Healthy gut microbiota and natural variability, stability and resilience – Identification of microbiota covariates and life style effects

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The discovery phase: microbiome association studies open up the promise for novel diagnostics and treatments

Reality check:
We *don't* even know what a *healthy* flora means!

Microbiome state-of-the-art:

MetaHIT, HMP + specific lab studies combined have profiled ±2000 individuals world-wide, still biased cut of the population

Genetics: 10-100.000s individuals

Variation in clinically relevant population = largely unknown
Temporal variation & stability of biomarkers = largely unknown
Factors influencing gut flora composition = largely unknown
Effect of host genetics = largely unknown
Effect environment = largely unknown

Clinical end points for functional foods, pre-/pro/synbiotics, pharma-/nutriceutical interventions etc are *unknown*

Flemish gut flora project: longitudinal study of +-5000 volunteers spread over a confined geographic region

FGFP sample collection

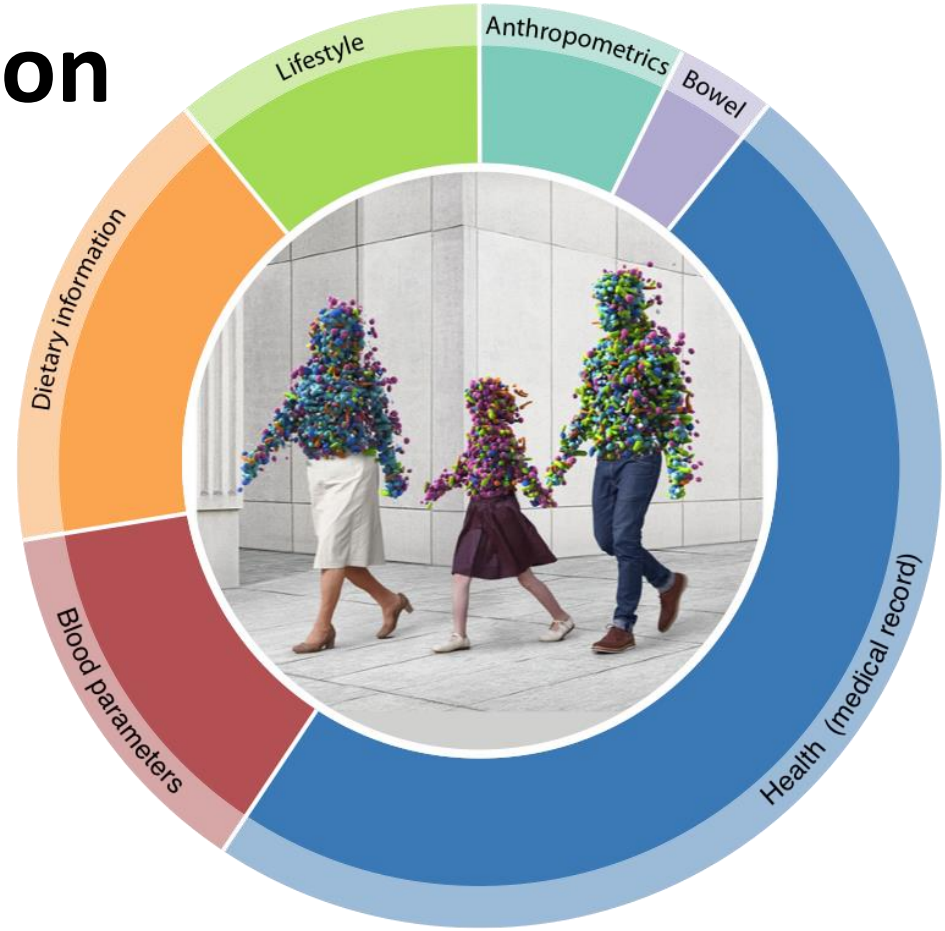
- Collection of faecal, blood (GP) and saliva samples

- Questionnaires:
  - Self-reported health
  - Detailed health (GP)
  - Diet (incl probiotics, drugs)
  - Wellbeing/QoL
  - Hygiene
  - Bowel habit/Bristol scale
  - Travel, Stress etc

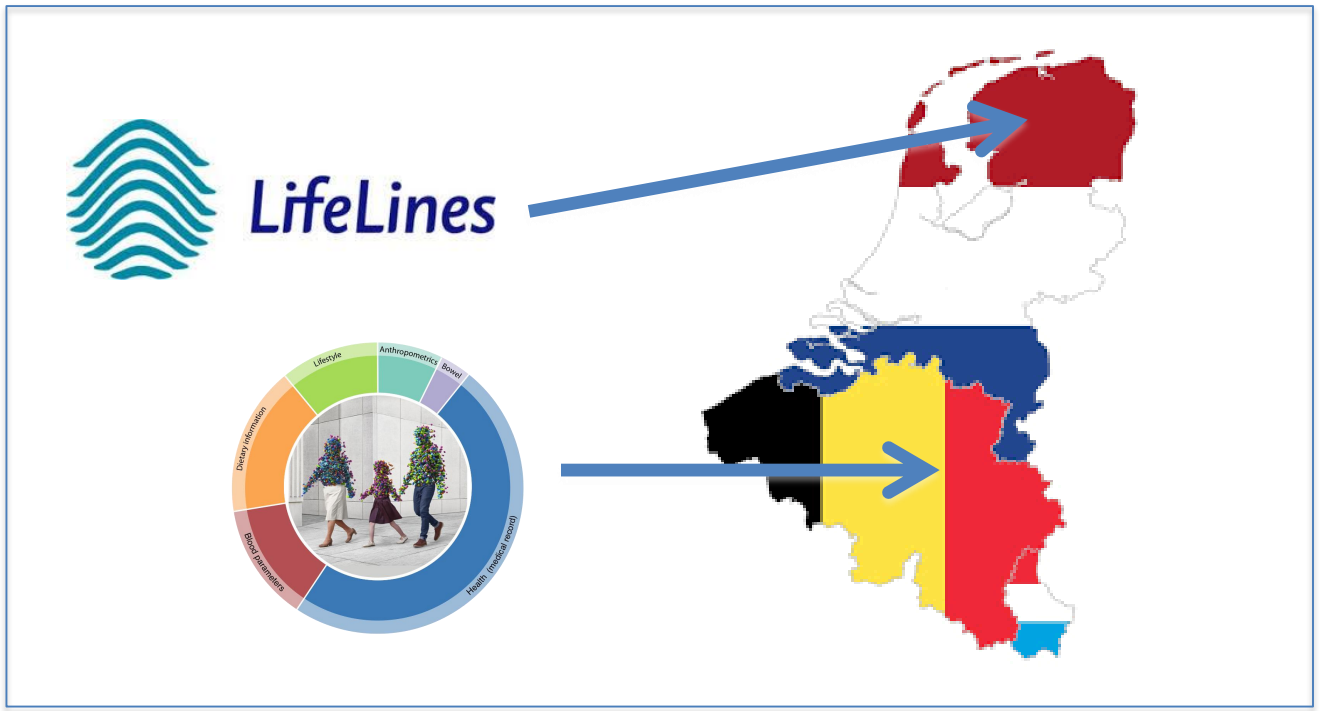
- Blood analysis: metabolic (e.g. glucose, HDL/LDL, triglicerides, insulin,…) and immunological/inflammatory readouts (cell counts, interleukins, CRP,…)

