Assessing the Human Gut Microbiota in Metabolic Disease

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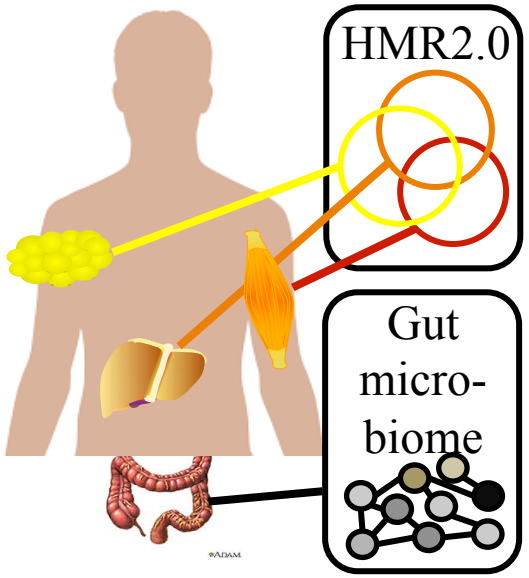
Genome-scale Metabolic Models

sys²bio
www.sysbio.se

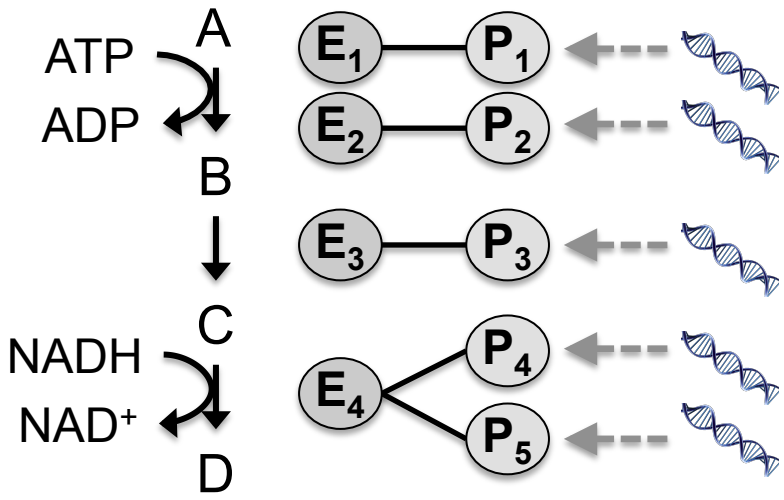
We have reconstructed a comprehensive model (GEM) for human metabolism HMR2.0 and this is used for analysis of tissue specific metabolism

Reactions	Metabolites	Genes
8170	5998 (3160)*	3763

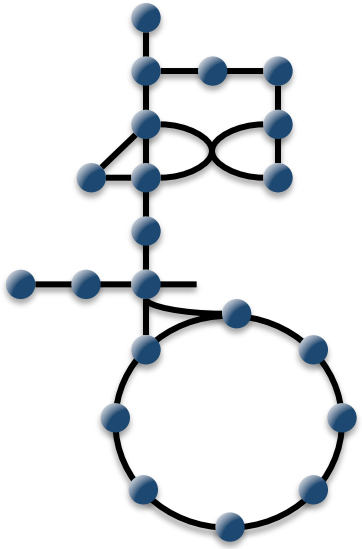
* Unique metabolites



Reaction Library



Metabolic Network



We are combining this with GEMs for the gut microbiome to obtain a complete description of human metabolism

GEMs for 32 Tissues and Organs

Using RNAseq from 32 tissue biopsies we generated GEMs for 32 tissues and organs and identified unique metabolic functions for each tissue

RESEARCH ARTICLE

PROTEOMICS

Tissue-based m… f th… human proteo…

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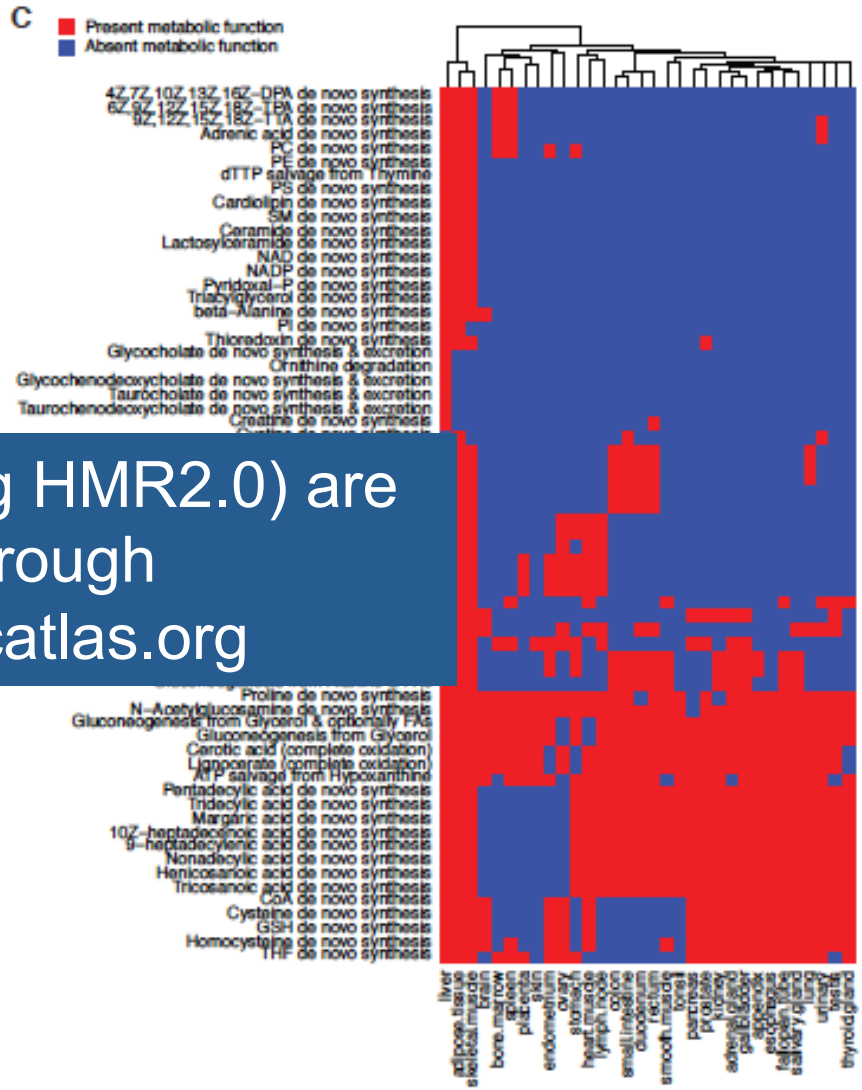
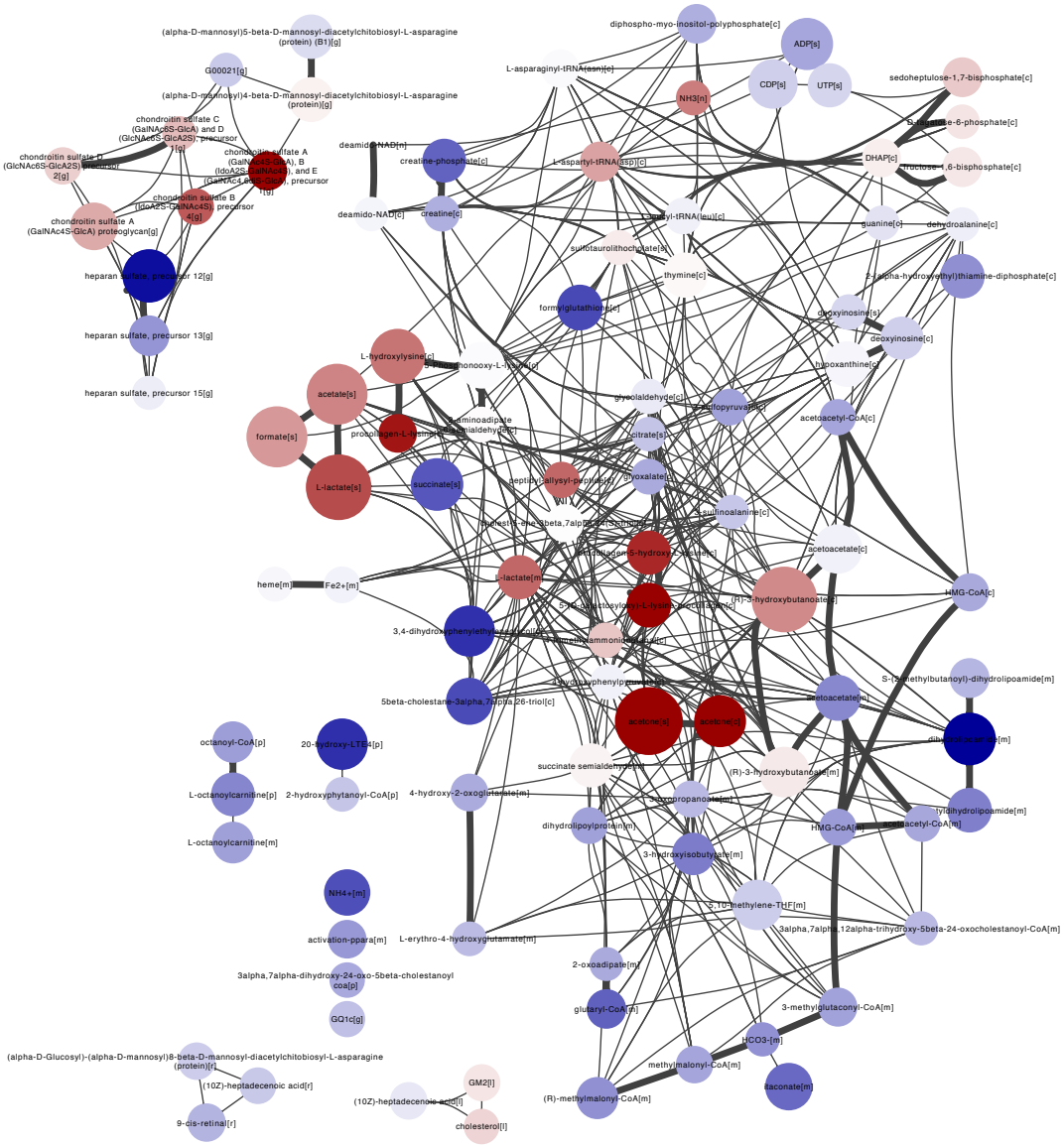
Jens Nielsen,[3,5] Fredrik Pontén[4]

Resolving the molecular details of proteome variation in the different tissues and organs of the human body will greatly increase our knowledge of human biology and disease. Here, we present a map of the human tissue proteome based on an integrated omics approach that involves quantitative transcriptomics at the tissue and organ level, combined with tissue microarray-based immunohistochemistry, to achieve spatial localization of proteins down to the single-cell level. Our tissue-based analysis detected more than 90% of the putative protein-coding genes. We used this approach to explore the human secretome, the membrane proteome, the druggable proteome, the cancer proteome, and the metabolic functions in 32 different tissues and organs. All the data are integrated in an interactive Web-based database that allows exploration of individual proteins, as well as navigation of global expression patterns, in all major tissues and organs in the human body.



