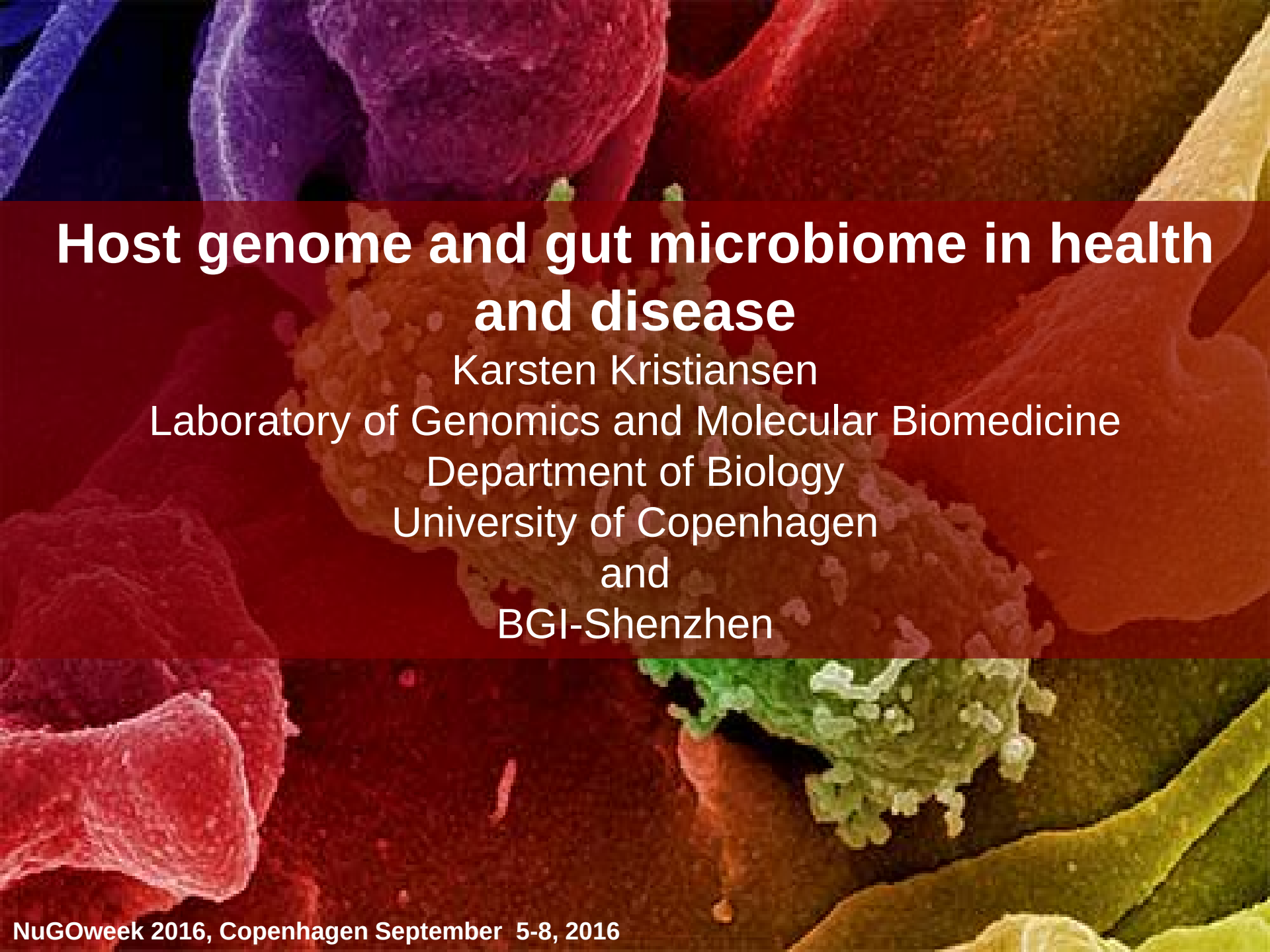
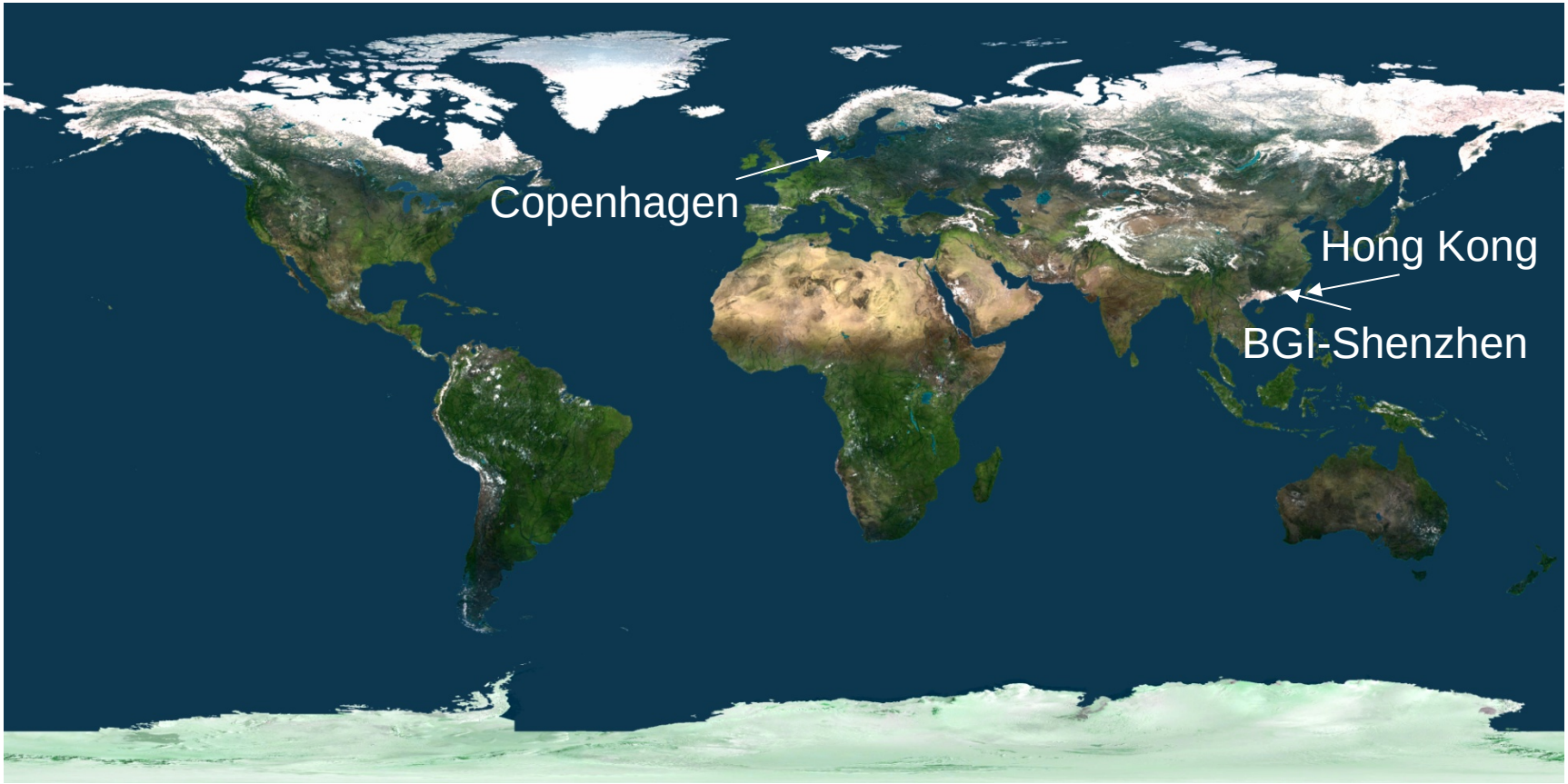


Host genome and gut microbiome in health and disease

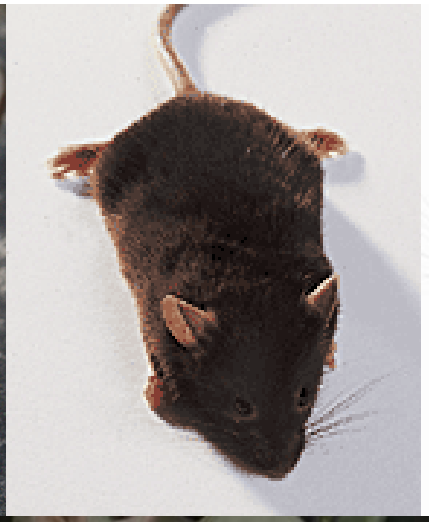
Karsten Kristiansen
Laboratory of Genomics and Molecular Biomedicine
Department of Biology
University of Copenhagen
and
BGI-Shenzhen

NuGOweek 2016, Copenhagen September 5-8, 2016

Where is Shenzhen?



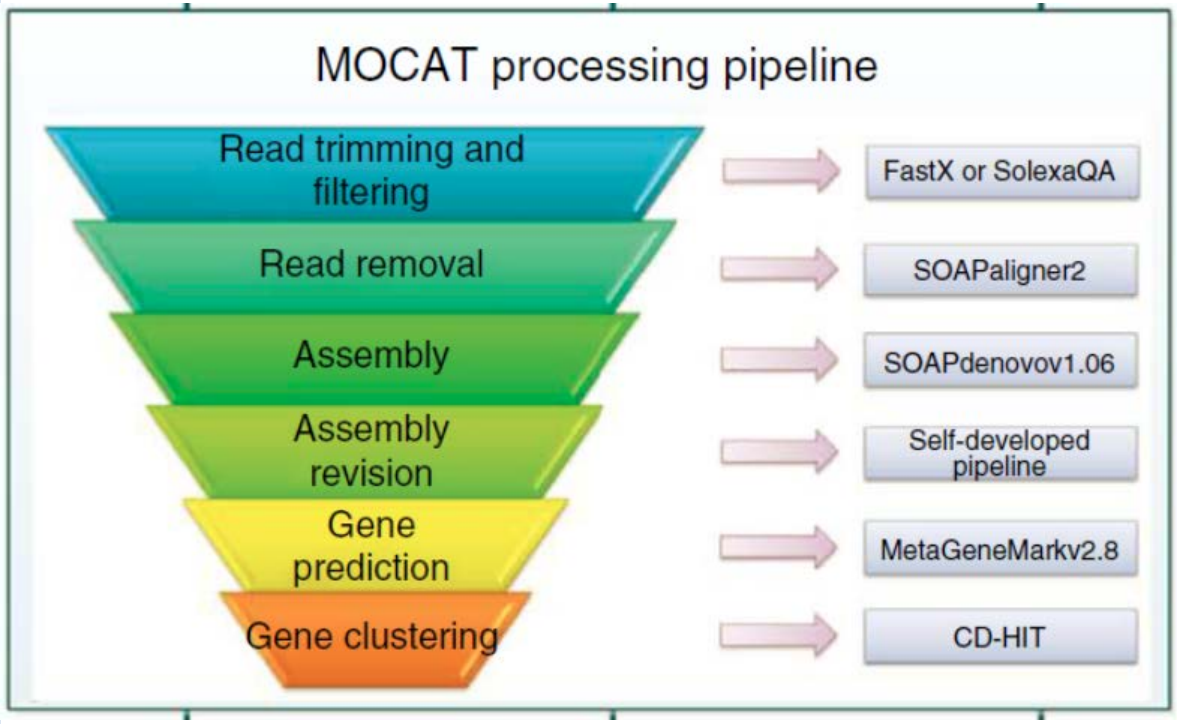
The mouse and the pig gut metagenome



Building a mouse gut microbial gene catalog

- 184 mice
  - different providers
  - different laboratories
  - different strains
  - different diets
  - males and females
- 1.13 Terabyte using Illumina HiSeq
- >5Gb on average
- 2.57 million non-redundant genes
- 541 metagenomic species