- Secured database, patient encoding

Current status:
3400 sample sets collected



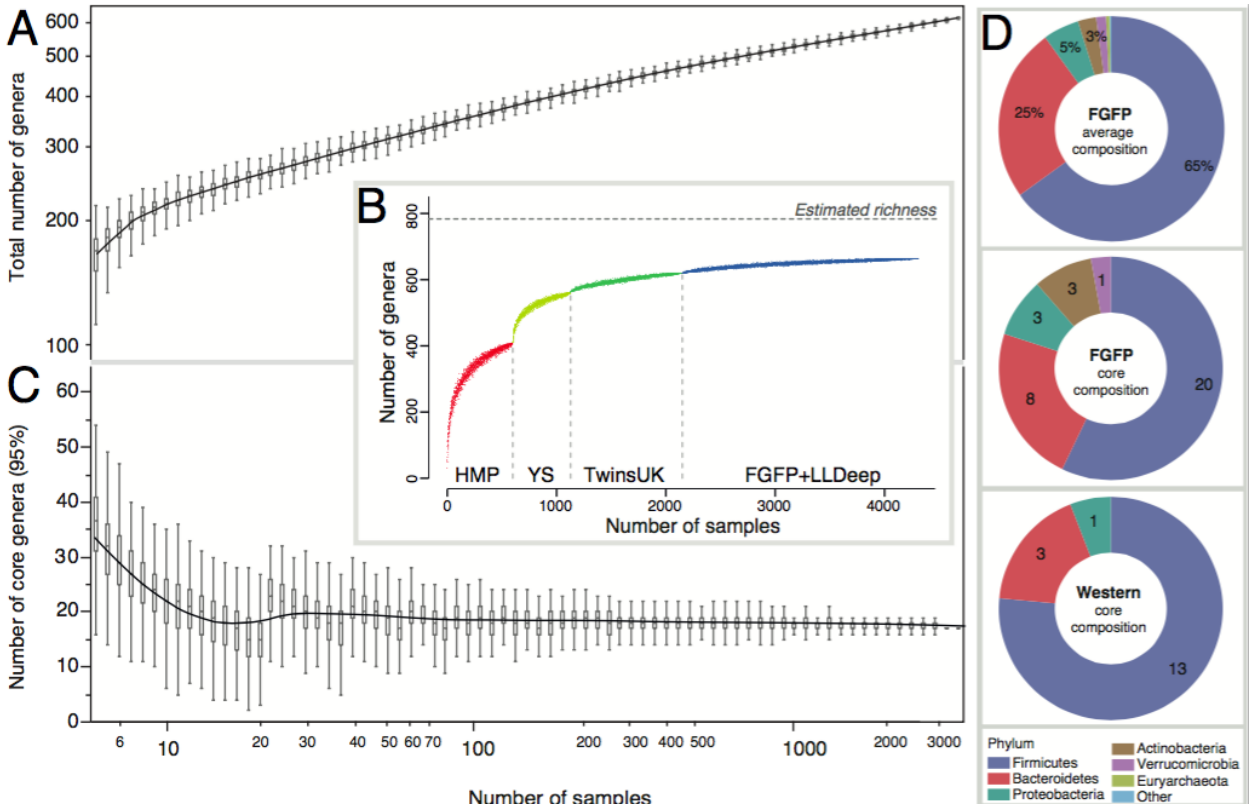
Cross-national collaboration to study population-level variation of the gut microbiota



Discovery cohort: FGFP first data freeze (N=1106)
Replication cohort: Lifelines Deep (Groningen, NL; N=1135)

Falony*, Joossens*, Vieira-Silva*, Wang* et al., **Science** 2016
Zhernakova et al., **Science** 2016

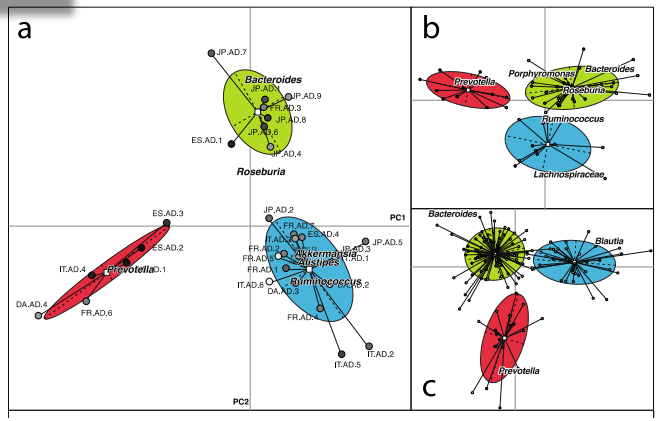
Integration with global datasets (N=3,948) reveals stable core microbiota, yet total gut diversity is still underexplored



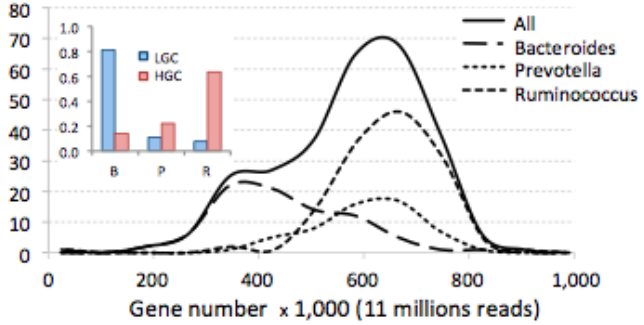
Western core = 17 genera; incl remote populations: 14