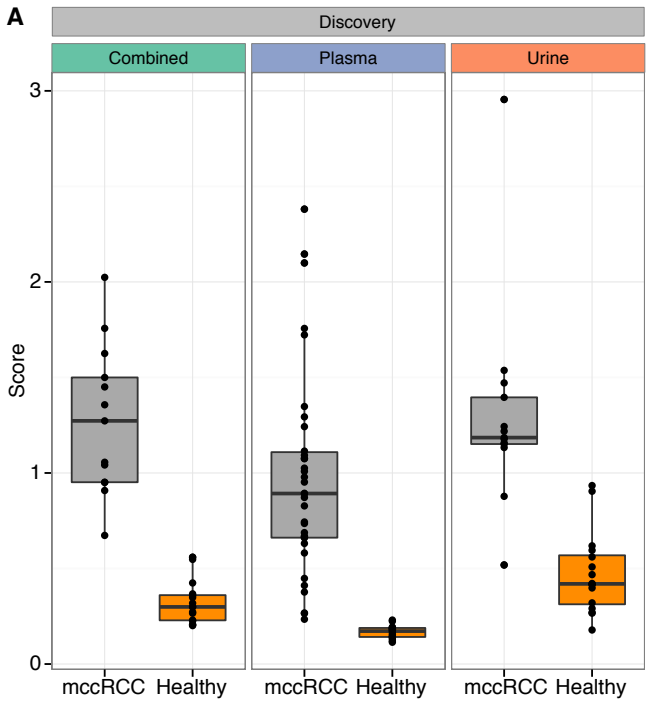
All models (including HMR2.0) are available through www.metabolicatlas.org

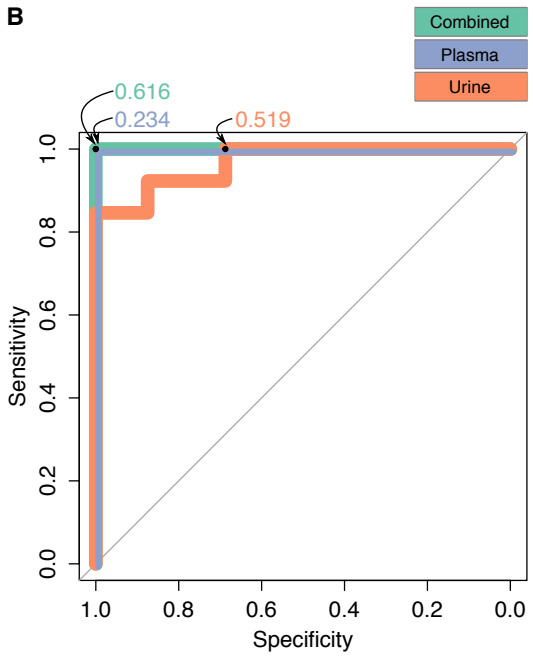
Identification of sub-networks

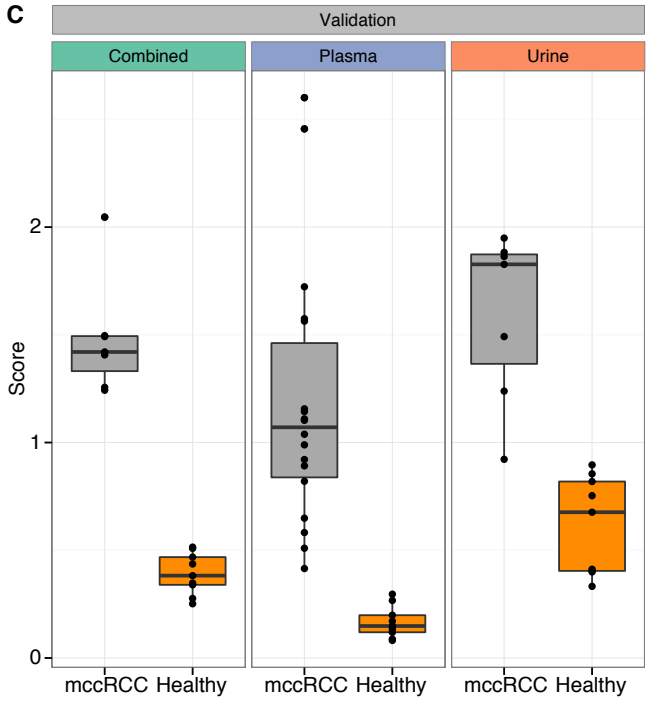


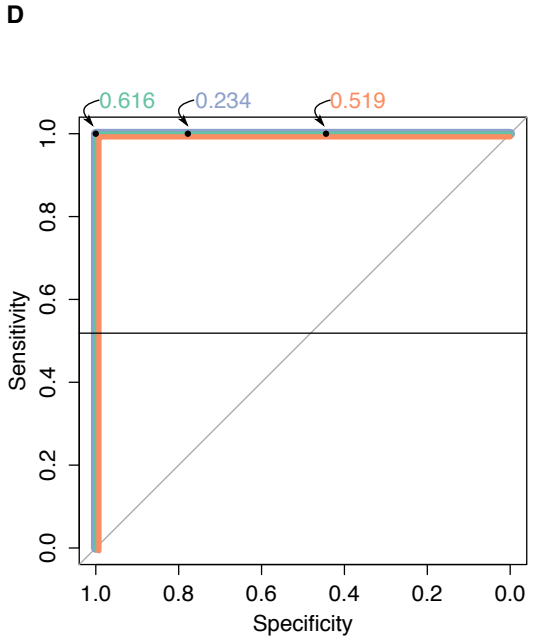
From comparative analysis of 481 ccRCC samples and 71 tumor adjacent normal samples we identified 6 sub-networks

The second largest networks is responsible for the biosynthesis of chondroitin sulfate (CS) and heparan sulfate (HS)









From analysis of CS and HS in 34 patients with metastatic ccRCC and 16 controls we identified a prognostic biomarker.

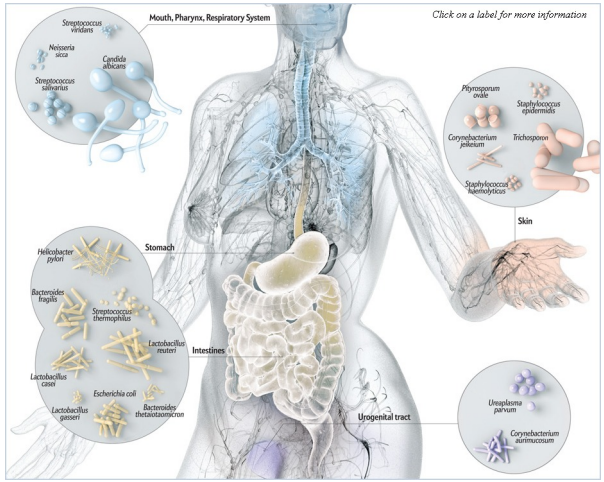
Evaluation of the prognostic biomarker in validation cohort of 18 ccRCC patients and 9 healthy controls shows that it has very strong predictive strength