Li et al. Nature Biotech. 32, 834-841, 2014

Xiao et al. Nature Biotechnology, 33, 1103-1108, 2015

Building a pig gut microbial gene catalog

7,685,872 non redundant genes
719 metagenomic species

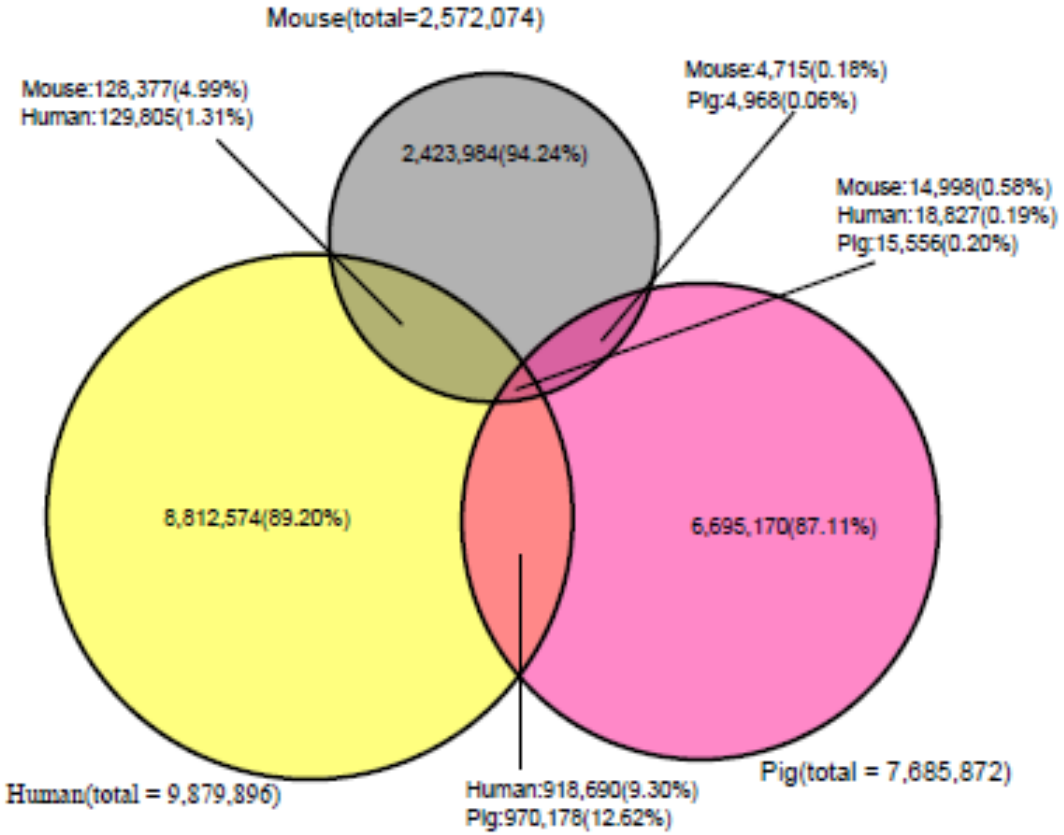
287 animals: 17 breeds or selected lines,
11 farms, various ages and diets, 3 countries

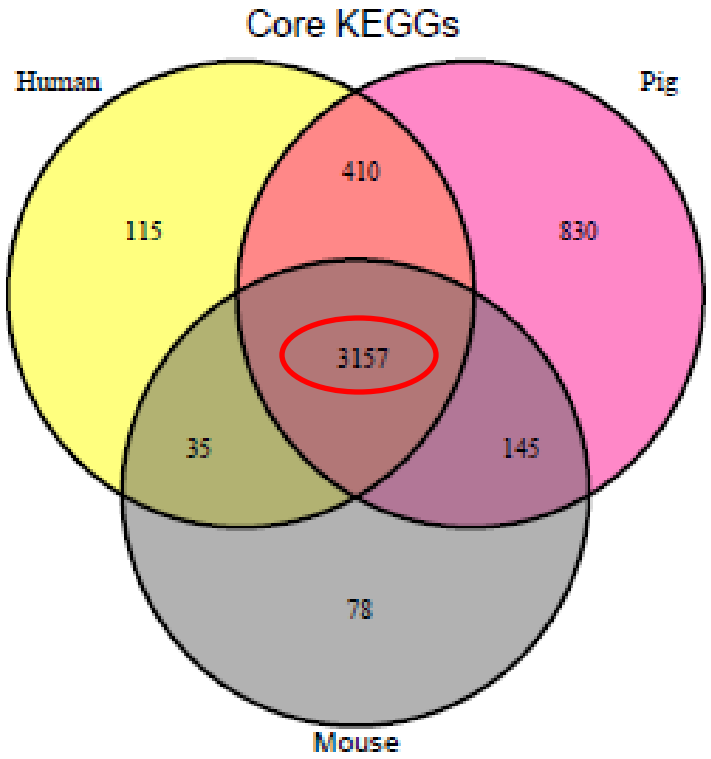
- Native pigs:
  - France ,West Indies: creole pigs
  - China: Bama, Ba Ring, Tibetan
- Selected breeds
  - France: Large White (LW), Large White X Landrace, (Large White X Landrace) X Pietrain, Pietrain, Meishan
  - Denmark: (Landrace x Yorkshire) X Duroc
  - China: Large White, binary mixed, tertiary mixed
- Miniature model pigs
  - Yucatan
  - Pitman-Moore
  - MeLiM
  - Vietnamese

Xiao et al. Nature Microbiology, in press

Of mice and men – and pigs

Comparison of catalogs





Pigs – pink
Human – yellow
Mice - grey

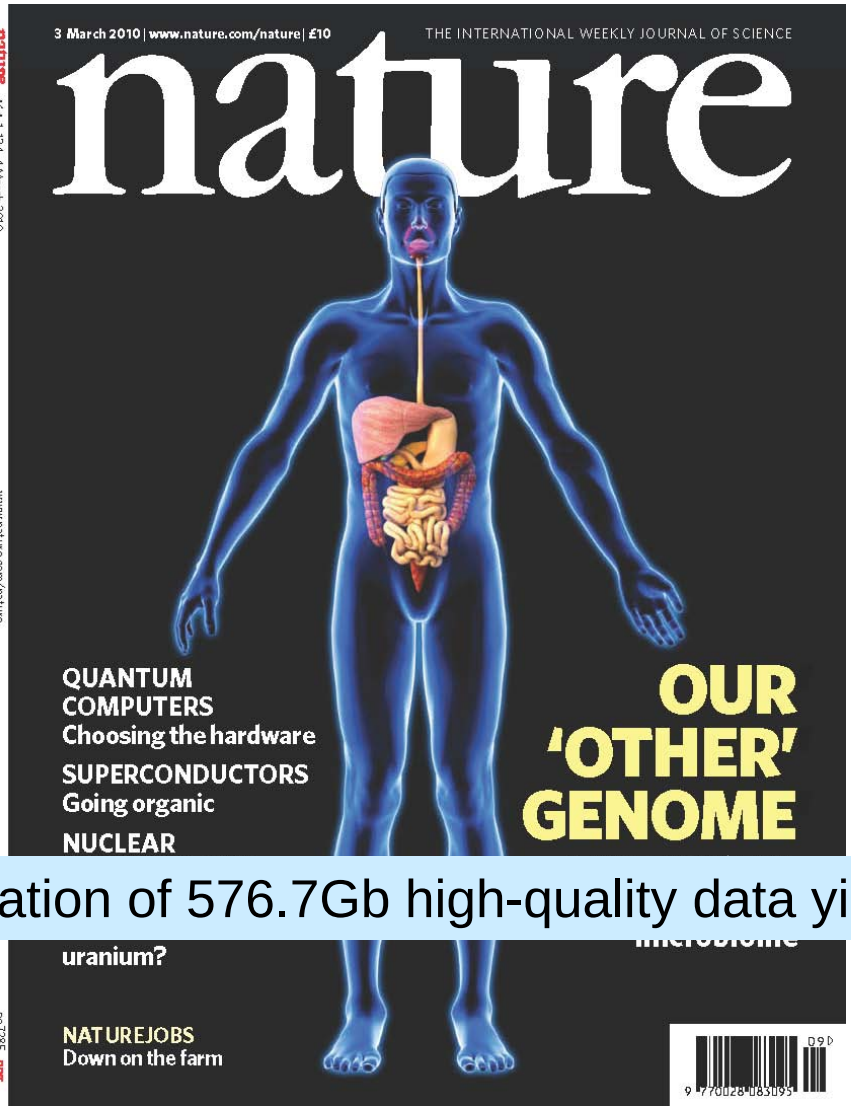
Xiao et al. Nature Microbiology, in press

Take home messages

- Comprehensive gene catalog of the gut microbiome of mice comprising 2.57 x 10^6 non-redundant genes and 541 metagenomic species
- Comprehensive gene catalog of the gut microbiome of pigs comprising 7.7 x 10^6 non redundant genes and 719 metagenomic species
- A larger set of genes shared between the pig and the human gut microbiomes than between the mouse and the human gut microbiomes, but overall limited overlap between species
- In spite of limited overlap of genes, substantial overlap between metabolic pathways and functions