Est. 40,000 individuals will need to be profiled to reach saturation

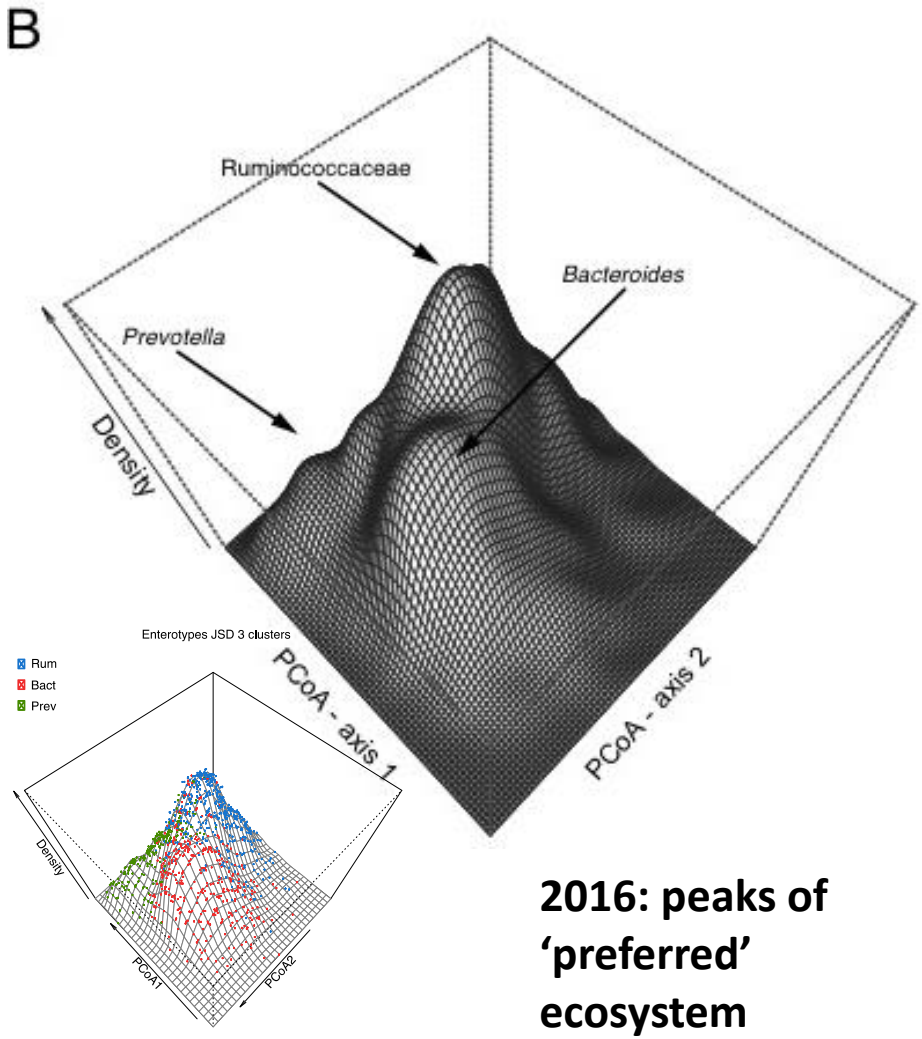
Enterotypes: from ‘blood groups’ to density landscapes



Arumugam*, Raes* et al. Nature 2011



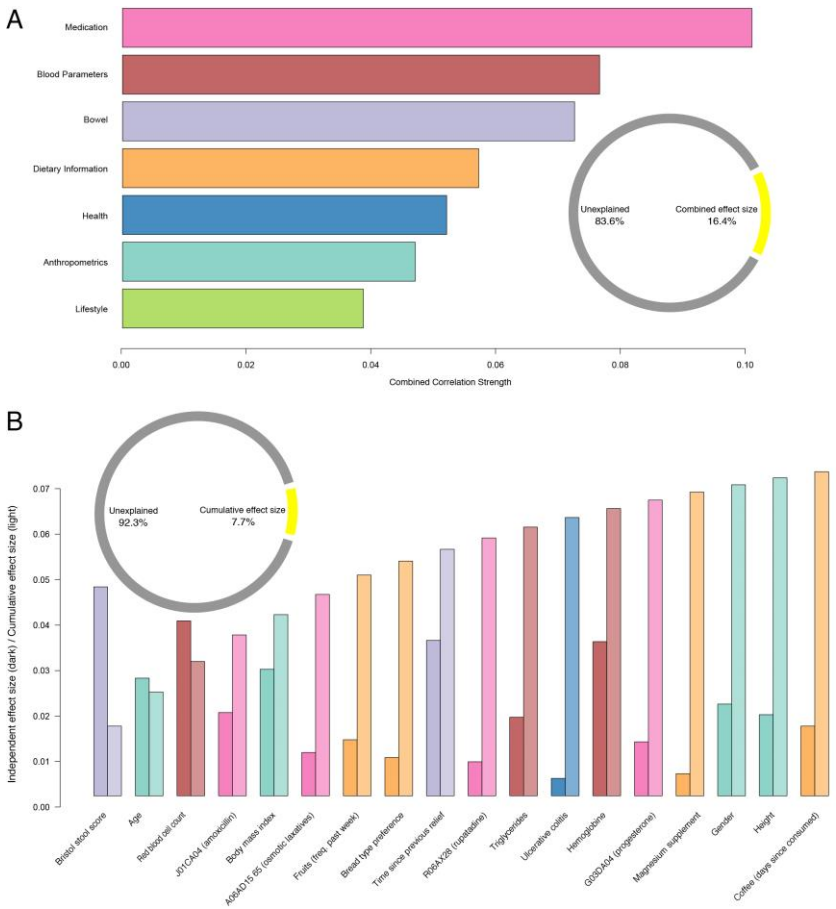
Enterotype association to metabolic syndrome risk markers; Le Chatelier et al Nature 2012