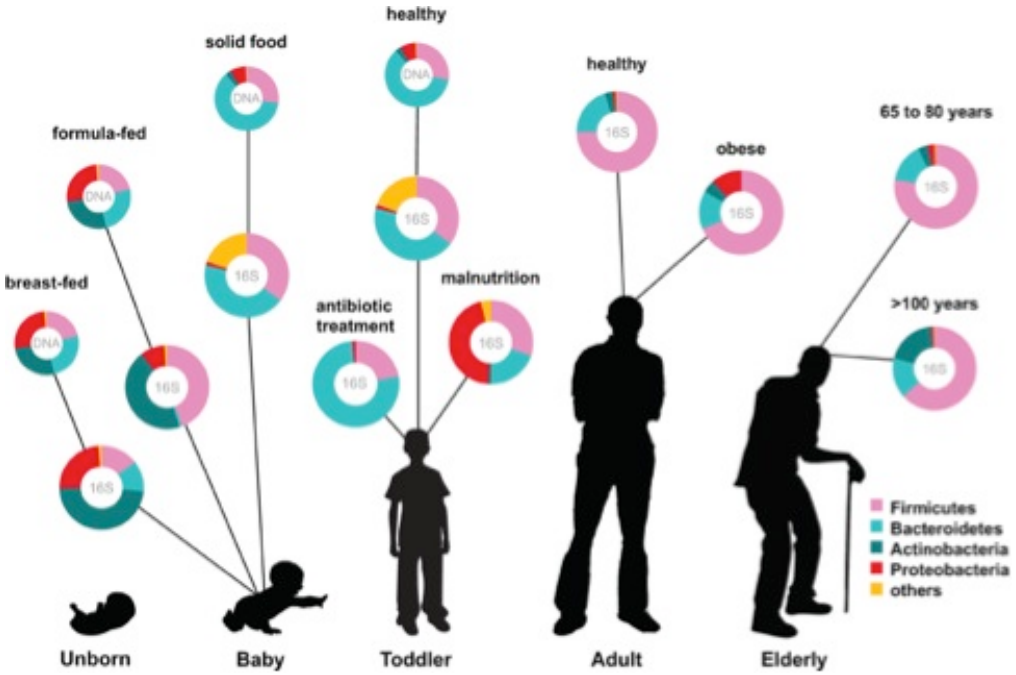
Cell Reports (2016) **15**:1-15

The Human Microbiome

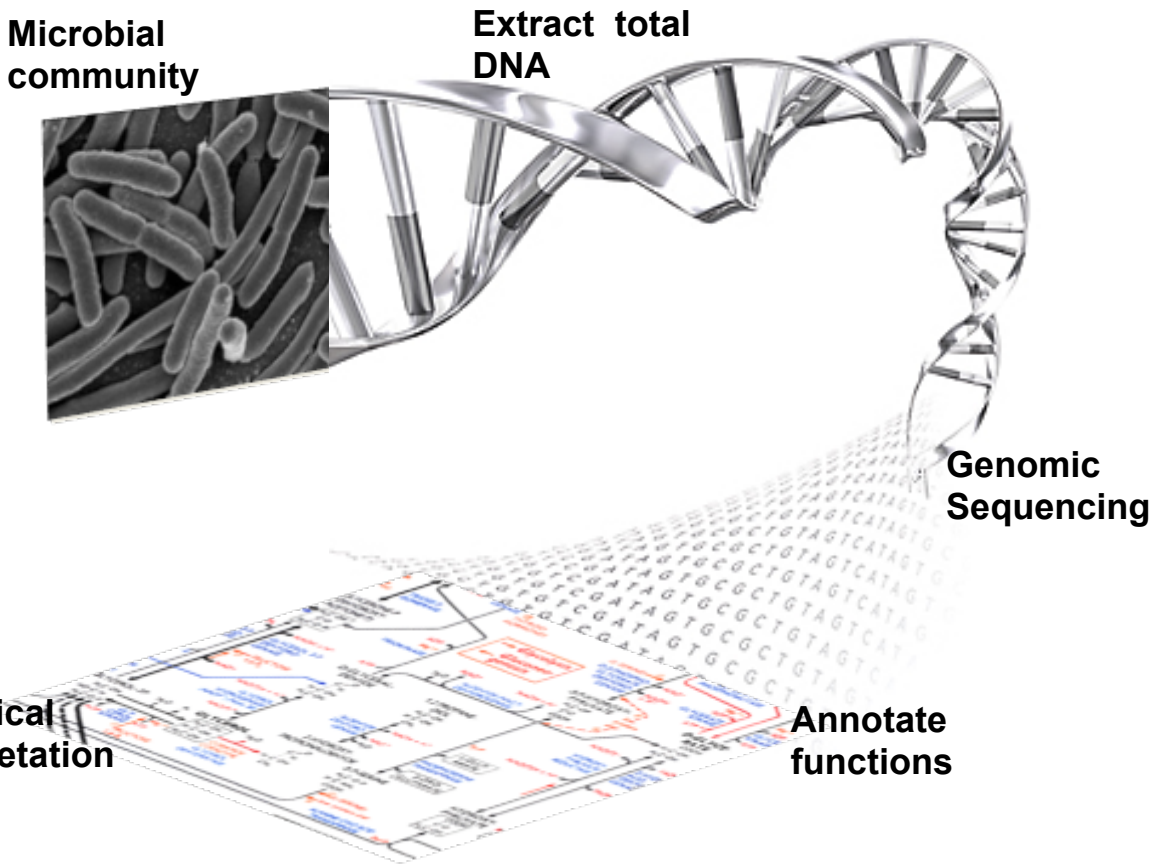
- over 30,000 microbial genome projects (1995-2014)
  cover < 20% of the diversity of cultured archaeal and bacterial species
  represent just 15% of the overall known prokaryotic diversity.) [3]

- The body contains 10 times more bacteria, archaea, fungi
  and other micro-organisms than human cells.

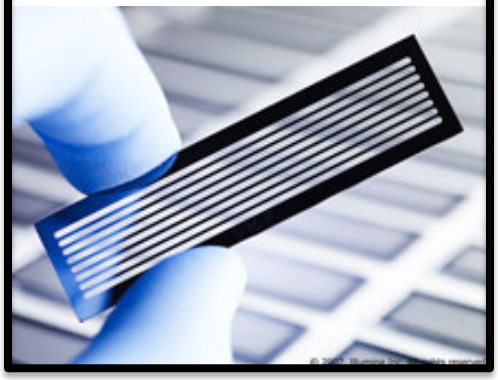




How to study the human microbiome?

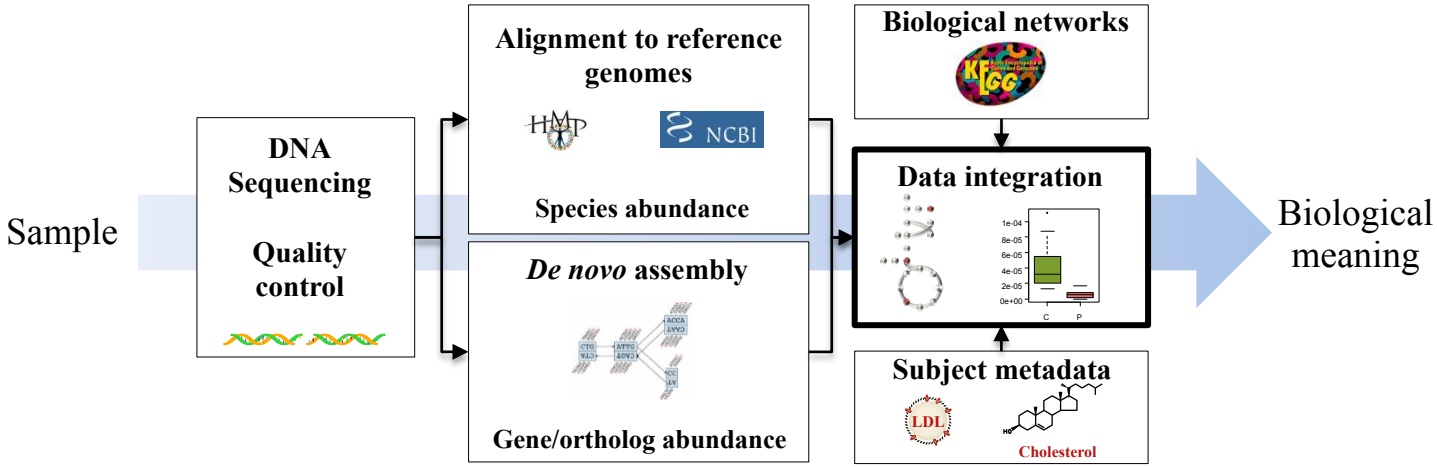


Total DNA extracted from fecal samples can be sequenced using NGD, e.g. with Illumina HiSeq2000



Very large data-sets are generated (Big-Data), typically 20 million reads per sample corresponding to >3 10^9 basepairs
Cohorts typically exceeds 100 subjects resulting in >3 10^{11} basepairs

MEDUSA: Bioinformatics pipeline for MEtagenomic Data UtiliSation and Analysis



Software and programming languages

SolexaQA

python™

MUSCLE multiple sequence alignment method with reduced time and space complexity

Bowtie
An ultrafast memory-efficient short read aligner

Velvet

R

HMMER

FASTX-Toolkit

Reporter features

Databases

MetaHIT

HMP

KEGG

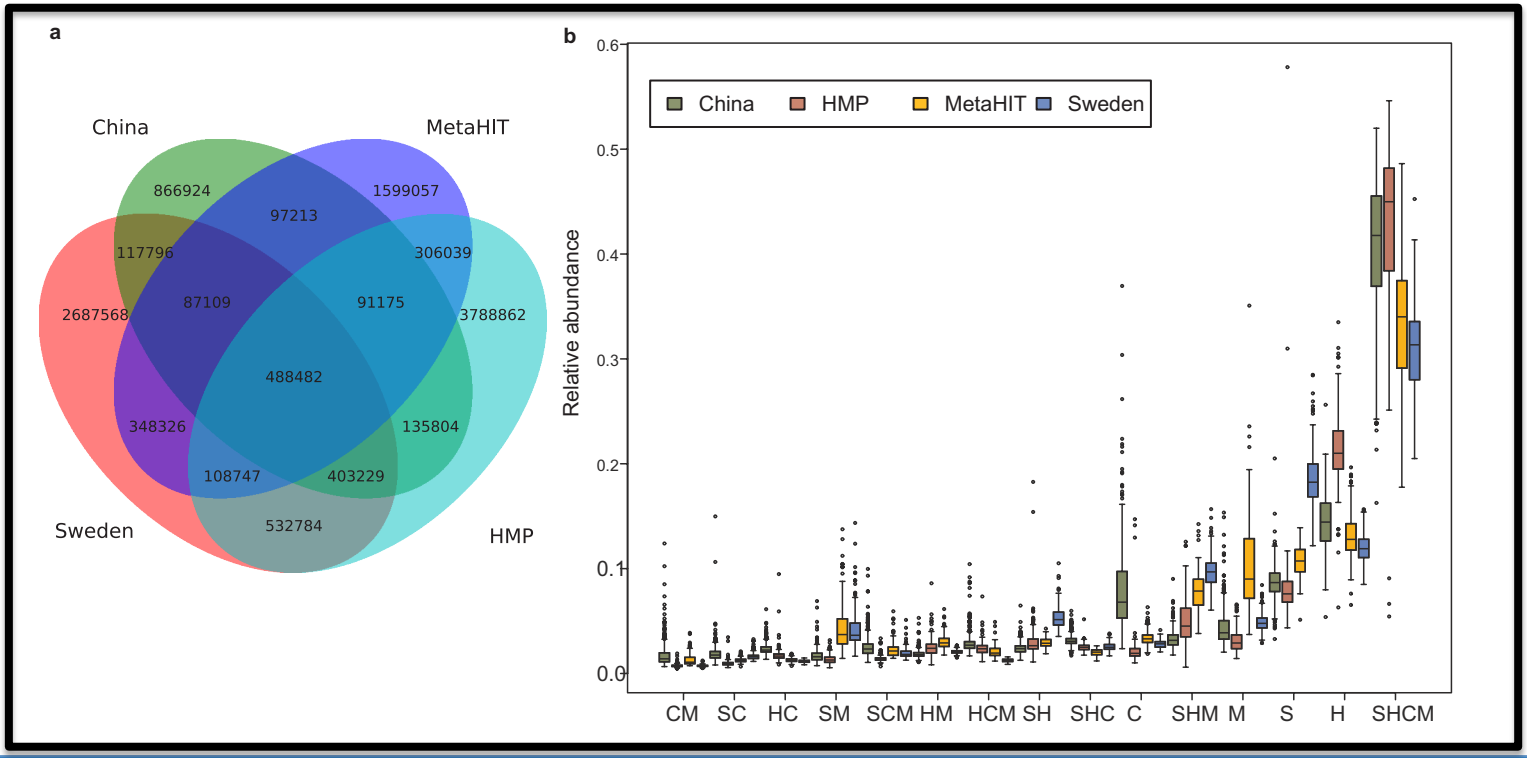
NCBI

CAZy Carbohydrate-Active enZYmes

A global gut microbiome gene catalogue

- 11.7 million genes identified
- 9 million genes unique to a single study
- About 500,000 genes were core in all four studies
- 2.7 million genes shared between at least two studies

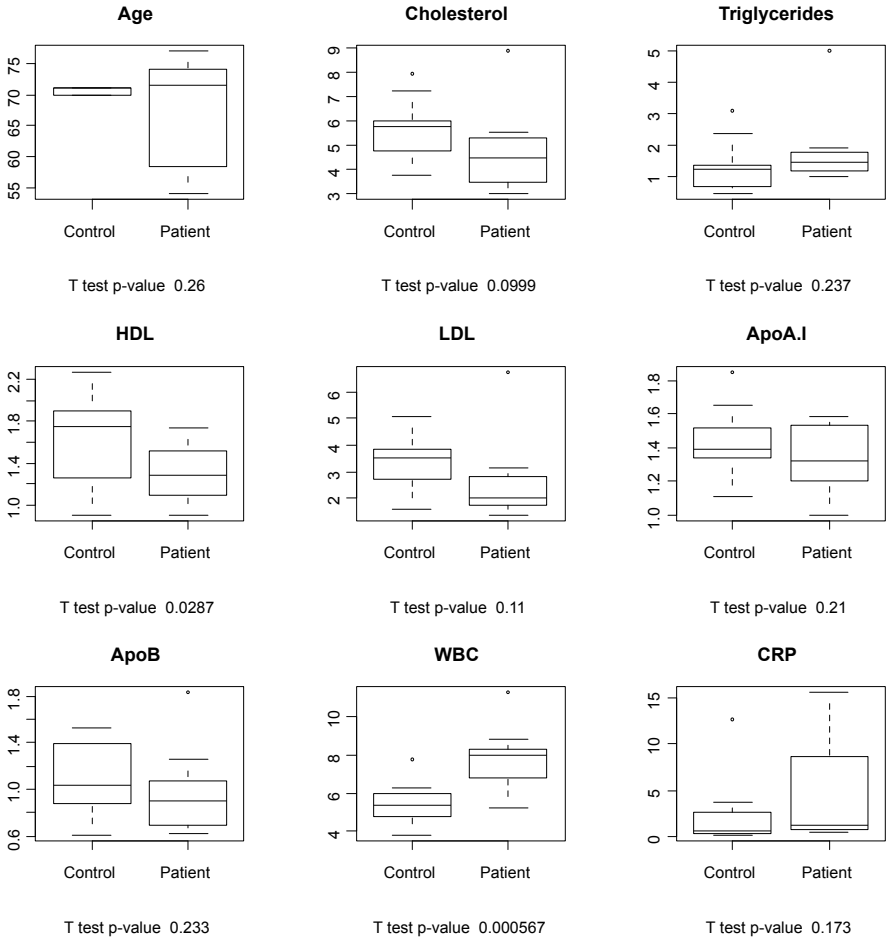
Shared genes were, however, the most abundant (with cores the most abundant)