Xiao et al. Nature Microbiology, in press

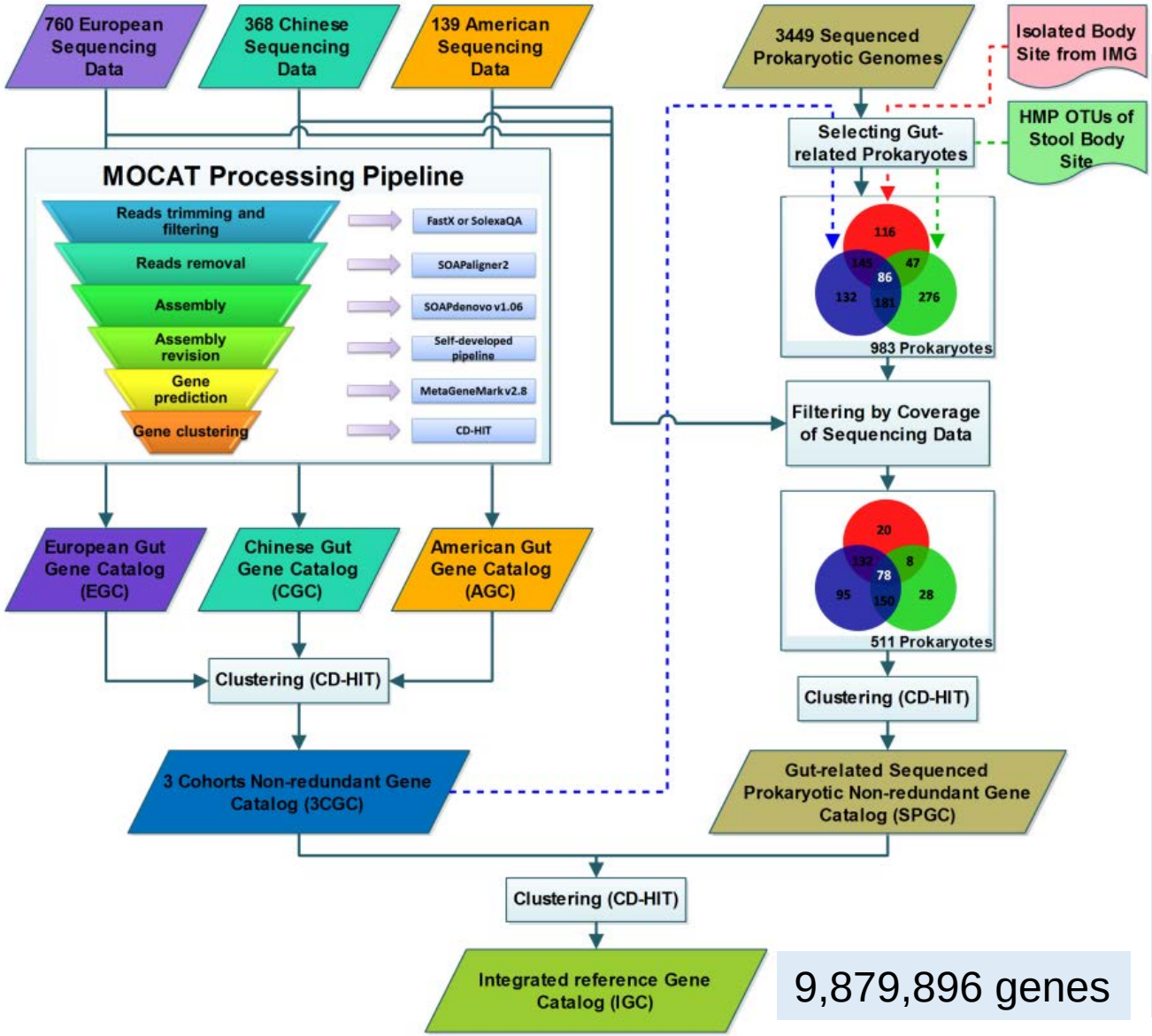
Towards the human gut metagenome



2010: In total, generation of 576.7Gb high-quality data yielding 3.3 mio. genes

Qin et al. Nature 464, 59-65, 2010

10 million human gut microbiome gene catalog



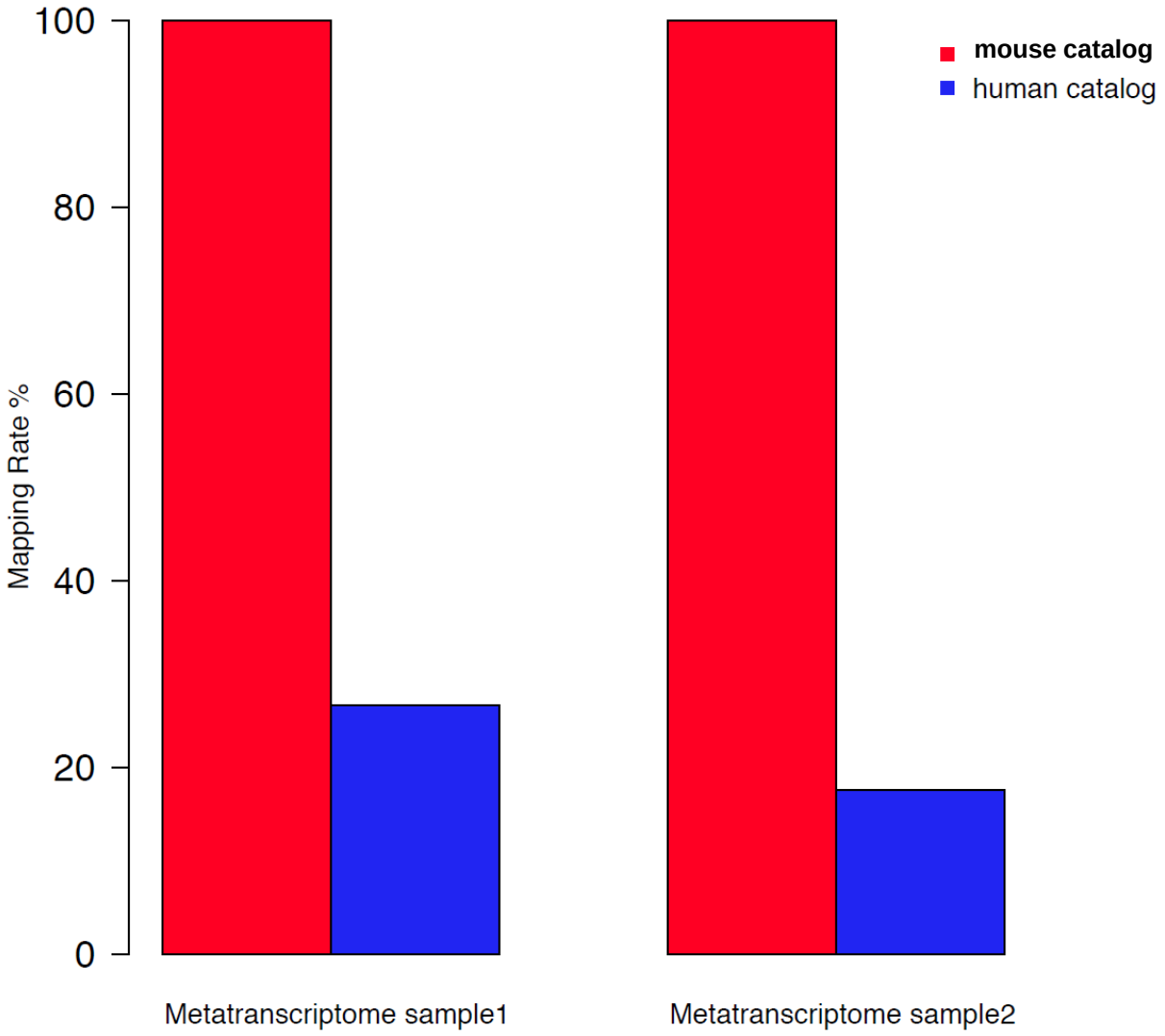
- 3 distinct cohorts
- 1,267 samples
- 6.4Tb data
- Illumina-based catalog construction pipeline
- Integrated with genomes from 511 gut-related prokaryotes
- ~10M genes in Integrated gene catalog
- IGC

Li et al. Nature Biotech. 32, 834-841, 2014

Why do we need gut microbiome gene catalogs?

- Better analyses of functional competence than possible based on 16S rDNA amplicon sequencing
- Important for comprehensive metatranscriptomics and metaproteomics
- Construction of metagenomic species/ metagenomic linkage groups
- Eventually cheaper analyses using Complete Genomics technology and mapping onto reference catalogs