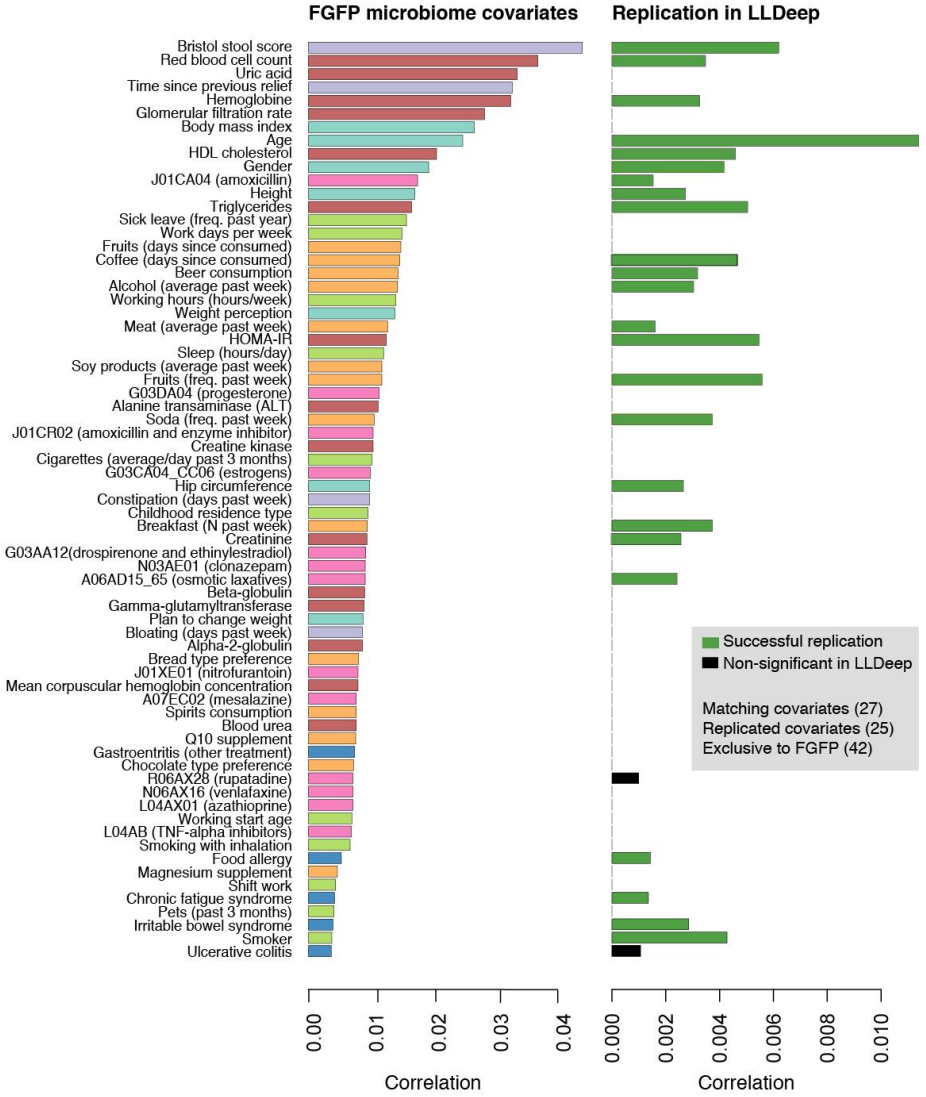


Falony et al. Science, 2016

2016: peaks of ‘preferred’ ecosystem constellations

Identification of 69 factors associated with microbiota variation





92% of comparable factors replicate in LLDeep

Falony et al. Science, 2016

Identification of multiple dietary covariates

Dietary interventions as potential microbiota modulation strategy

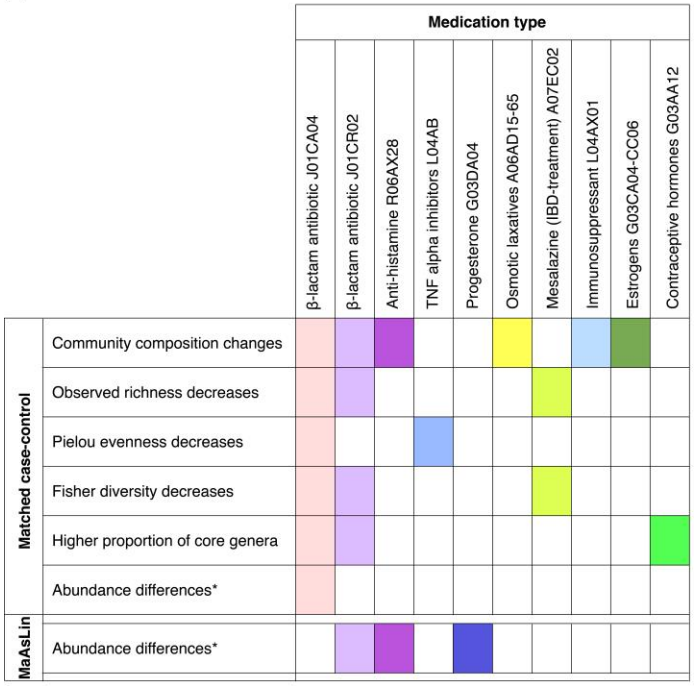
Fruit consumption
Meat consumption
Bread type preference
Soy products/yoghurts

Go Belgium!

Beer consumption
Dark chocolate preference

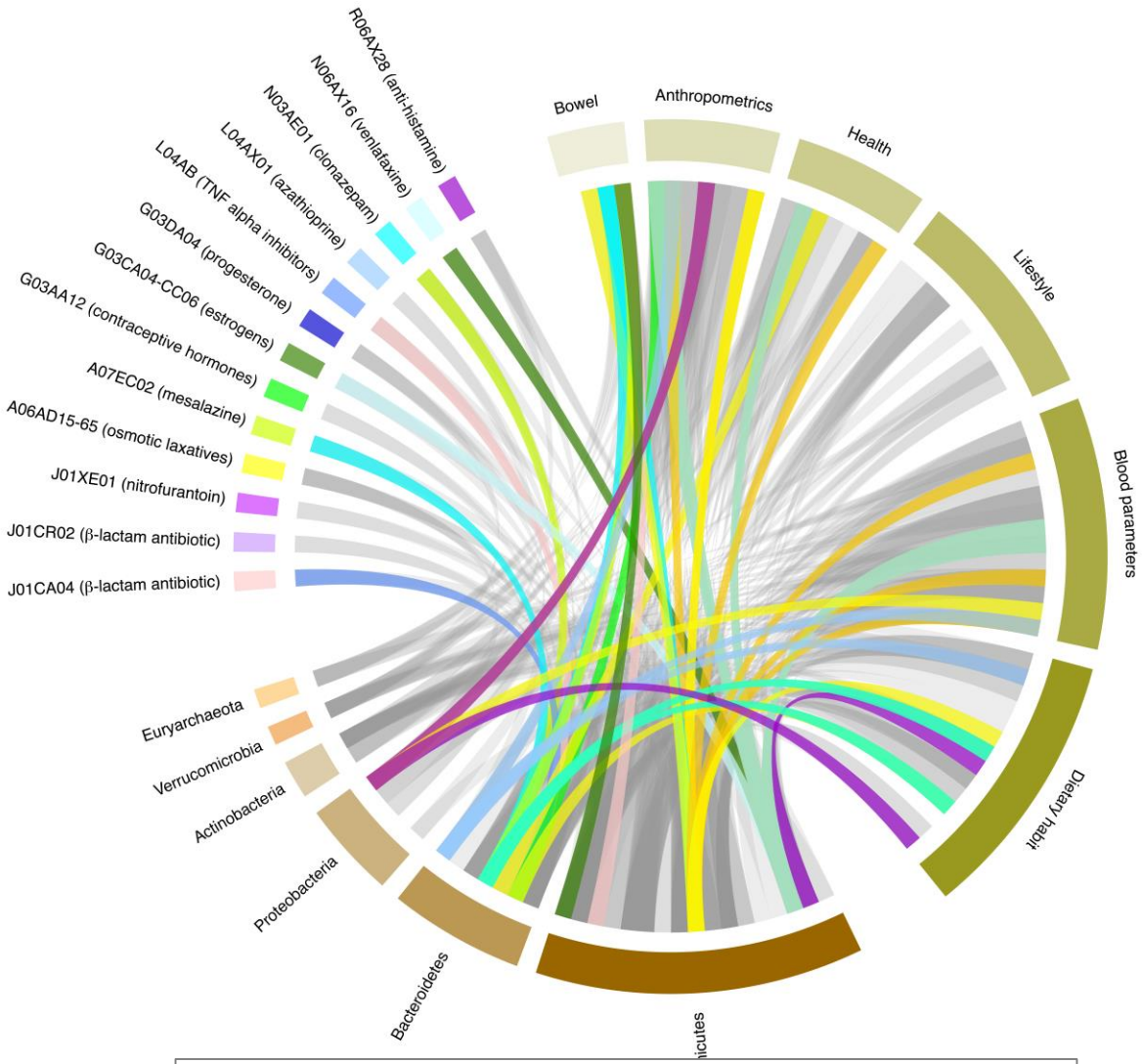
Microbiota-drug associations as primary confounder category

