pro-inflammatory species

A decrease in butyrate producing bacteria

More opportunistic pathogens
Metformin is the first-line drug in treatment of elevated blood glucose in type 2 diabetes. It is known for its side effects:

- Gastrointestinal pain, bloating, meteorism, etc
- Vitamin B12 deficiency

Does the gut bacteria adapt to drug treatment and in this case cause side effects?
The antidiabetic drug metformin has a major impact on gut bacterial composition in patients with type 2 diabetes.
The antidiabetic drug metformin impacts **gut bacterial function** in patients with type 2 diabetes pointing to an explanation of the known gastrointestinal side effects.
• Focus on human **pregnancy** and **early life** to learn lessons about opportunities for **disease prevention**

• **Prospective quantitative metagenomics** in large cohorts of well-phenotyped and initially treatment-naive cases

• **Intervention** studies

• **Integration of microbiomics** with outcomes of complementary omics: genomics, epigenomics, metabolomics, lipidomics and peptidomics of the host and meta-transcriptomics and meta-proteomics of the microbiota

• **Mechanistic** studies in rodents, including stool transplantations from discordant twins

• **In vitro functional** studies
Virus as a part of the gut microbiota

The human gut virome in health and disease?
Fungi as a part of the gut microbiota

The human gut mycome in health and disease?
PERSPECTIVES - Gut Bacterio-Therapy

- Combinations of complexes of hundreds of probiotic bacterial species and a ‘healthy diet’ with specific disease therapeutic effects or general health sustaining effects

- Bioactive bacterial compounds with therapeutic or disease preventive effects
Incorporation of modified bacteria that express therapeutic factors into the gut microbiota

$N$-acyl-phosphatidyl-ethanolamines (NAPEs) are precursors to the $N$-acyl-ethanolamide (NAE) family of lipids, which are synthesized in the small intestine in response to feeding and reduce food intake and obesity.

Administration of engineered NAPE-expressing $E. \text{coli}$ Nissle 1917 bacteria in drinking water for 8 weeks reduced the levels of obesity in mice fed a high-fat diet.

Treatment with pNAPE-EcN, but not pEcN, inhibits gain in body weight and adiposity

Effects of pNAPE-EcN persist for more than 6 weeks after ending administration.

Gut microbiota-based intervention – CHALLENGES

• Proof that an altered gut microbiota is causative for a given specific disorder
• Proof that a TEST gut bacterial community or a TEST bacterial bioactive compound has the desired therapeutic effect
• Need of novel preclinical models to deconstruct mechanisms, stability, safety etc
• Need of novel surrogate clinical endpoints, longterm efficacy, etc…….
• Can naturally occurring bacteria and their compounds be patented and commercialized?
The gut microbiota and human biology

It is early days and "a science in making"

Are we heading towards a medical revolution?

OR

Are we dealing with a science with too much trust in GUT?

Will it turn out that the gut microbiota only plays a marginal role in man’s part of the integrated supraorganism?

The answers are unknown –

.........blowing in the wind.............
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Metagenomics of the Human Intestinal Tract
MetaHIT
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