Humans: not good enough for mice
A meta-transcriptomics example

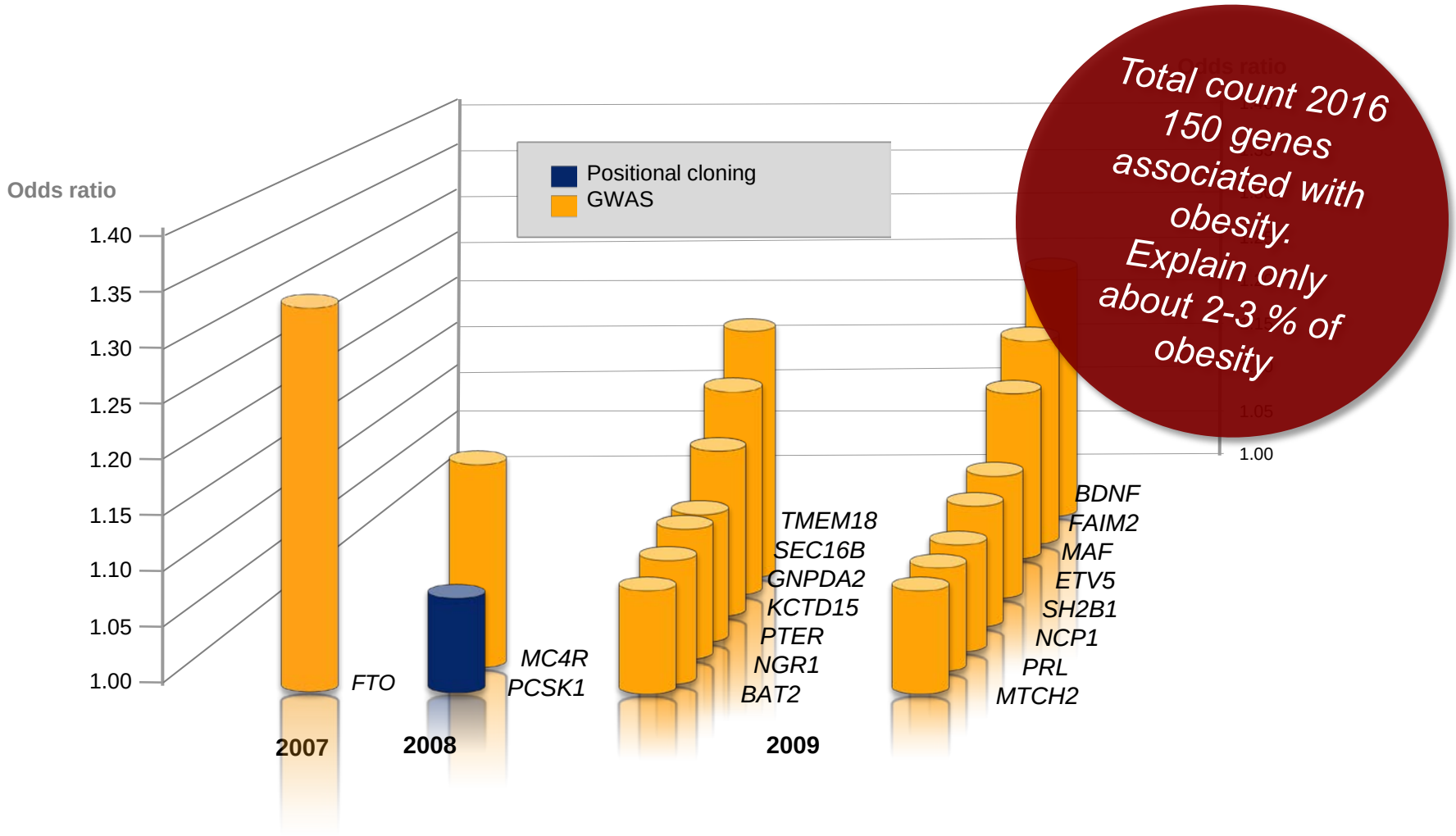


Xiao et al. Nature Biotechnology, 33, 1103-1108, 2015

Photograph by Karen Kasmauski © National Geographic 2007

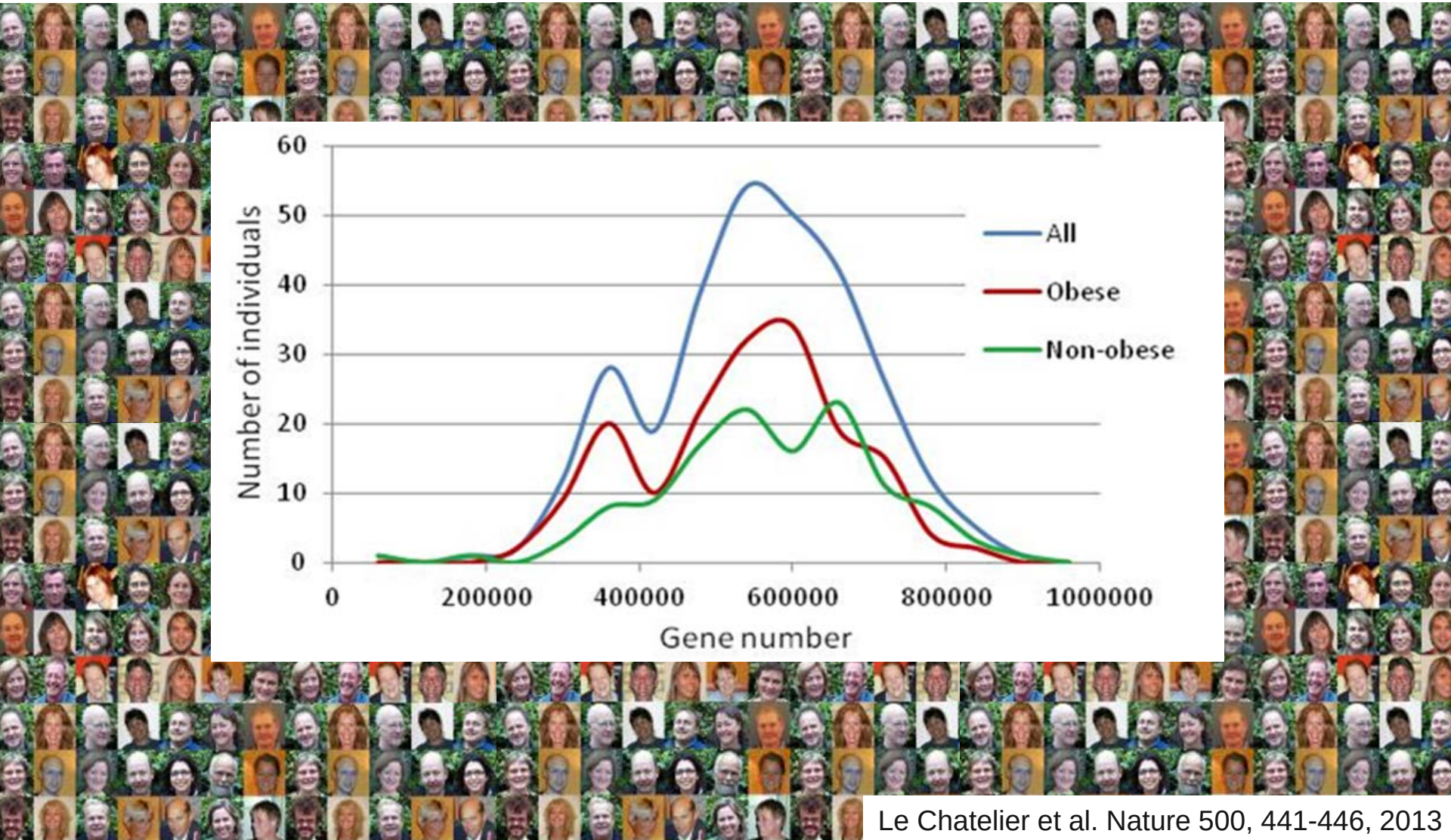
50 risk loci associate with obesity/increased BMI with genome-wide significance

Each of them are common but only increase risk of obesity with 8-33%



The two faces of obesity – healthy or unhealthy

Bimodal distribution of 292 non-diabetic Danish individuals

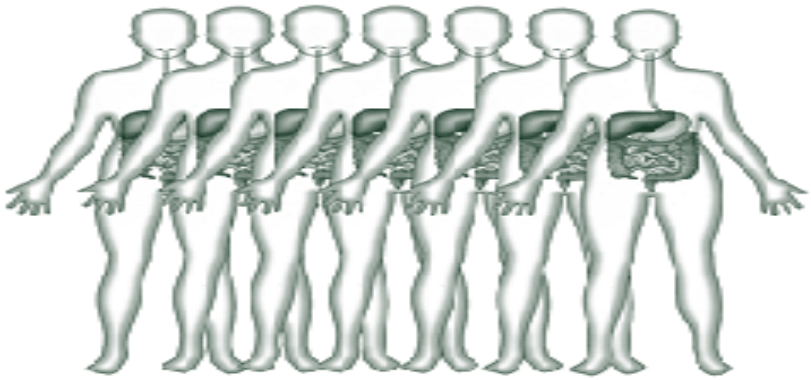


Le Chatelier et al. Nature 500, 441-446, 2013

LGC individuals are more obese, more insulin resistant and more proinflammatory than HGC individuals

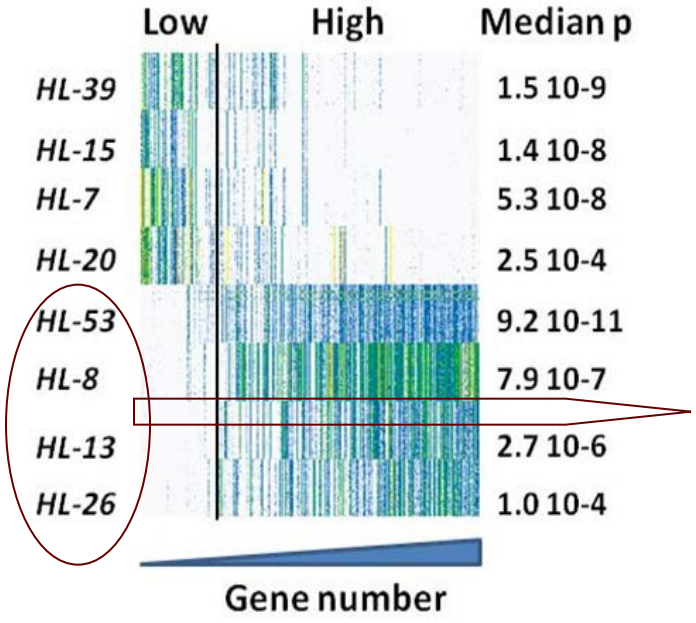
Low gene count individuals constitute about 23% of the population in Denmark. Compared with high gene count individuals they are characterized by:

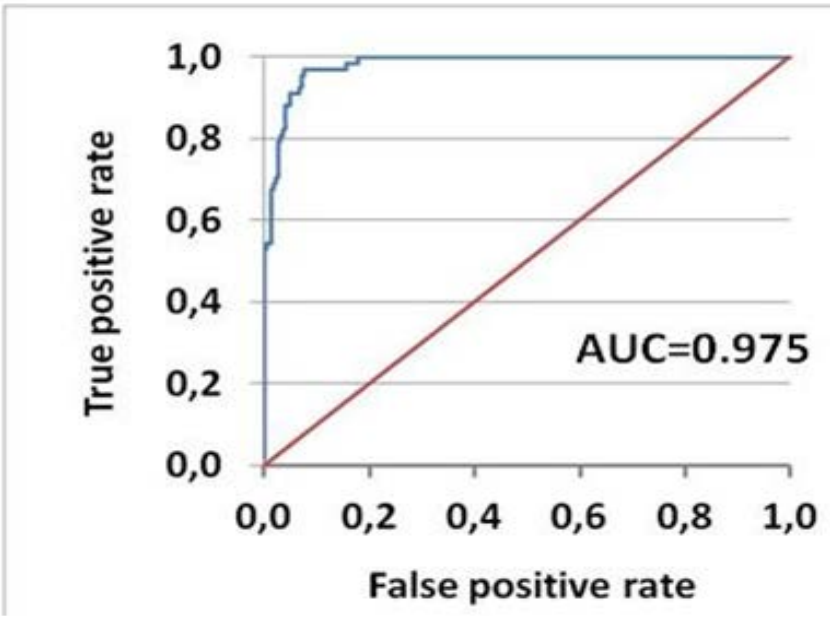
- overall adiposity
- elevated serum leptin,
- decreased serum adiponectin,
- insulin resistance and hyperinsulinaemia
- dyslipidaemia
- a more marked inflammatory phenotype