A



Direct associations
e.g. Antibiotics, laxatives, Immunosuppressants, Hormones

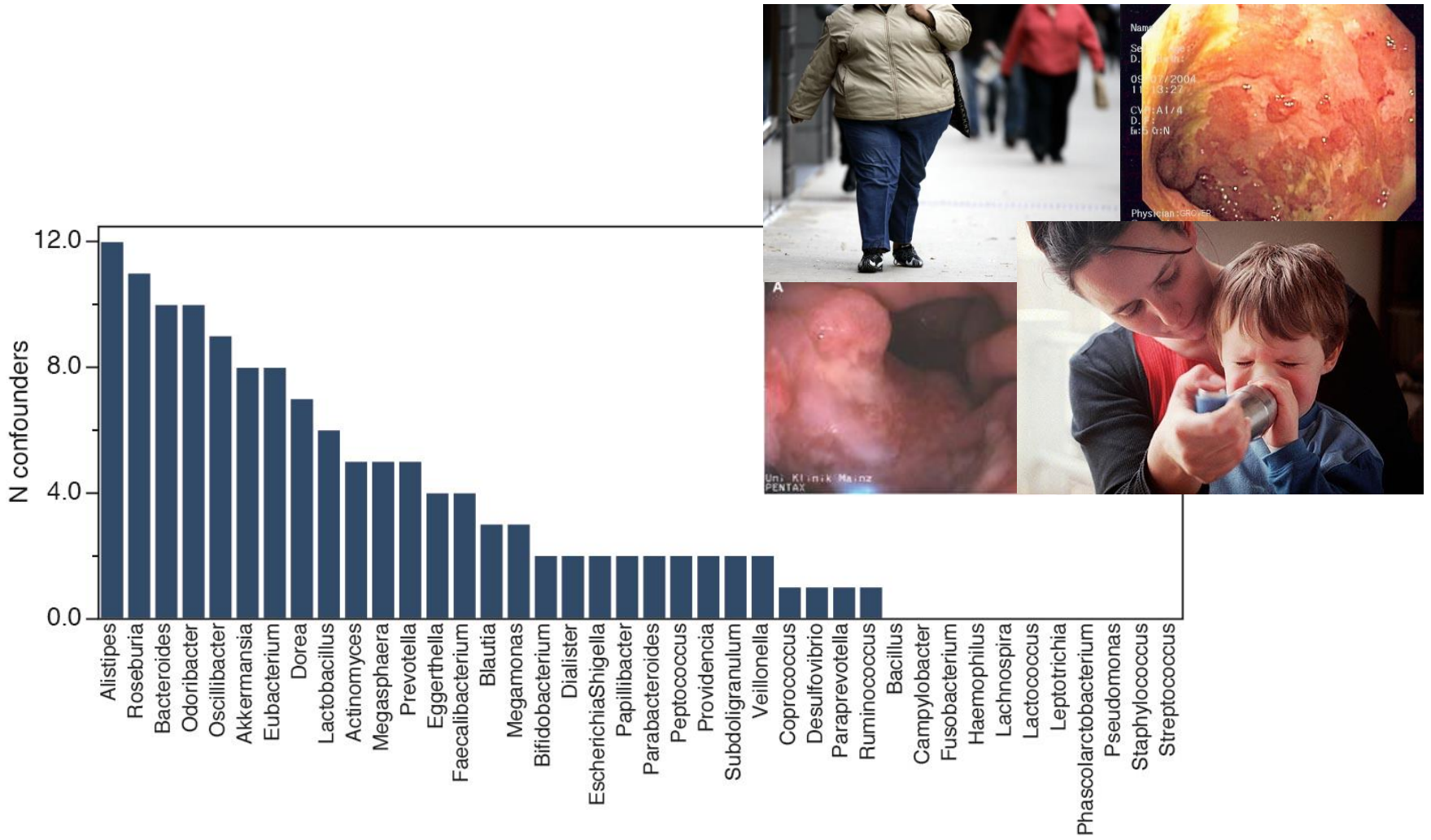
B



Drugs interacting with cofactor-associations

See also: Forslund, Hildebrand*, Nielsen*, Falony* Nature 2015*

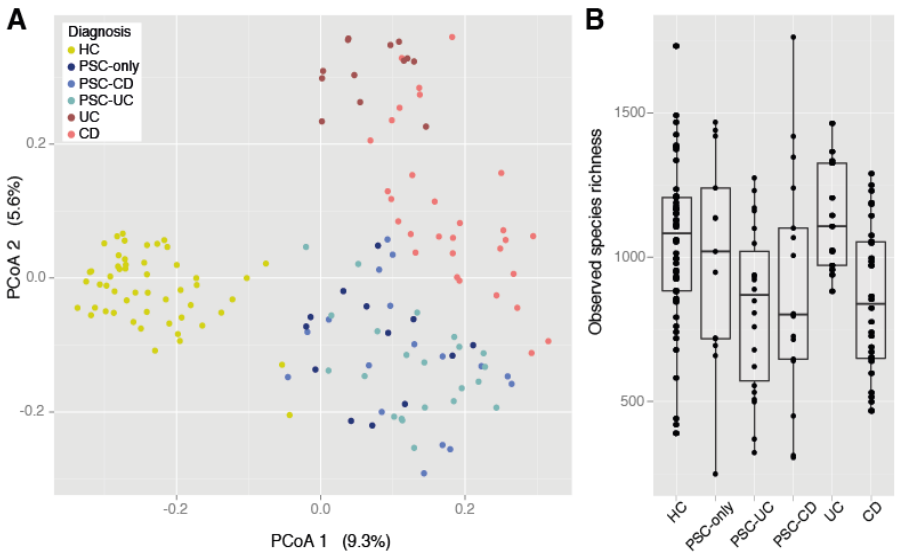
Majority of genera thusfar associated to disease are also confounded by unrelated host factors