Gut Microbiome and Symptomatic Atherosclerosis

- 12 Patients
  - Amaurosis fugax (3)
  - Transient ischemic attack (4)
  - Minor ischemic stroke (5)
- 15 Controls age and sex matched
- Serum chemistry (cholesterol, Triacylglycerides, HDL, LDL, inflammation markers …)

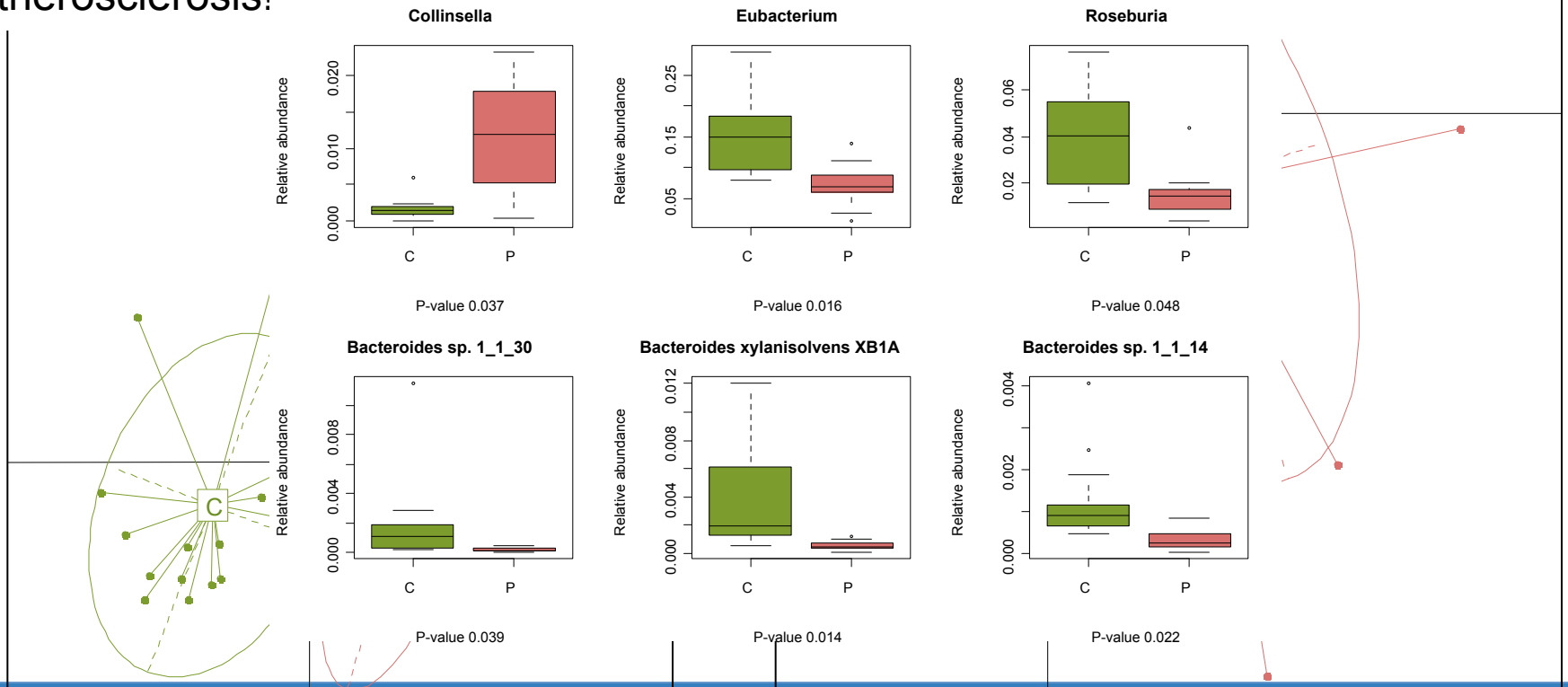
Extensive Metadata



Atherosclerosis is associated with an altered gut micrbiota

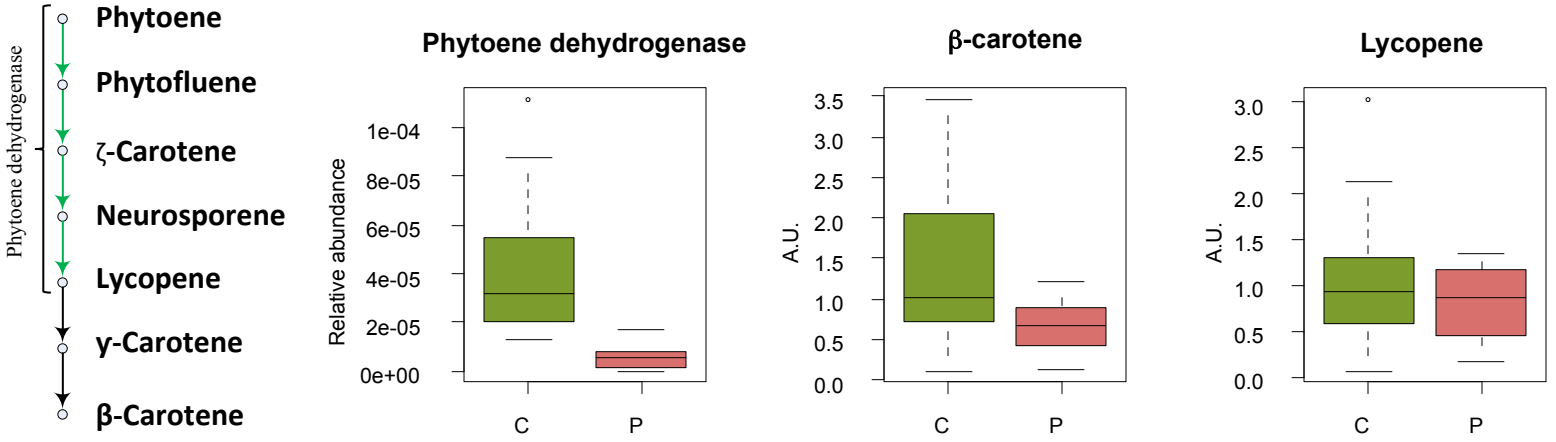
Two controls were clustering with the patients. Checking their medical record s that they subsequent atherosclerosis!

Atherosclerosis is associated with certain bacterial species



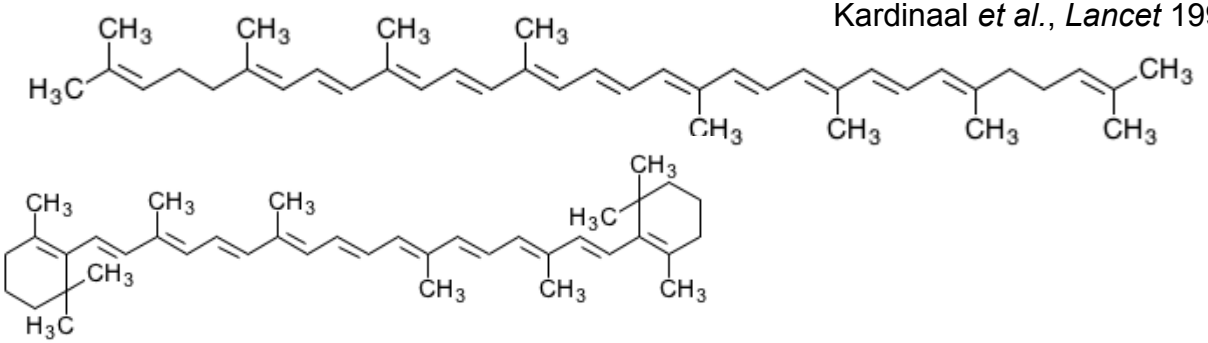
P-value for separation between groups: 1e-04

Phytoene dehydrogenase is enriched in controls



High levels of lycopene and β-carotene are associated with a reduced risk of cardiovascular disease.