Le Chatelier et al. Nature 500, 441-446, 2013

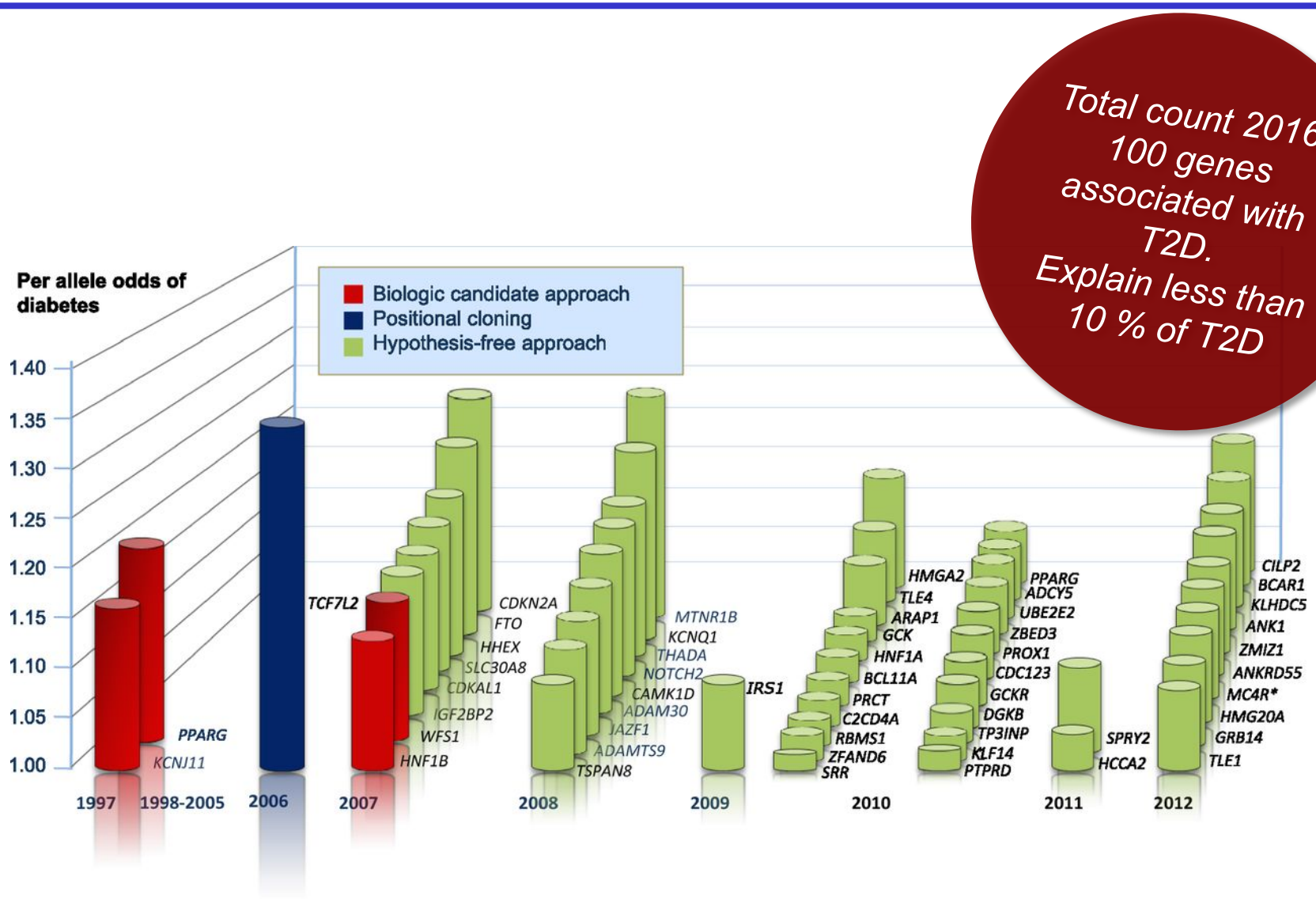
Four metagenomics linkage groups characterize low and high gene count individuals





Le Chatelier et al. Nature 500, 441-446, 2013

The evolving landscape of established type 2 diabetes-associated genetic loci



Franks et al. Diabetes Care 36:1413-1421, 2013

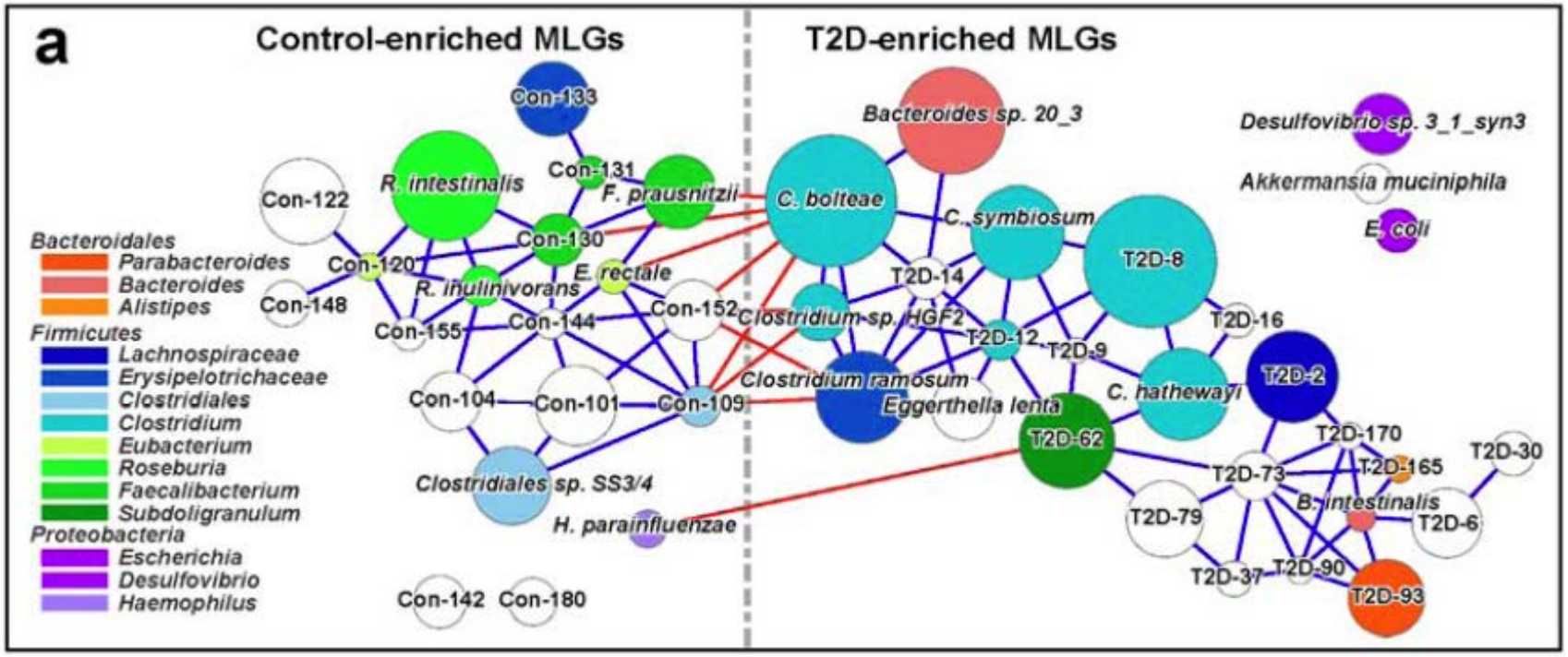
The genetic architecture of type 2 diabetes

A list of authors and affiliations appears in the online version of the paper

The genetic architecture of common traits, including the number, frequency, and effect sizes of inherited variants that contribute to individual risk, has been long debated. Genome-wide association studies have identified scores of common variants associated with type 2 diabetes, but in aggregate, these explain only a fraction of the heritability of this disease. Here, to test the hypothesis that lower-frequency variants explain much of the remainder, the GoT2D and T2D-GENES consortia performed whole-genome sequencing in 2,657 European individuals with and without diabetes, and exome sequencing in 12,940 individuals from five ancestry groups. To increase statistical power, we expanded the sample size via genotyping and imputation in a further 111,548 subjects. Variants associated with type 2 diabetes after sequencing were overwhelmingly common and most fell within regions previously identified by genome-wide association studies. Comprehensive enumeration of sequence variation is necessary to identify functional alleles that provide important clues to disease pathophysiology, but large-scale sequencing does not support the idea that lower-frequency variants have a major role in predisposition to type 2 diabetes.