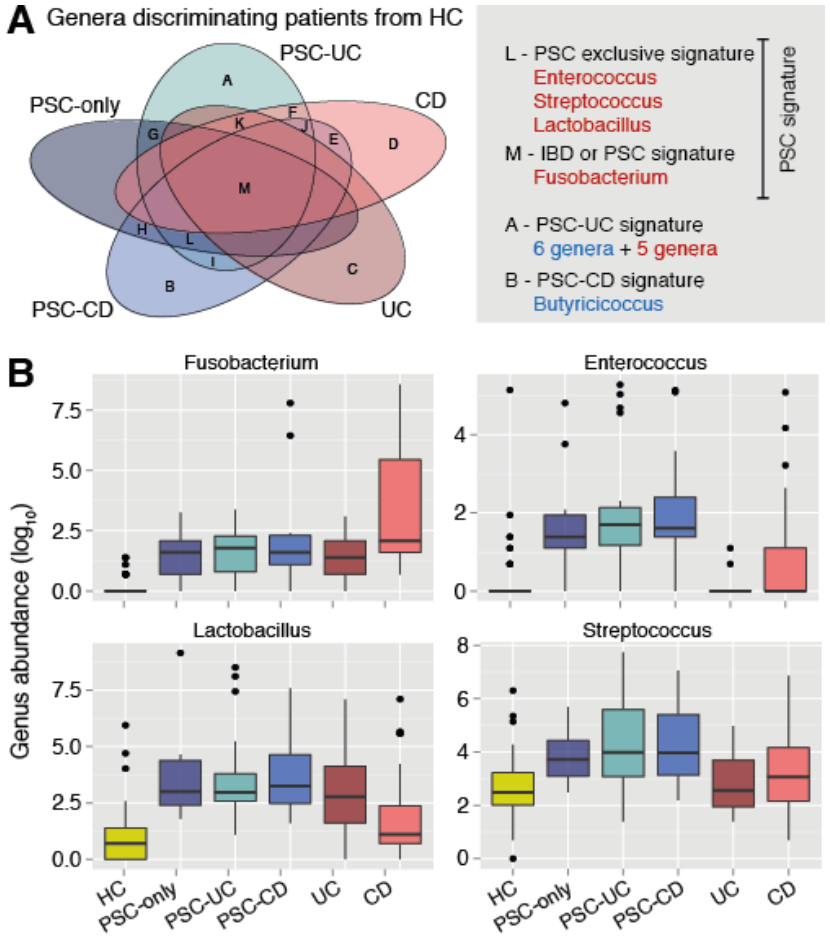


Falony et al. Science, 2016

Using matched FGFP controls & confounder knowledge increases robustness of clinical microbiome studies

Identification of Primary Sclerosing Cholangitis (PSC) signature independent from IBD and drug usage





Sabino*, Vieira-Silva* et al **Gut** 2016

FGFP: next steps

Longitudinal sampling

- Whole cohort sampled every 2 years
- 500 participants sampled every month for 24 months
- 50 participants sampled every week for 24 weeks
- 50 participants sampled every day for 45 days

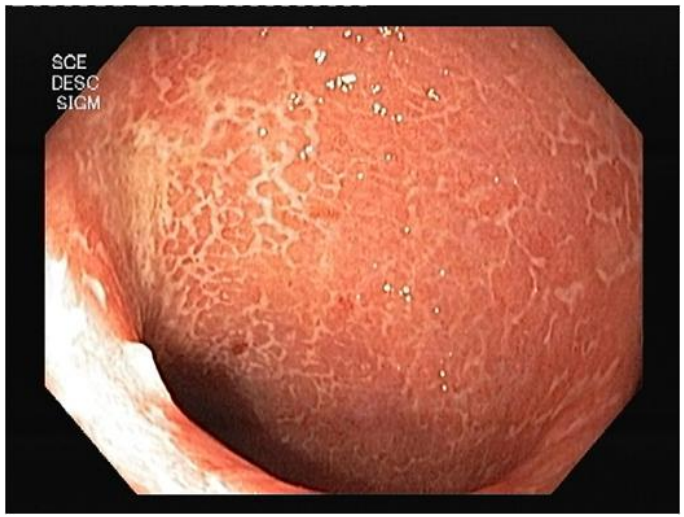
Data generation:

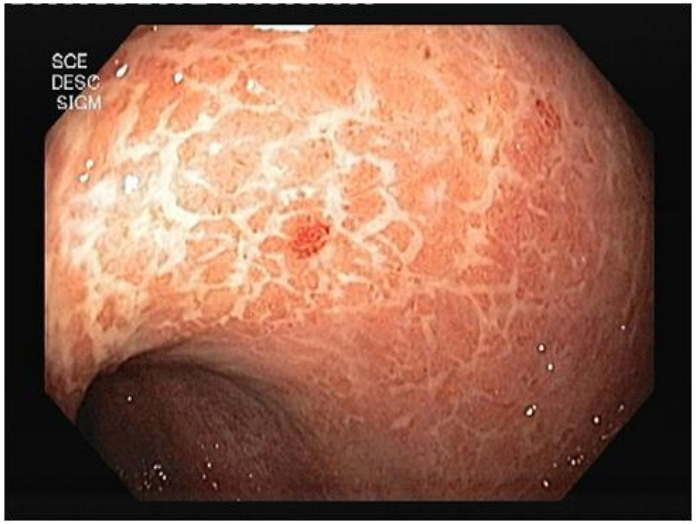
- From 16S sequencing to metagenomic shotgun sequencing → phylogenetic & functional profiling
- Host genotyping
- Metatranscriptomics, proteomics, metabolomics
- Target strain culture and characterization

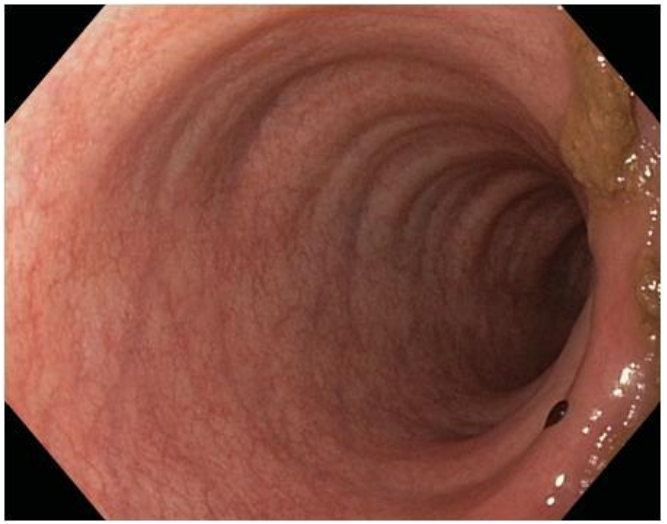
The healthy microbiota as a drug: Faecal bacteriotherapy in Ulcerative Colitis