Kardinaal *et al.*, *Lancet* 1993, Kohlmeier *et al.*, *Am J Epidemiol 1997*



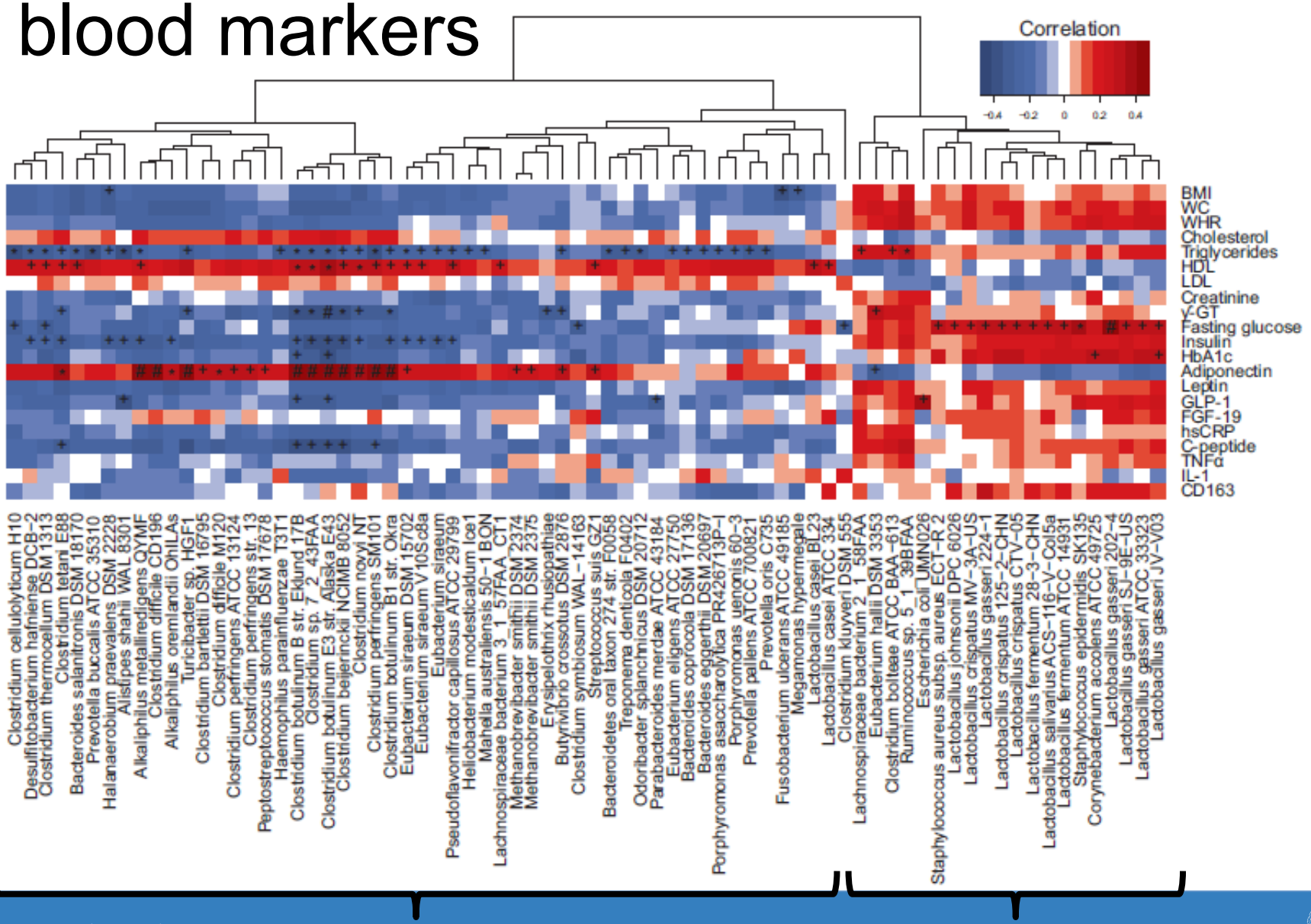
DIWA study

Large Swedish study on development of T2D in elderly women

	T2D (n=53)	IGT (n=49)	NGT (n=43)	P-value
Age	70	70	70	1
BMI	28.4±0.672	26.9±0.576	25.8±0.664	0.017
Waist	94.2±1.44	88.8±1.18	84.1±1.41	3.7e-06
HbA1c	5.52±0.1240	4.60±0.0508	4.53±0.0353	2.6e-16
Statin treatment, n (%)	26(49)	16(33)	10(23)	0.027
Insulin treatment, n (%)	6(11)	0(0)	0(0)	0.0044
Oral antidiabetic medication, n (%)	22(41)	0(0)	0(0)	1.7e-10

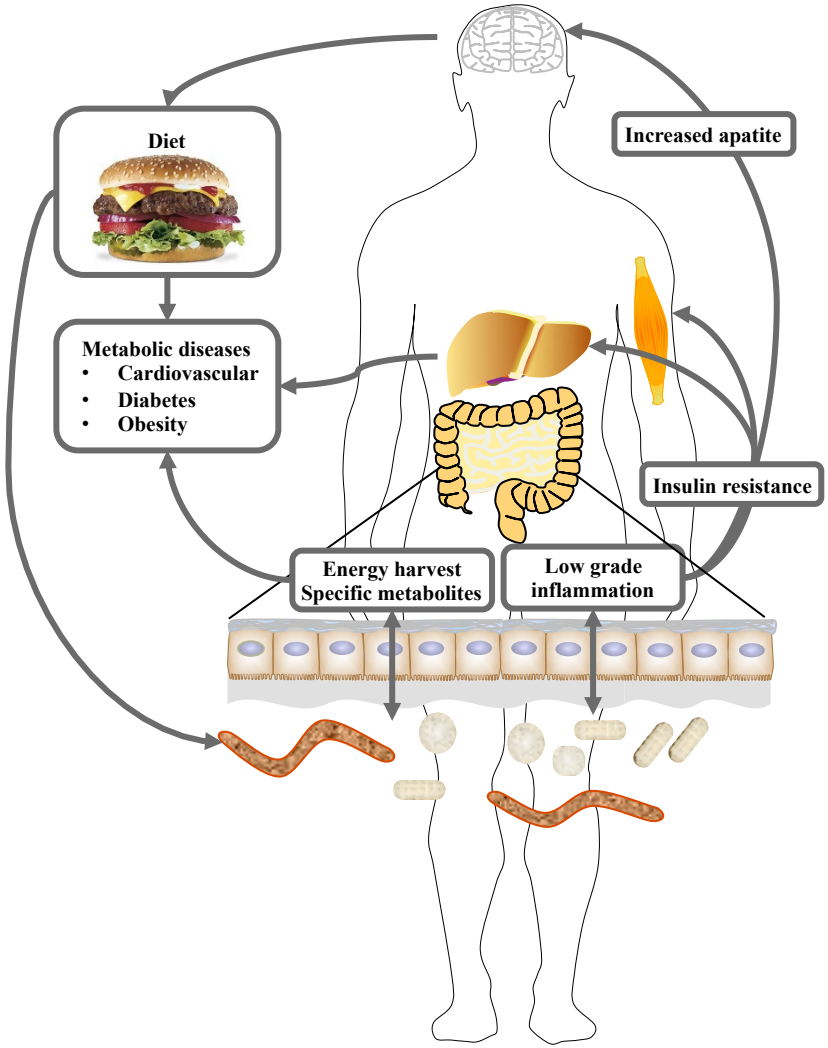
Type 2 diabetes mellitus (T2D)
fasting glucose ≥6.1 mM OR 2H-OGTT ≥11.1 mM
Impaired glucose tolerance (IGT)
fasting glucose < 6.1 mM AND 7.8≥ 2H-OGTT < 11.1 mM
Normal glucose tolerance (NGT)
fasting glucose < 6.1 mM AND 2H-OGTT <7.8 mM

Species abundance correlates with blood markers



Nature (2013) **498**:99-103

A working model for microbiota, diet and host interactions



Metagenomics can identify associations and biomarkers, provide a catalogue of genes and generate hypotheses.

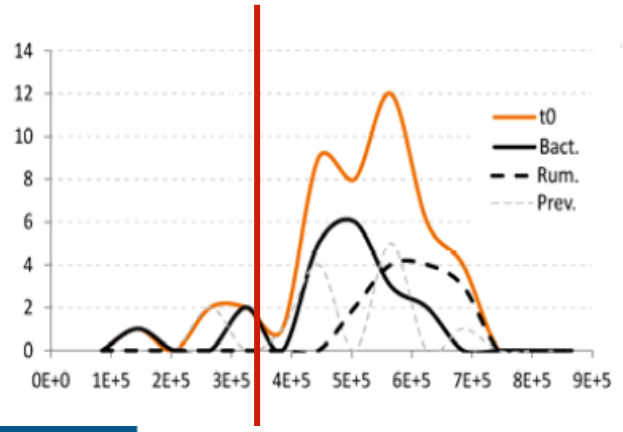
Modeling can generate and test hypotheses, provide detailed mechanistic understanding and be used as scaffold for data analyses.

Provide biomarkers and new interventions in the **medical field**

Aid in design of interventions in terms of drugs and probiotics in **pharmaceutical and food industry**

MicroObese Study

A study lead by Prof. Karine Clement identified that over-weight subjects have varied gut microbiome composition (Nature (2013) **500**:585-588



- Subjects with high gene count (HGC)
- Subjects with low gene count (LGC)

Low gene count suggests low bacterial diversity and LGC subjects had high HOMA index and other disease risk markers

CellPress

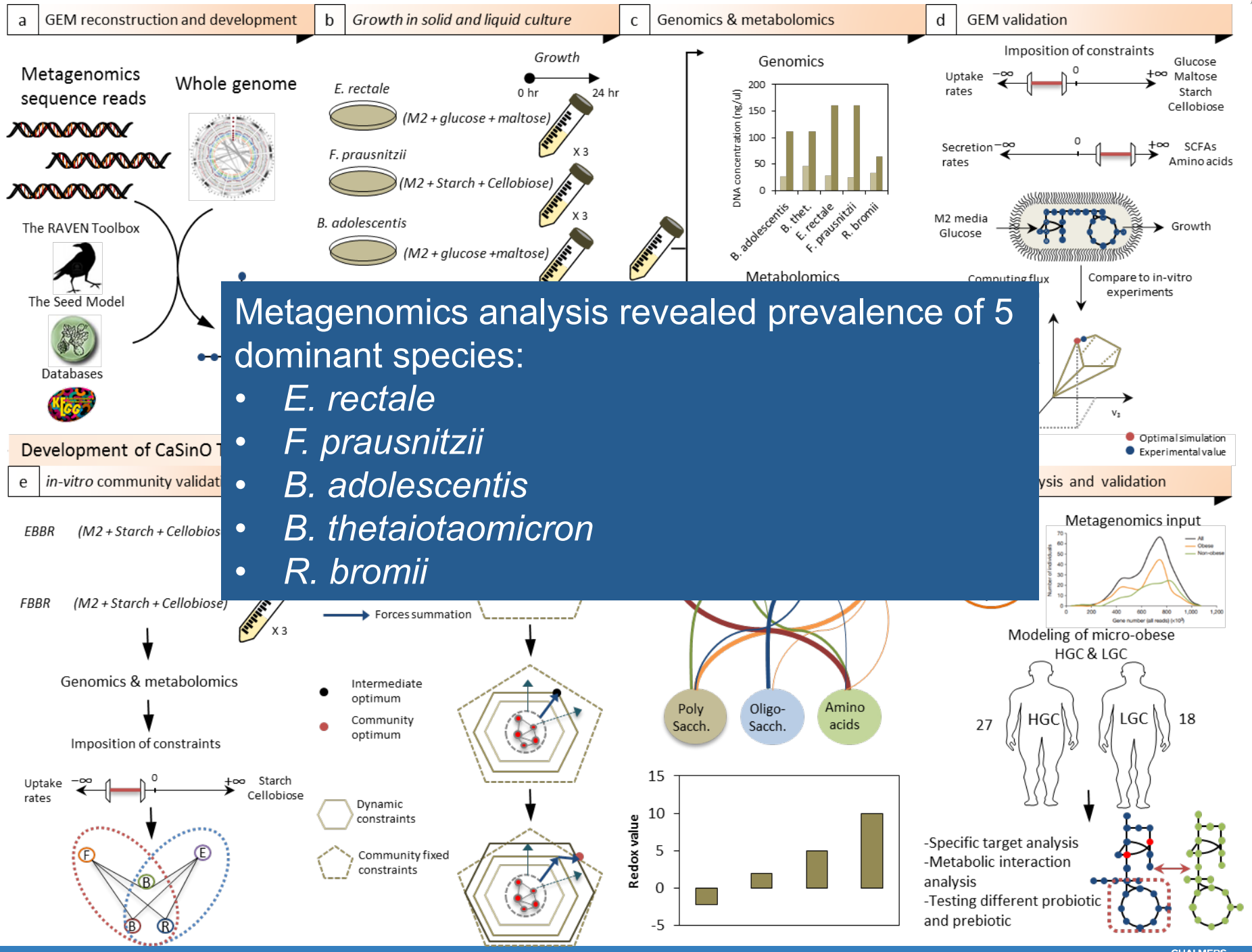
Cell Metabolism

Resource

6

Quantifying Diet-Induced Metabolic Changes of the Human Gut Microbiome

Saeed Shoaie,[1] Pouyan Ghaffari,[1] Petia Kovatcheva-Datchary,[2] Adil Mardinoglu,[1] Partho Sen,[1] Estelle Pujos-Guillot,[3] Tomas de Wouters,[4] Catherine Juste,[4] Salwa Rizkalla,[5,6] Julien Chilloux,[7] Lesley Hoyles,[7] Jeremy K. Nicholson,[7] MICRO-Obes Consortium, Joel Dore,[4] Marc E. Dumas,[7] Karine Clement,[5,6,8] Fredrik Bäckhed,[2,9] and Jens Nielsen[1,*]