The gut microbiota and type 2 diabetes

Metagenomic Linkage Groups (MLGs) enriched in controls and T2D patients



Qin et al, Nature 490, 55-60, 2012

Bacteria associated with an increased risk of type 2 diabetes

	Increased risk
Highest risk gene *TCF7L2* (Inuit specific TBC1D4	1.47 X 10.3 X)
Clostridium bolteae	5.89 X
Clostridium hatheway	23.1 X

Qin et al, Nature 490, 55-60, 2012

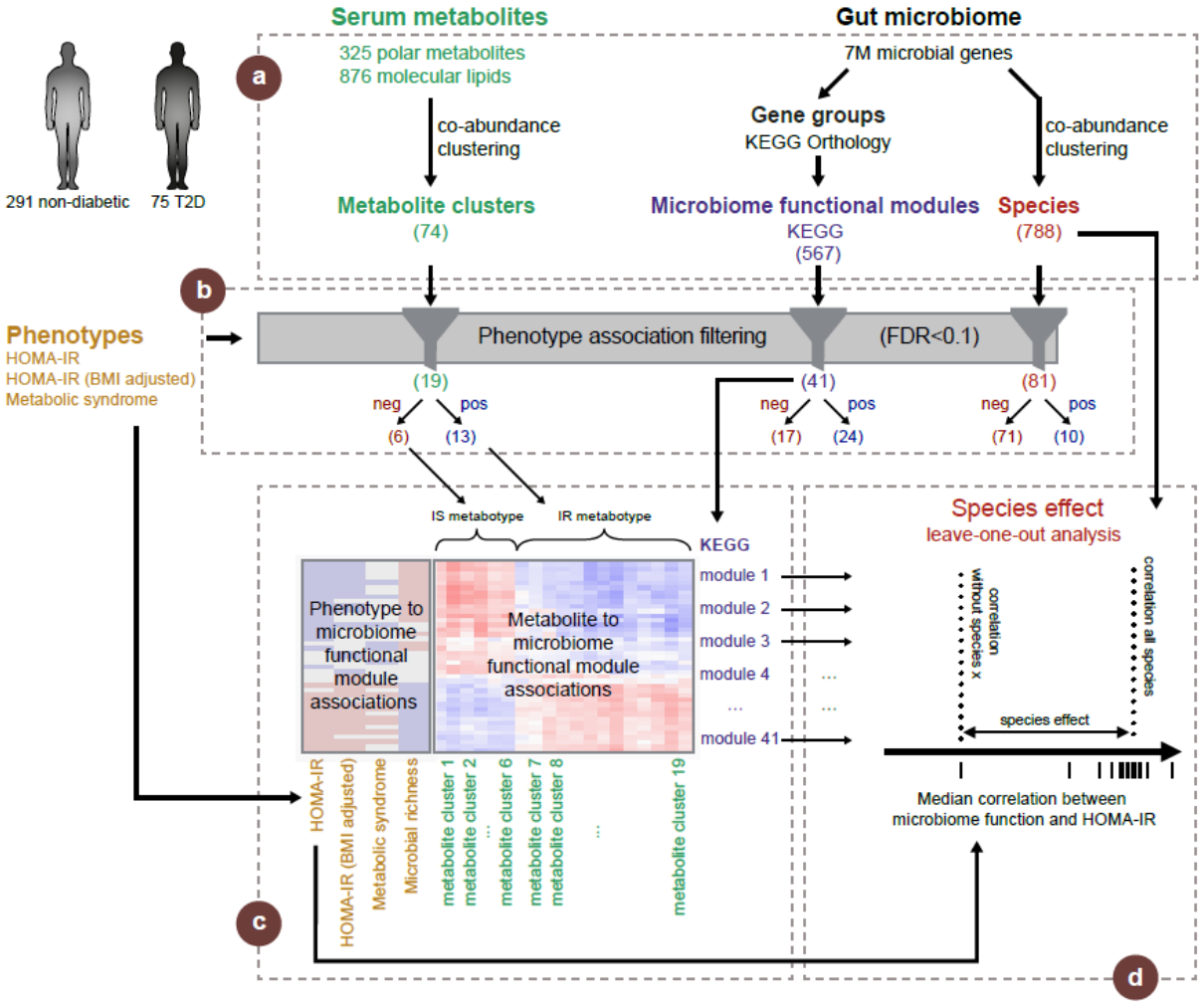
Branched-chain amino acids (BCAAs) and insulin resistance

Branched-chain amino acids and metabolism – a complex interplay

- Fatty acids (FA) and FA-derived metabolites have long been implicated in the development of insulin resistance and type 2 diabetes
- Branched-chain amino acids (BCAA) and related metabolites are more strongly associated with insulin resistance than many common lipid species
- BCAA-related signature is predictive of incident diabetes and intervention outcomes and uniquely responsive to therapeutic interventions
- BCAA supplementation requires the background of a high-fat diet to promote insulin resistance

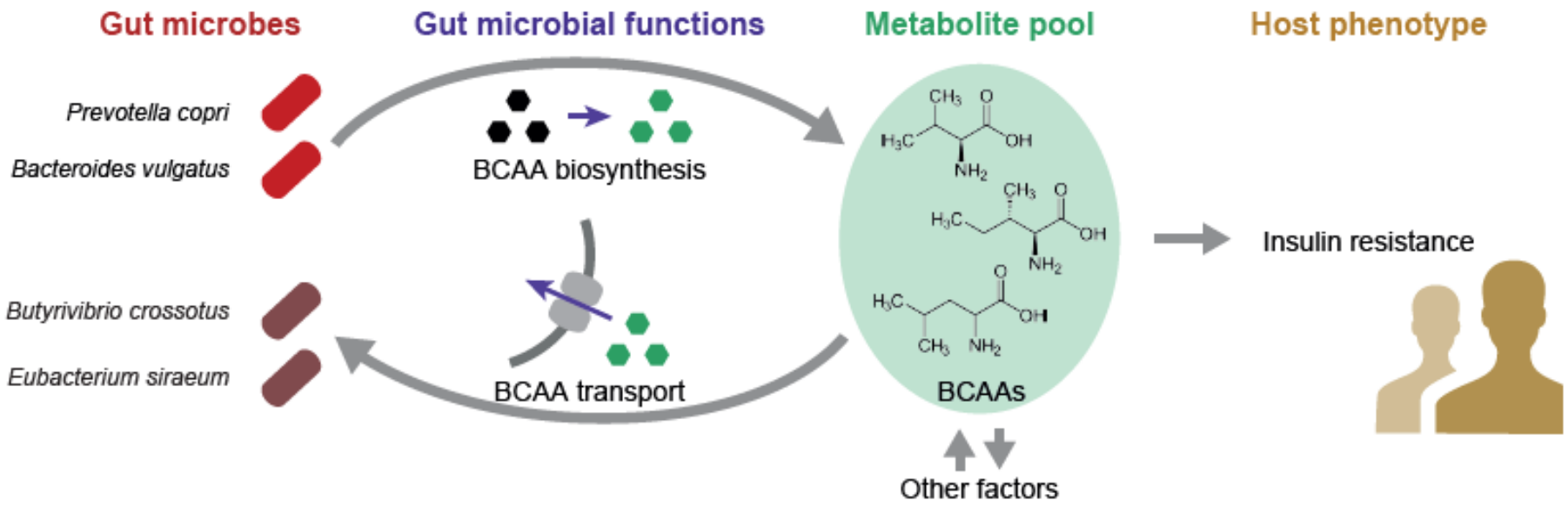
Christopher B. Newgard Cell Metab. 15, 606-614, 2012

Insulin resistance correlates with increased levels of serum BCAAs and fecal *Prevotella copri*



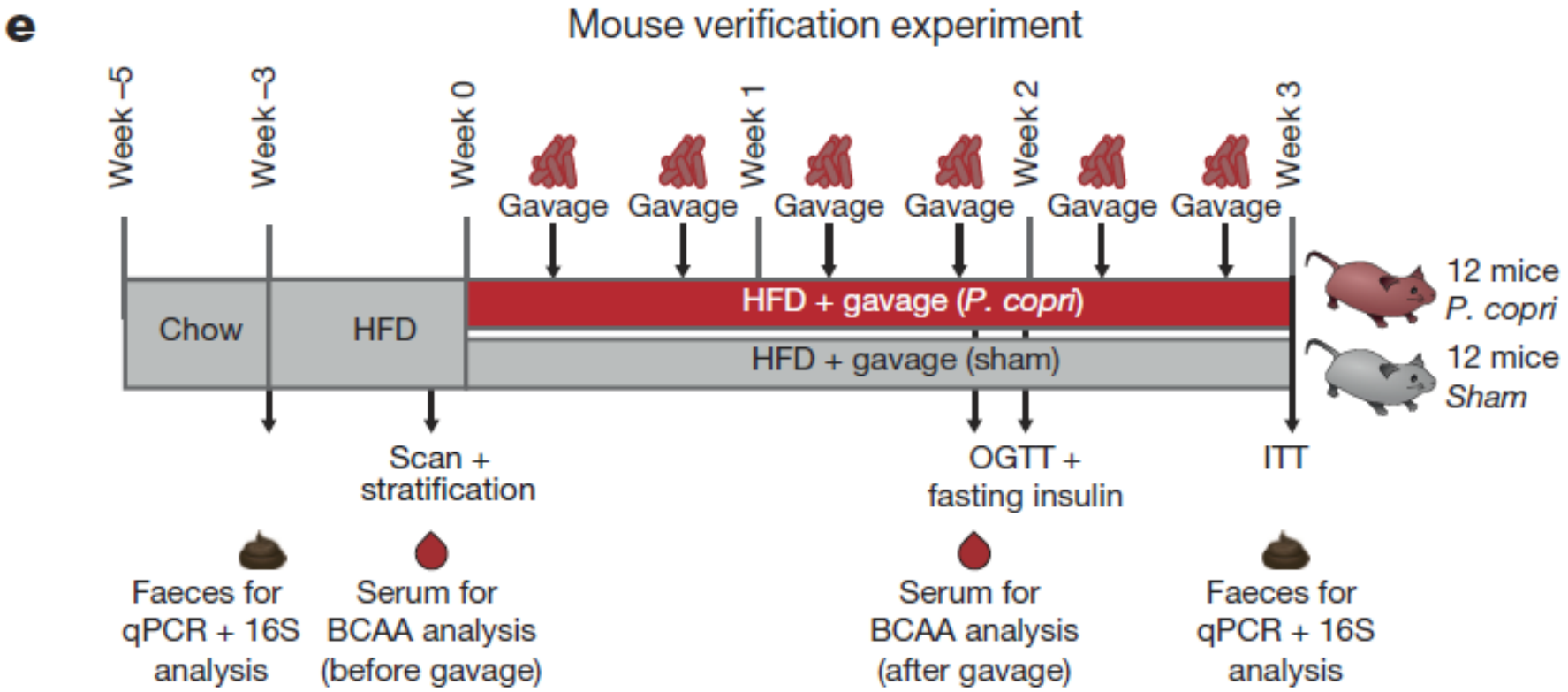
Pedersen et al. Nature 535, 376-381, 2016

Insulin resistance correlates with increased levels of serum BCAAs and fecal *Prevotella copri*



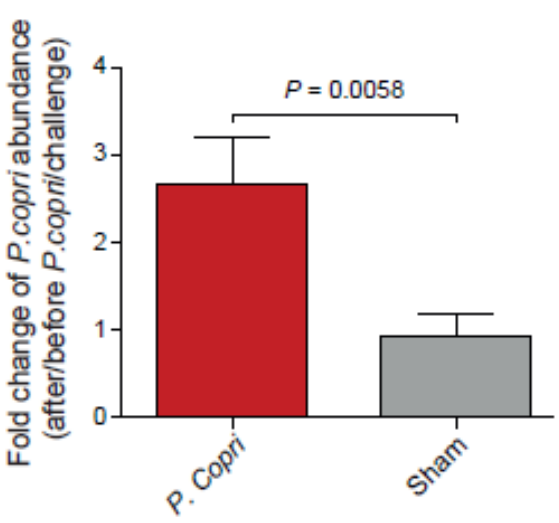
Pedersen et al. Nature 535, 376-381, 2016