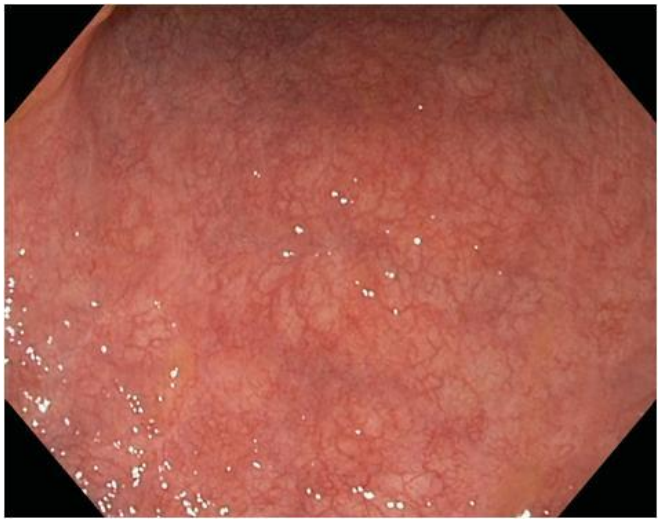
February 7th, 2012

March 30th, 2012





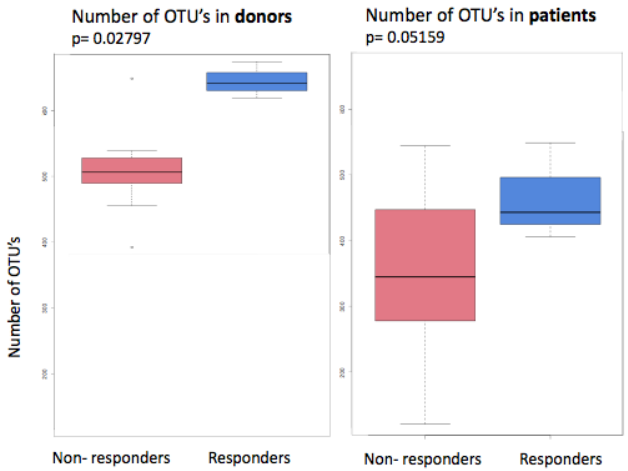




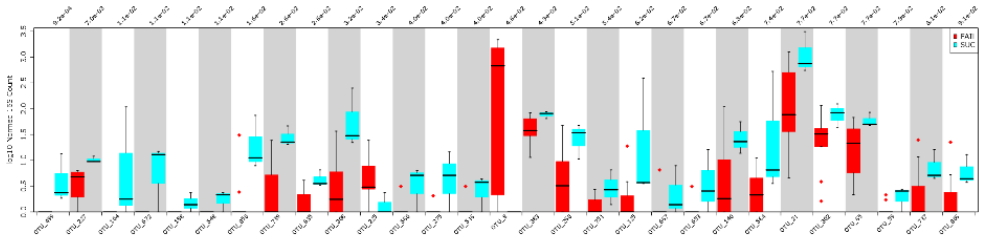
Collab S. Vermeire, KU Leuven, B

FMT in UC: 25% success rate

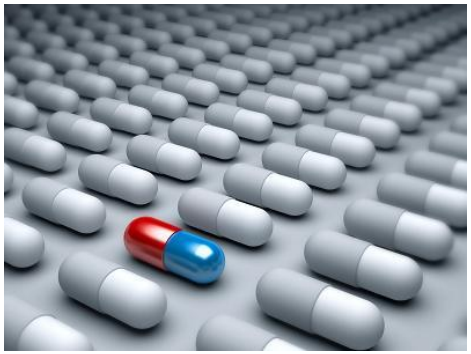
Microbiome monitoring allows treatment optimisation



"Donor" biodiversity determines treatment outcome



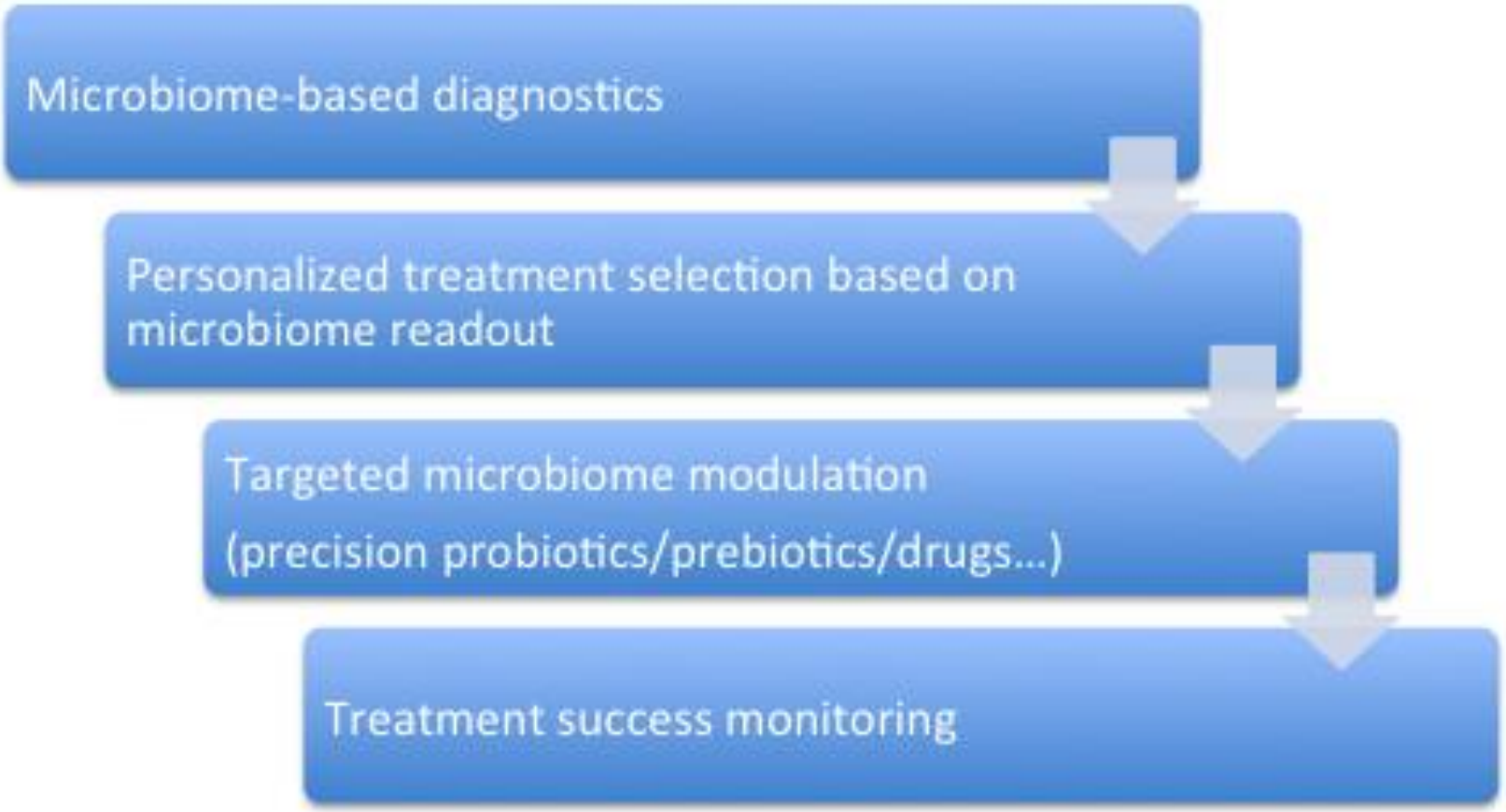
Patient microbiome predictors for treatment success