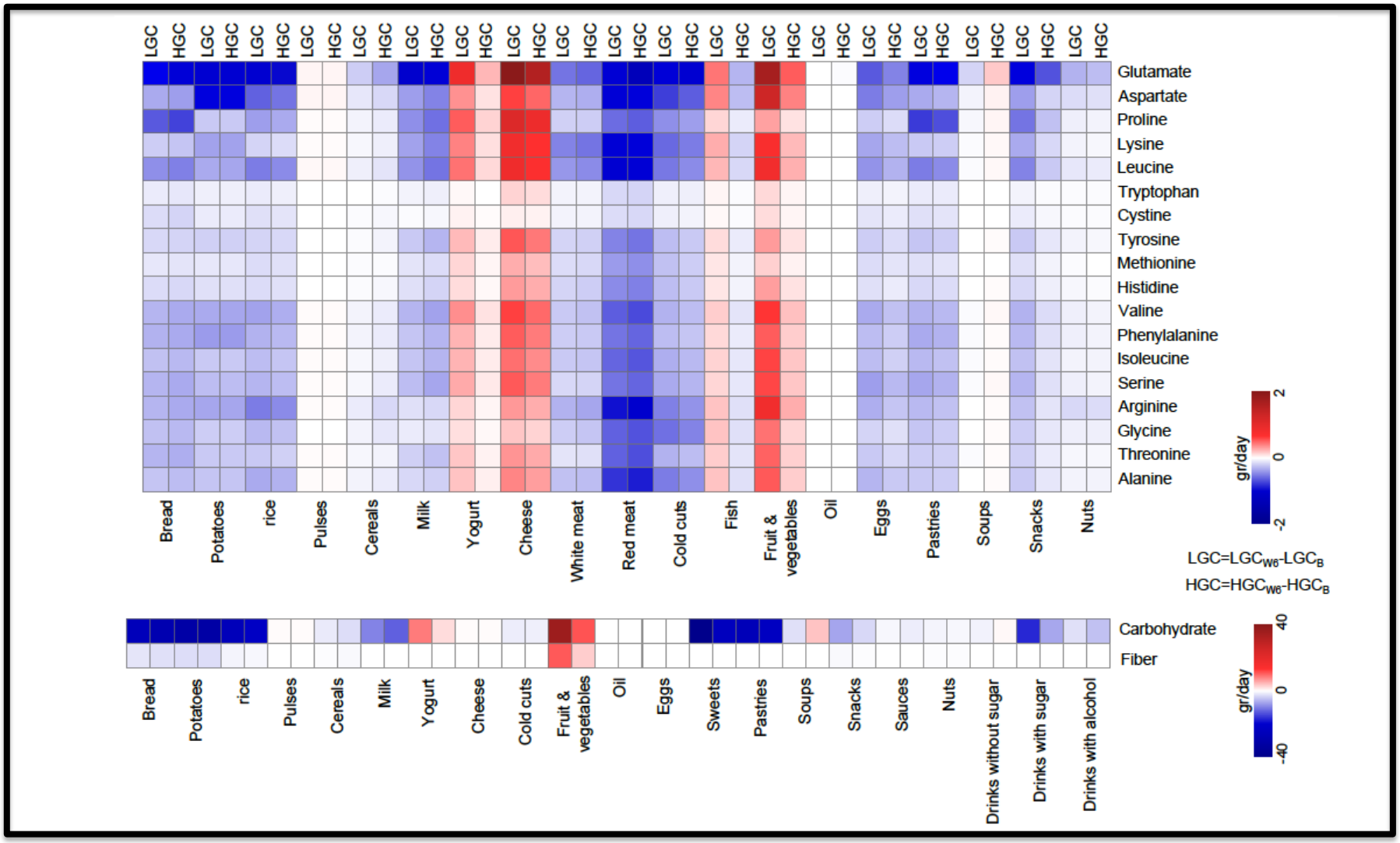


Metagenomics analysis revealed prevalence of 5 dominant species:

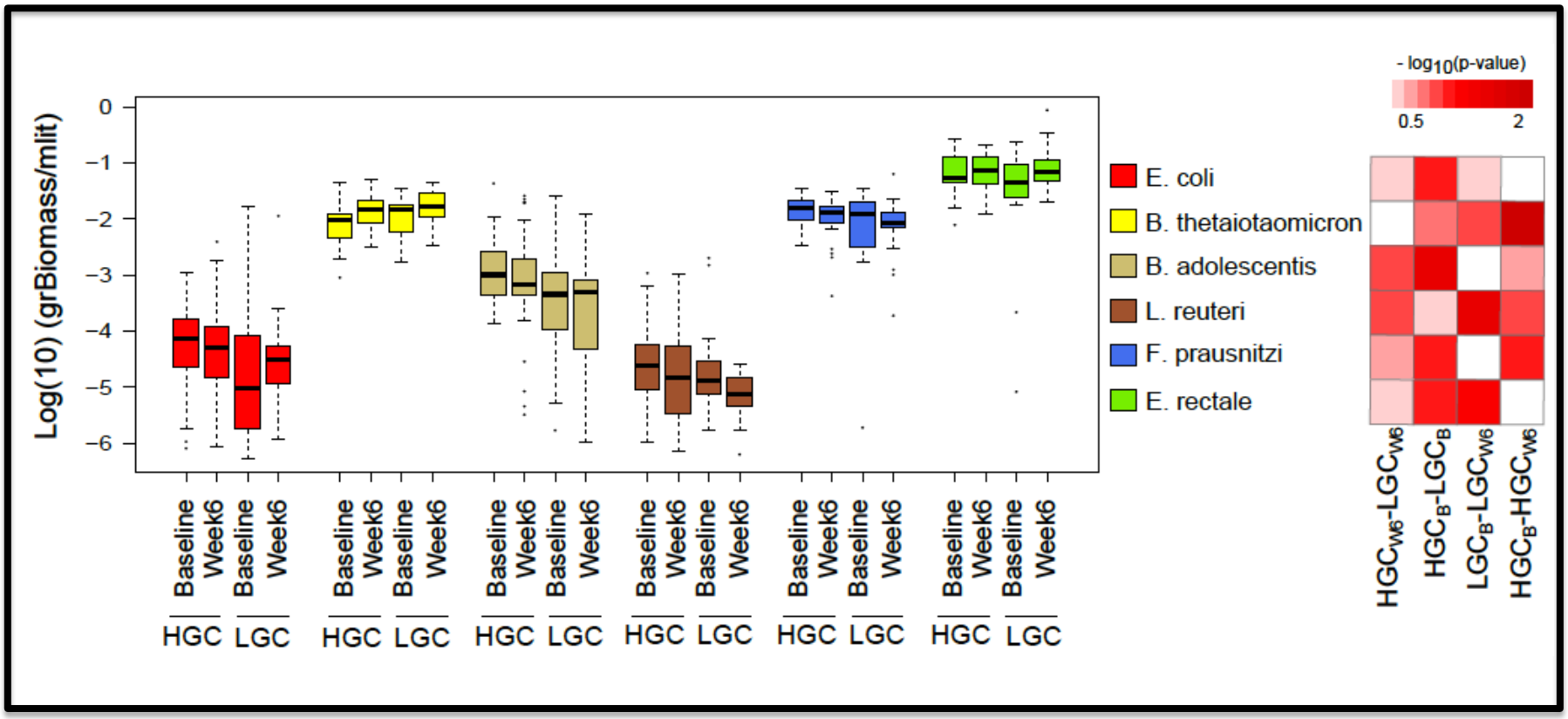
- *E. rectale*
- *F. prausnitzii*
- *B. adolescentis*
- *B. thetaiotaomicron*
- *R. bromii*

MicroObese Study

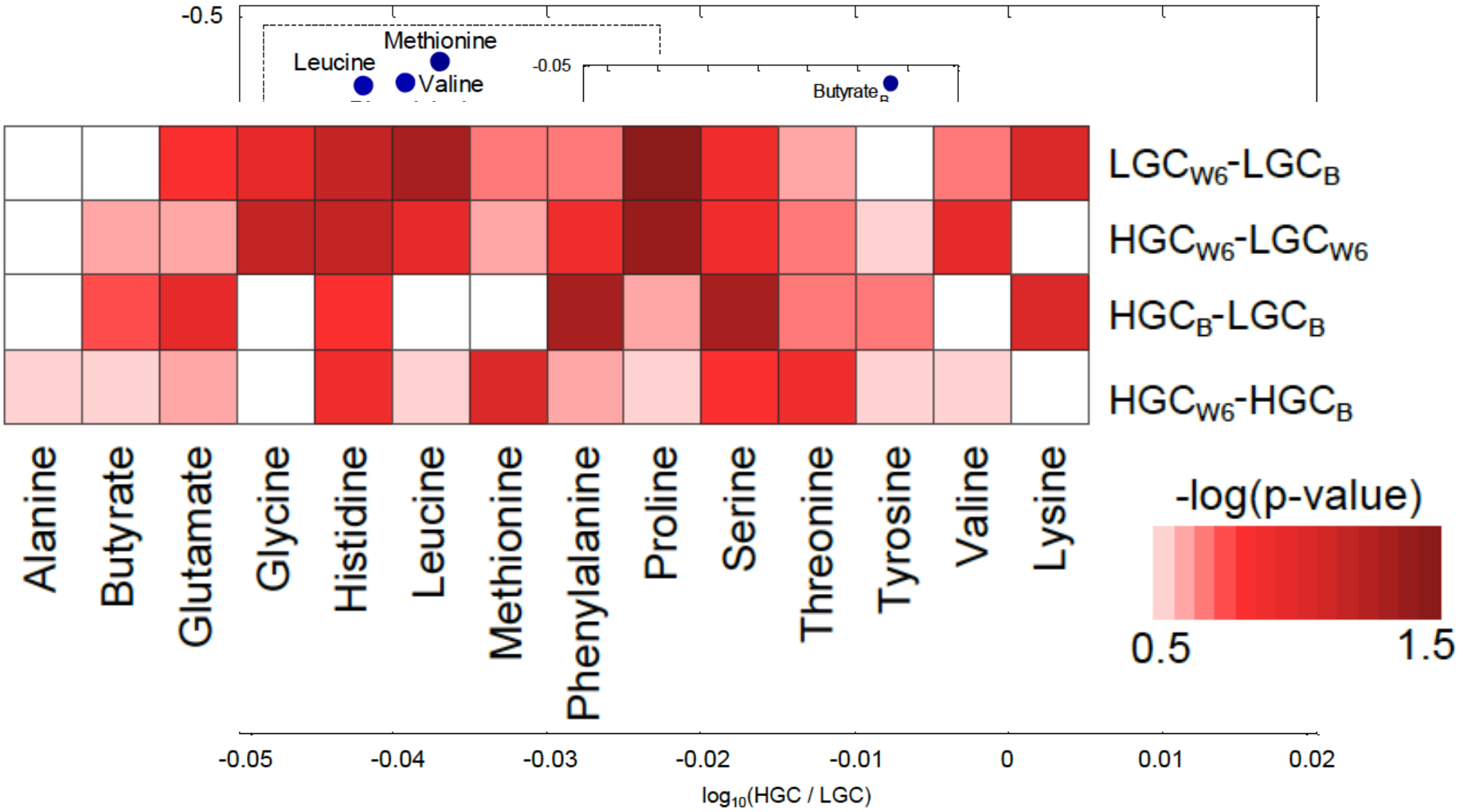
Changes in the diet between HGC and LGC according to time points



