Le Microbiote Intestinal

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Le microbiote intestinal:

Microbiota acquisition and early development

Microbiota composition – a metagenomics view

Dysbiosis – towards risk biomarkers

Microbiota functions – towards new bioactives
Microbiota acquisition and early development

• Intestinal colonization is affected by:
  – Gestational term
  – Mode of delivery (vaginal or Cesarean)
  – Hygiene of neonatal environment
  – Maternal microbiomes and maternal nutrition
  – Food & feeding mode (breast milk vs formula; weaning)

• Early colonisation and hygiene hypothesis:
  early exposure to **low microbial diversity** would prevent or delay maturation of the mucosal immune system and thereby promote aberrant responses to allergens or autoantigens and development of associated diseases

Is species richness affected by long term changes?

Impact, over generations, of:

- Nutritional transition?
- Repeated antibiotherapy?
- …

Yatsunenko et al. Nature 2012
Microbiota composition – a metagenomics view

The metagenome, made of the combined genomes of all dominant microbes within a given ecosystem

DNA extraction → Metagenomic DNA → Whole Genome shotgun sequencing → Assembly and annotation → Reference gene catalog and gene counts
Human microbiomes differ by gut bacterial genes, species and enterotypes

Reference gene catalogs:
- 3.3 million bacterial genes/ 124 subjects
- 10 million bacterial genes/1267 subjects
- 500,000 bacterial genes/individual

Comparable gene catalog for Europeans, Americans, Japanese, Chinese
- 50% bacterial genes of each microbiome shared by >50% surrounding individuals: metagenomic core

Qin et al, Nature 2010
Arumugam et al, Nature 2011

‘Density plots’ for ~400 individuals
- ecological landscape

Impact of diet
Wu Science 2012

Bacteroides
Prevotella
Ruminococcus

Scheffer, Nature 2001
Human microbiomes differ by gut bacterial gene counts

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

Microbiome: source of biomarkers for stratification and monitoring
Chronic immune diseases associated with dysbiosis of the 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
Pouchitis  Lim et al., 2009, Kühbacher et al., 2006
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
Cœliac 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
Cancer colorectal  Mai et al., 2007; Scanlan et al., 2008
Cardiovascular  Wang et al. 2011
others....

Prevention = risk detection + risk alleviation
Metagenomic signatures of dysbiosis

inflammatory bowel diseases

and obesity

Diagnostic genes and genomes are specific of the microbiome of patients
Microbiome biomarkers are more discriminant than clinical assessment and the human genome in Type 2 diabetes

ROC analysis:

AUC = 0.84

Host genetic markers from
GWAS AUC=0.6
Metagenomic signatures of risk: diversity is a key stratifier?

Low gene count individuals (low bacterial richness, 23%) have less healthy prognosis.

In obesity (O. Pedersen & K. Clément), with:
• less healthy metabolic & inflammatory traits
• higher weight gain over the past 10 years
• more biomarkers of risk of aggravation and comorbidities
• worse response to a calories-restricted diet

In Ulcerative Colitis (F. Guarner), with:
• higher relapse rate of chronic acute phases
• non-responders to probiotic-induced microbiota stabilization

Note: Low bacterial gene count microbiomes are enriched in Bacteroides enterotype and often lack core-species of dominant firmicutes.

Prognostic genes and genomes are biomarkers of risks of comorbidities
A low gene count microbiome predicts a worse response to nutritional intervention.

A low diversity microbiome is predictive of a worse response to calorie-restriction; especially obesity-associated inflammation.

**intervention**

- 1200-1500 Kcal
- Low fat, high protein diet, rich in low glycemic index sugars;
- In diverse plant-based dietary fibres.
Microbiota functions – towards new bioactives

The metagenome; a window to unexplored microbiota functions – acting at interfaces:

- Metabolism (food-microbe)
- Mucosal barrier integrity & beyond (cell-microbe)
  - Mucus; Tight junctions; Trophicity of epithelium and cell renewal
  - Immuno-modulation; Dendritic cells, Paneth cells and defensins
  - Gut-brain crosstalk
- Barrier against pathogens – colonization prevention (microbe-microbe)
Microbiota functions – food-microbe

- vitamin production (Riboflavin)
- plant polysaccharides degradation (Tasse GenomRes 2010)
- beta-glucuronidase activity (Gloux et al PNAS 2010)
Large homogenous metagenomic DNA library

Metagenomic DNA

Bacterial fraction

DNA extraction

Fosmid Vectors

Cloning

human reporter-cell-lines

Cultured strains & Cultured strain DNA library

Large homogenous metagenomic DNA library

Recombinant clones

clones

genes ➔ molecules..

9 metagenomic libraries :
340 000 clones

screening of bacteria-cell crosstalk
Microbiota functions – microbe-host

Bioactive metagenomic clone F4 protects against Salmonella (FB62)-induced tissue destruction

Tissue with control medium control Ecoli clone F4

without Salmonella
CTRL EPI300 F4

with Salmonella FB62
FB62 EPI300 + FB62 F4 + FB62

Collab. with Maria Rescigno et al, IEO - Milan
Microbiota functions – microbe-microbe

In vivo elimination of *Clostridium perfringens* by *Ruminococcus gnavus* E1

This RumC region is part of a 220 kb ICE; a transferable “colonisation island”
Fons and col. *Crost 2010, 2011; Pujols 2011*
Microbiota as a neglected organ

- Relevant to the push for personalized and digital medicine
- Relevant for health, preventive nutrition and medical applications
  - Predict responders / non-responders (to nutritional supplements or therapeutics)
  - Predict relative risk of disease onset in healthy subjects
  - Predict risk of aggravation and co-morbidities in patients
- Signatures to assist in diagnosis/prognosis and clinical management of patients
- Biomarkers to provide rationale targets and strategies for microbiota modulation
- Bioactives with potential for translation in the clinic
Merci de votre attention

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http://www.gutmicrobiotaforhealth.com/