Development of next-gen probiotic cocktails

Vermeire et al., **JCC** 2015

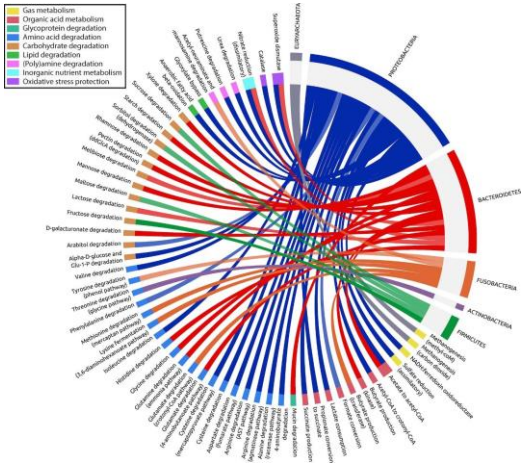
Microbiome therapeutic model

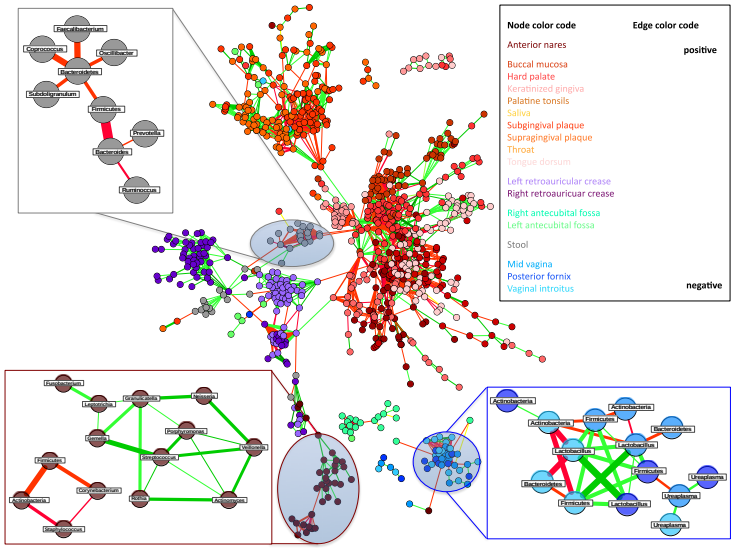


Raes, **Gut** 2016

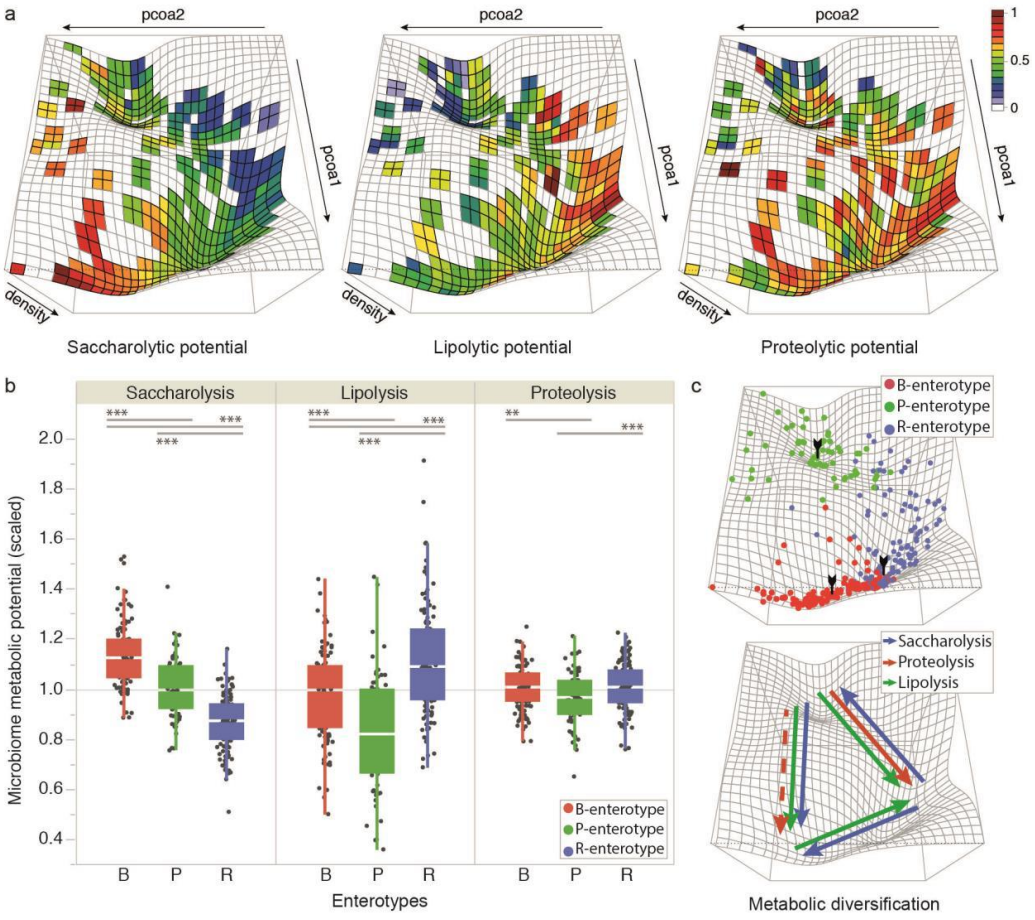
From parts lists to system-level understanding

"who-does-what" map of the intestinal ecosystem indicates lowered perturbation resilience in the Bacteroides enterotype



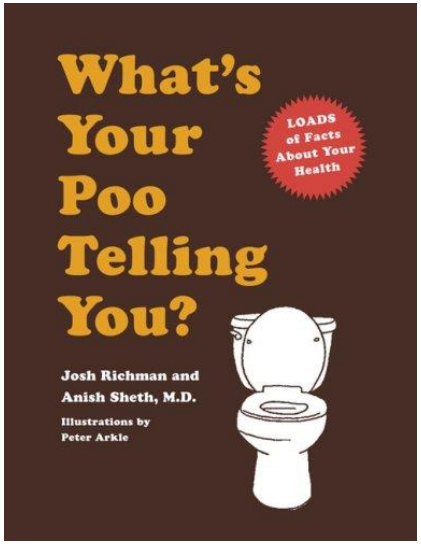


HMP, **Nature** 2012



Vieira-Silva*, Falony* et al **Nature Microbiology** *in press*

Conclusions



- Definition of normal variation and confounders is essential towards robust microbiome diagnostics and preventive care
- Microbiome as drug and/or treatment guidance
- Systems approaches provide insights in biology behind dysbiotic states
- Multi-national collaboration essential for generalization and validation of results
- Ongoing: long-term variation and health outcomes

Policy suggestions

- Structural, long-term funding of national microbiome initiatives essential for survival
- Establishment of international integration mechanisms between cohorts: towards a global microbiome monitoring effort (incl. remote populations!)
- Human intervention studies are the ultimate proof: tight integration between microbiome data crunchers and clinical groups
- Public-private partnerships crucial for translation of findings to products

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