Healthy gut microbiota and natural variability, stability and resilience – Identification of microbiota covariates and lifestyle 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

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, triglycerides, 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)

Zhernakova et al., Science 2016
Integration with global datasets (N=3,948) reveals stable core microbiota, yet total gut diversity is still underexplored.

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


2016: peaks of ‘preferred’ ecosystem constellations
Identification of 69 factors associated with microbiota variation

92% of comparable factors replicate in LLDeep

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

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

See also: Forslund*, Hildebrand*, Nielsen*, Falony* Nature 2015
Majority of genera thusfar associated to disease are also confounded by unrelated host factors

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

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

Microbiome-based diagnostics

Personalized treatment selection based on microbiome readout

Targeted microbiome modulation (precision probiotics/prebiotics/drugs...)

Treatment success monitoring

From parts lists to system-level understanding

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

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

HMP, Nature 2012
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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