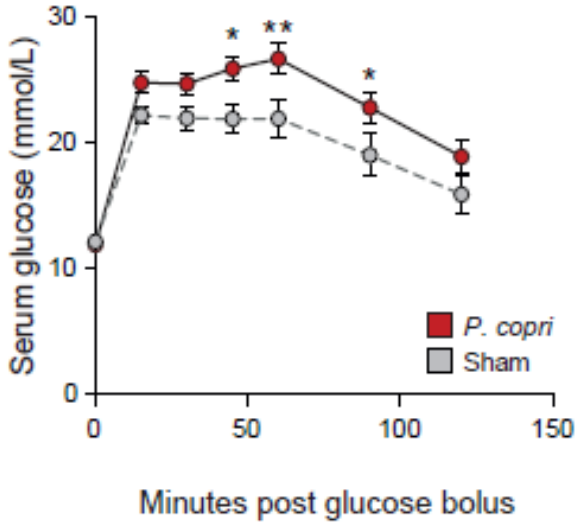
Causal relationship between *P. copri* and plasma BCAAs, glucose intolerance and insulin resistance

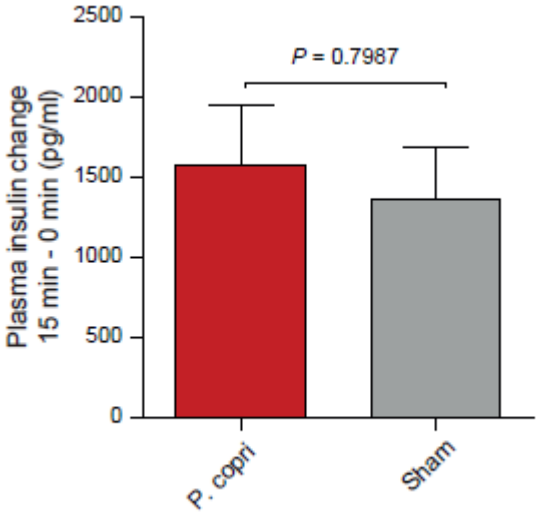


Pedersen et al. Nature 535, 376-381, 2016

Gavage with *P. copri* increases the relative abundance in fecal samples and impairs glucose tolerance





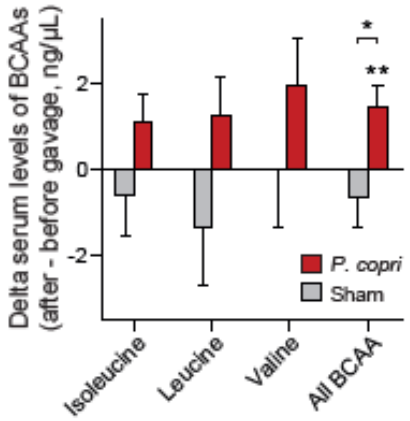


Pedersen et al. Nature 535, 376-381, 2016

Gavage with *P. copri* increases serum BCAAs and decreases inslun sensitivity

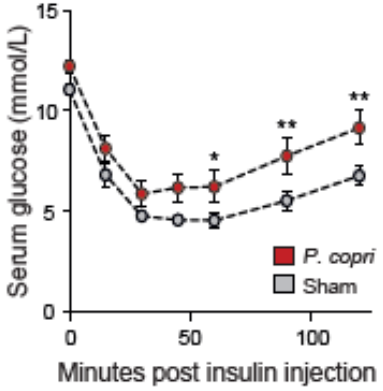
f

Serum BCAA levels are increased in *P. copri* compared to Sham gavaged mice



g

Insulin sensitivity is diminished in *P. copri* compared to Sham gavaged mice



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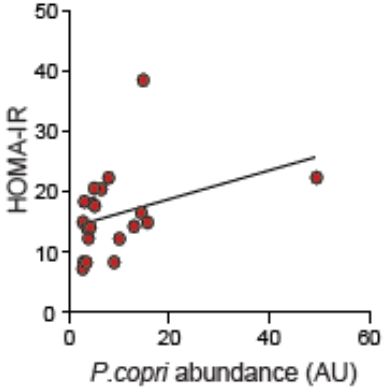
Fecal *P. copri* abundance correlates with HOMA-IR



Pedersen et al. Nature 535, 376-381, 2016

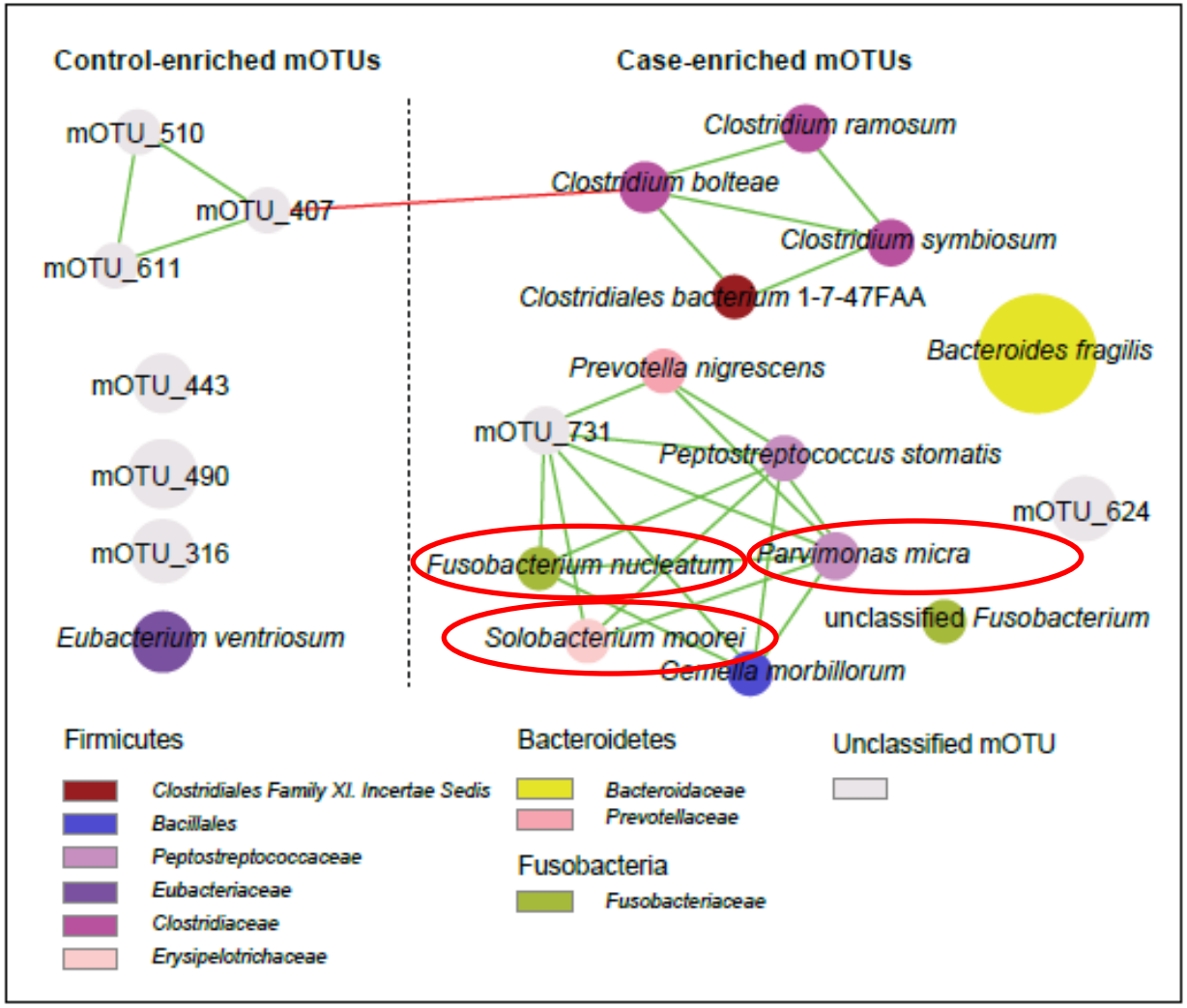
Take home message

Microbial synthesis of branched chain amino acids seems to be causally associate with insulin resistance