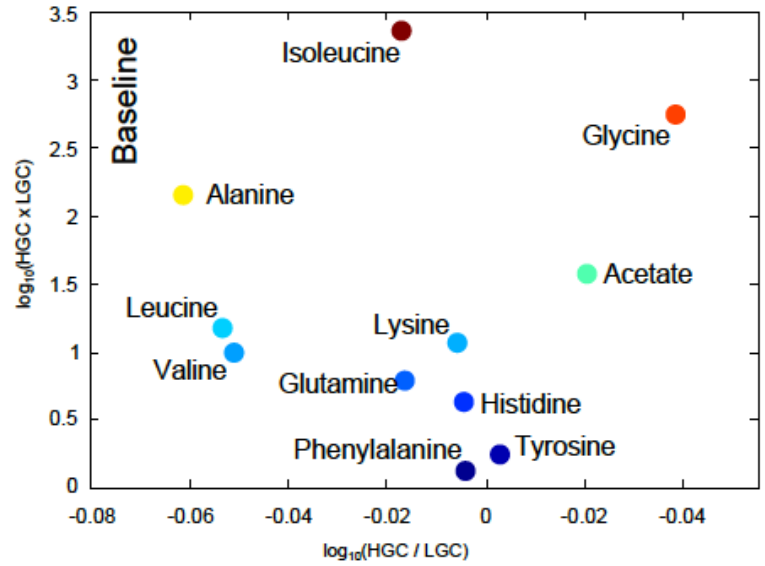
Abundance of the bacteria before and after diet interventions in HGC and LGC subjects

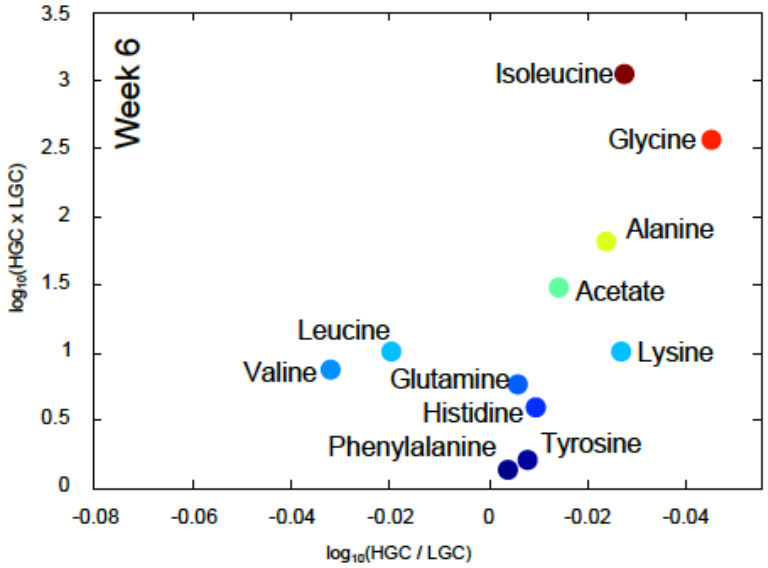


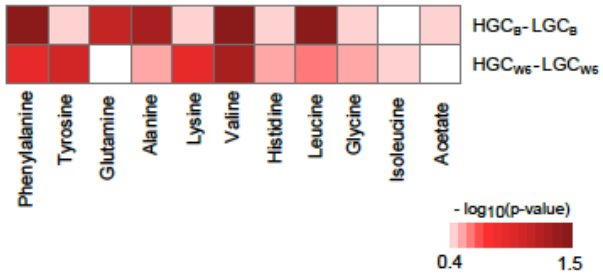
Summary of average predictions for fecal metabolites at baseline and after 6 weeks

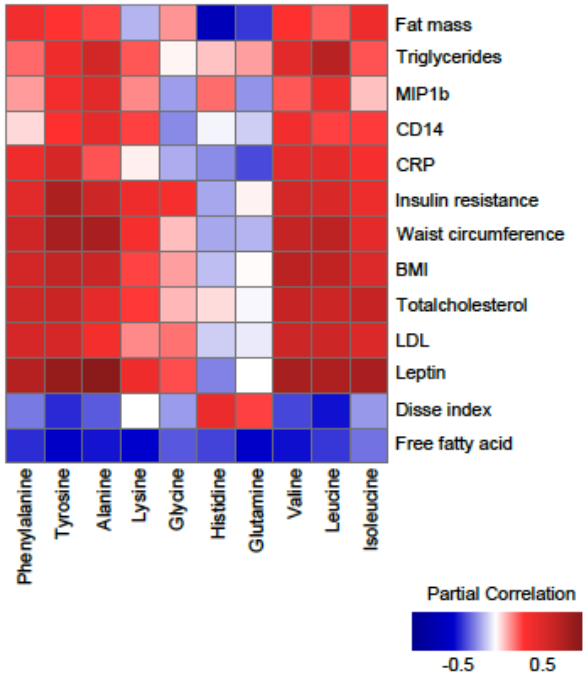


Also prediction of plasma metabolomics





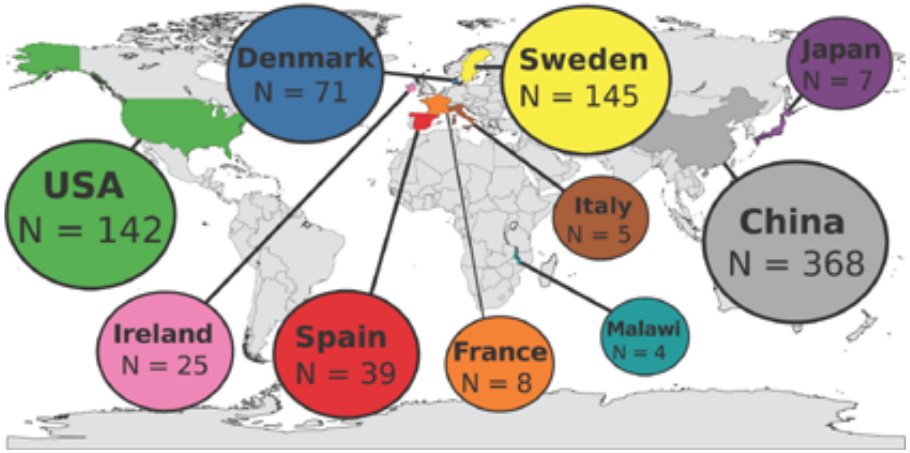




Both groups observe a decrease in plasma amino acids in response to diet intervention

LGC subjects have a larger relative reduction in plasma amino acid levels compared with the HGC subjects

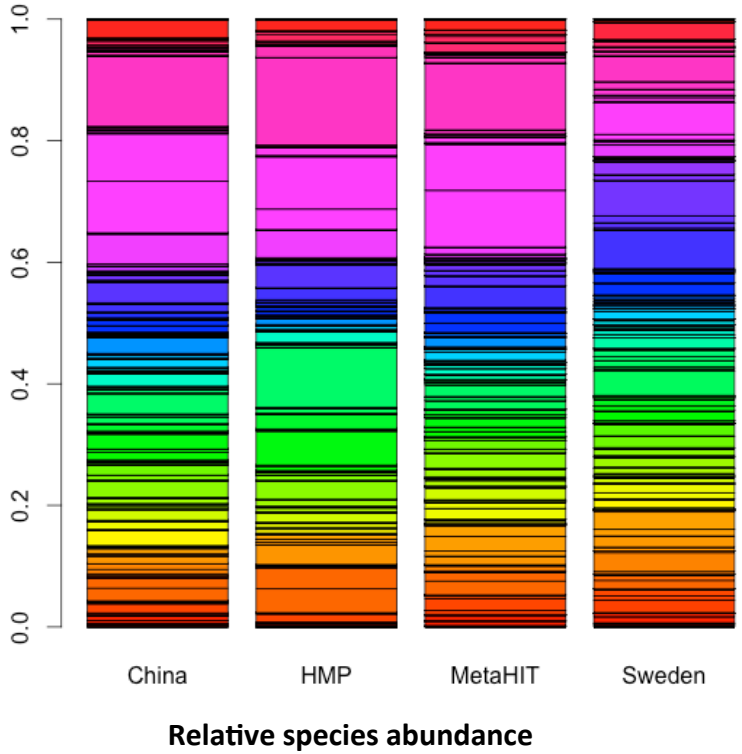
Using GEMs to study interaction between all key gut microbes



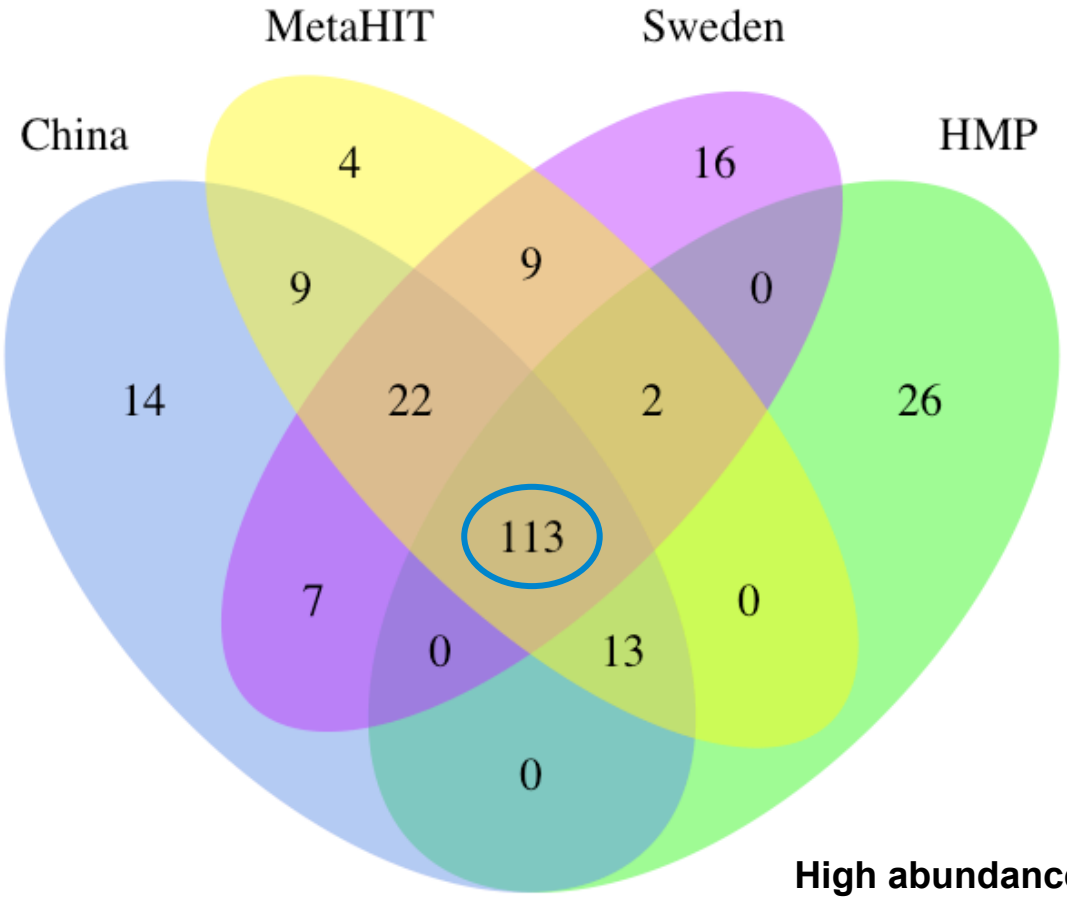
We used metagenomics data from:

- HMP (USA)
- MetaHIT(Denmark)
- China
- Sweden

to identify key species abundance



Selected Genomes for Model Reconstruction

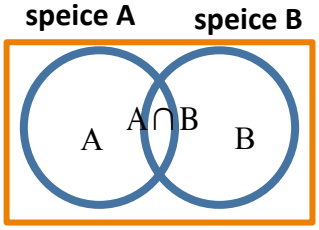


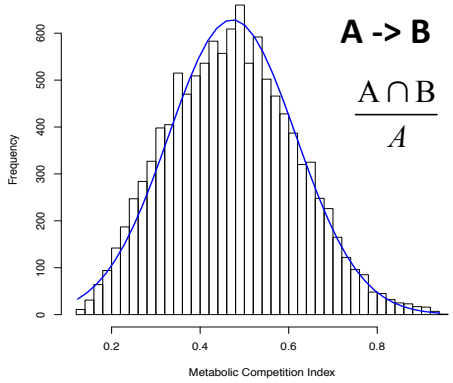
High abundance

113 species were identified to be of high abundance (>0.01%) in all four studies

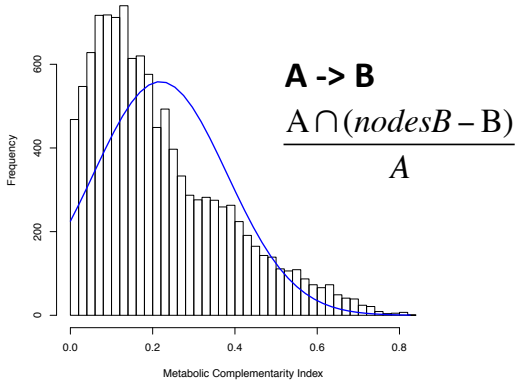
107 are available in the SEED database and these were analyzed further

Metabolic complementarity analysis

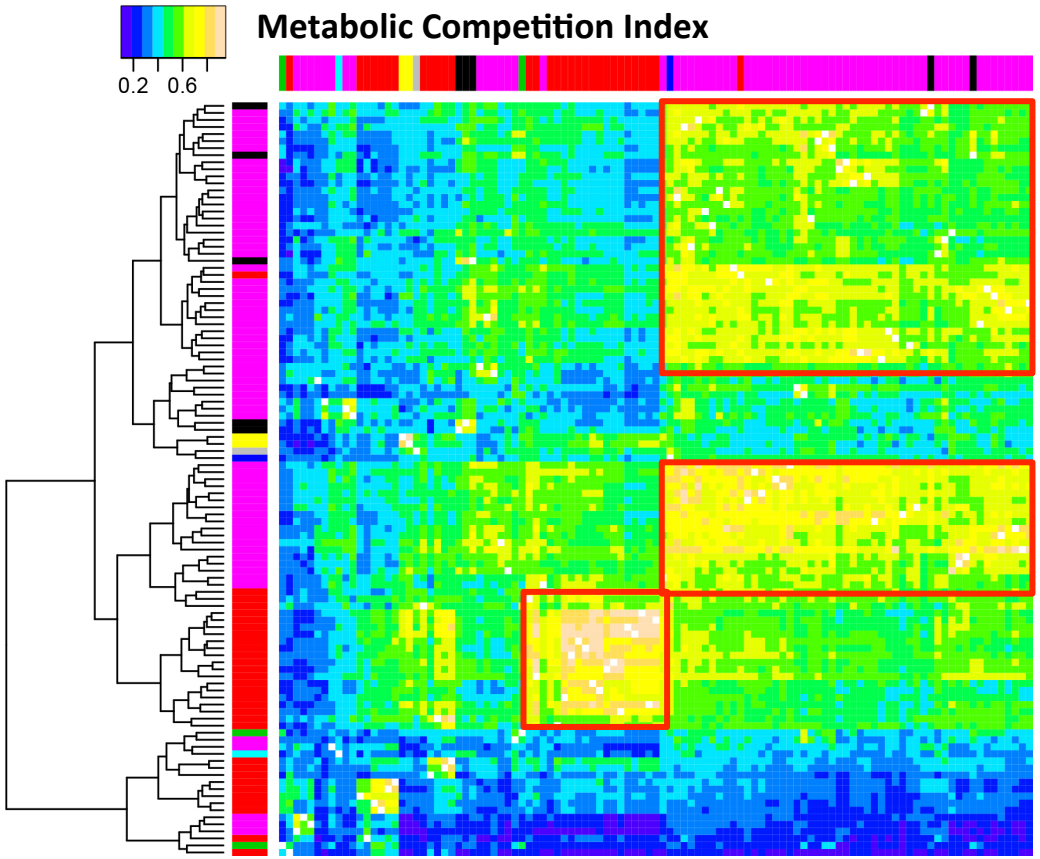




Metabolic Competition Index



Metabolic Complementarity Index

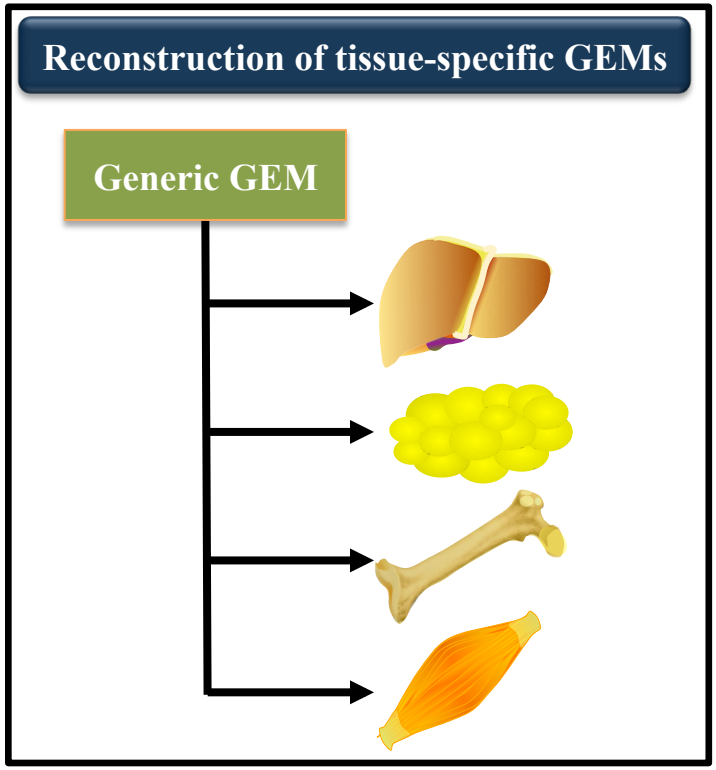


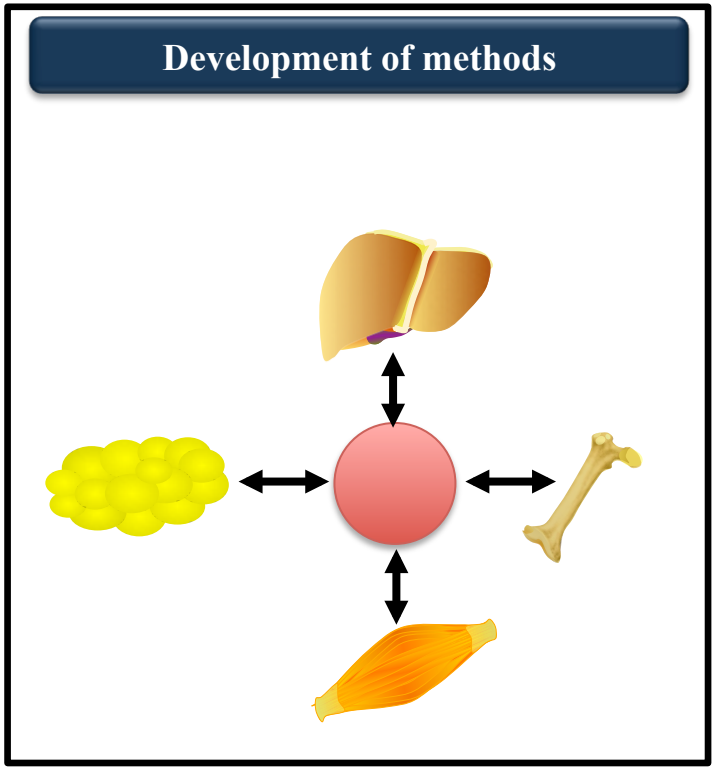
Strong Inter-Phylum Competetion

Indicates that competition, not cooperation, dominates metabolic interaction between gut microbes.

Improving Child Growth

We are collaborating with ***Bill & Melinda Gates Foundation*** for using our GEMs for finding innovative solutions to the problem of child development in Africa.
We are analyzing how metabolism responds to nutrition and **malnutrition in children.**





Prospects for Personalized Medicine

Use of gut metagenomics for stratification

- e.g. for responders and non-responders to immuno-therapies in cancer treatment

Development of second-generation probiotics for disease treatment

- T2D diabetes
- Combination drugs for cancer treatment

As for early days in human genomics we are still only learning, but we may see new therapies within the next 3-5 years

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Karine Clement, ICAN (France)
Marc Dumas, ICL (UK)

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