Colorectal cancer

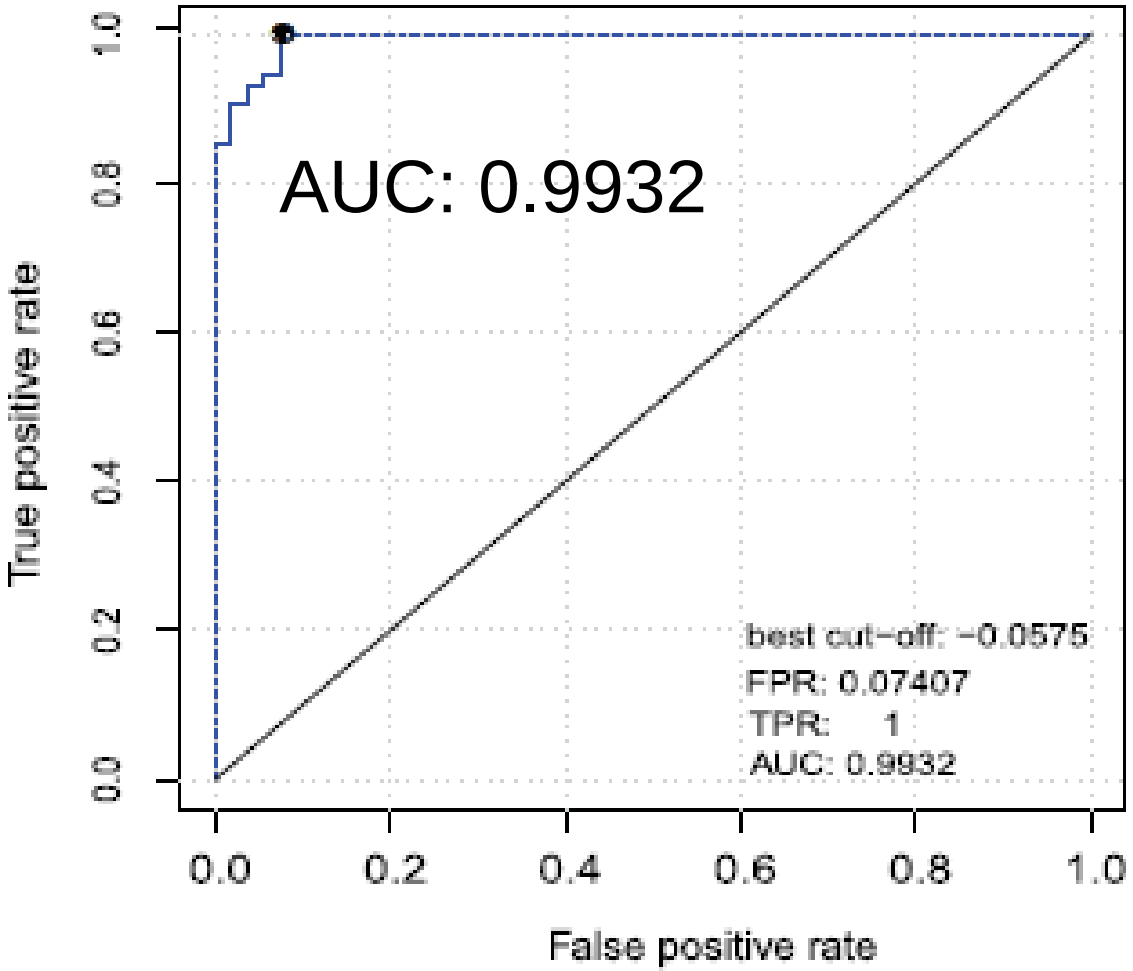
- 74 Han Chinese colorectal cancer patients
- 54 Healthy Han Chinese individuals
- 16 Danish colorectal cancer patients
- 24 Healthy Danish individuals
- Discovery phase using Chinese samples
- Validation phase using Danish samples supplemented by published data on Austrian and French patients

Co-occurrence network deduced from relative abundance of 21 mOTUs associated with CRC



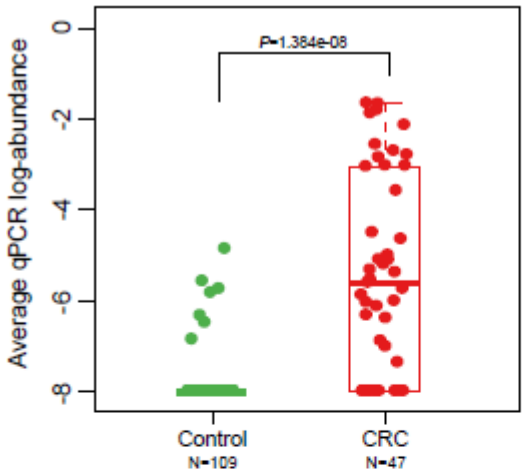
Yu et al. Gut 2015, doi: 10.1136/gutjnl-2015-309800

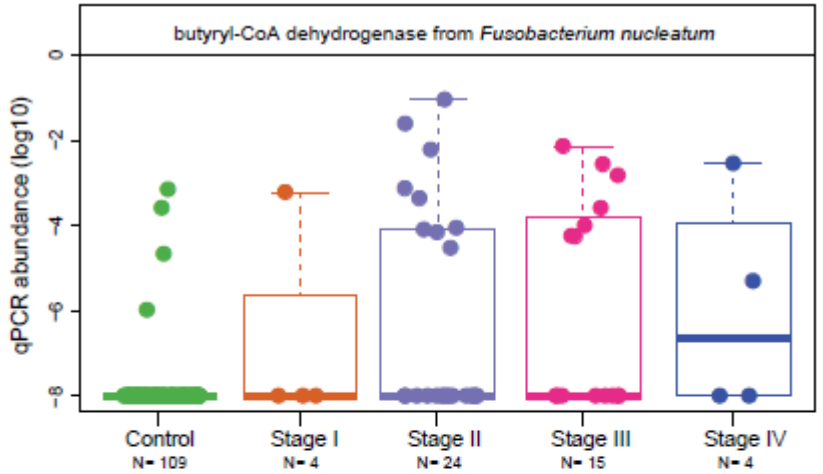
Discovering gut microbial gene markers associated with CRC based on 31 markers



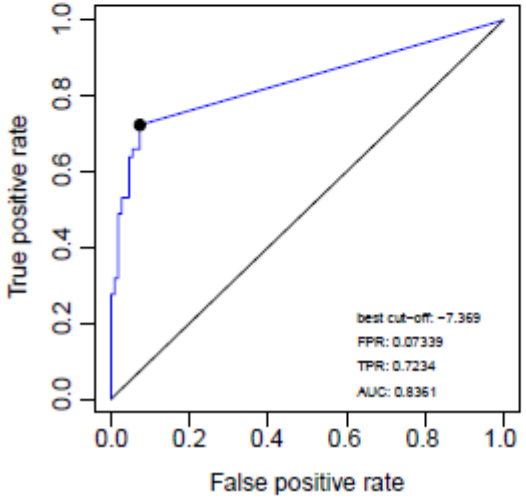
Yu et al. Gut 2015, doi: 10.1136/gutjnl-2015-309800

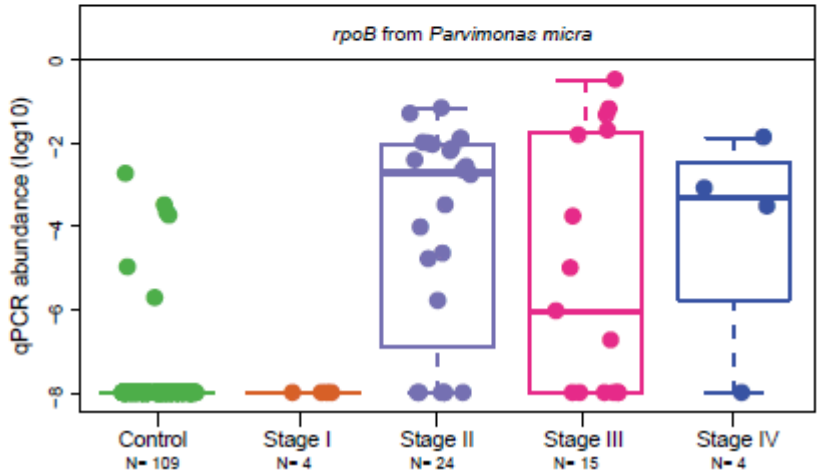
Validating a few robust gene markers associated with CRC





D

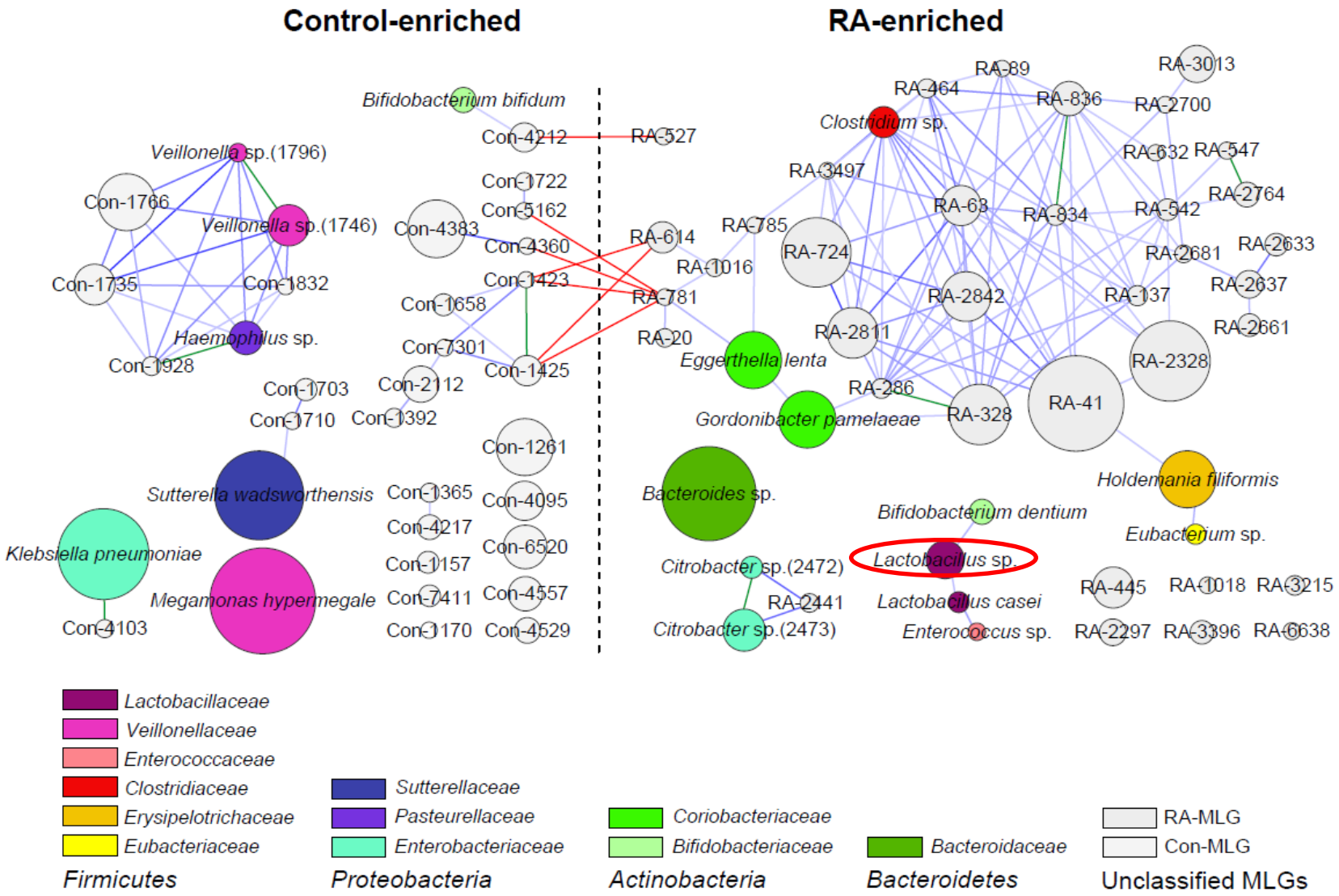




Yu et al. Gut 2015, doi: 10.1136/gutjnl-2015-309800

Rheumatoid arthritis

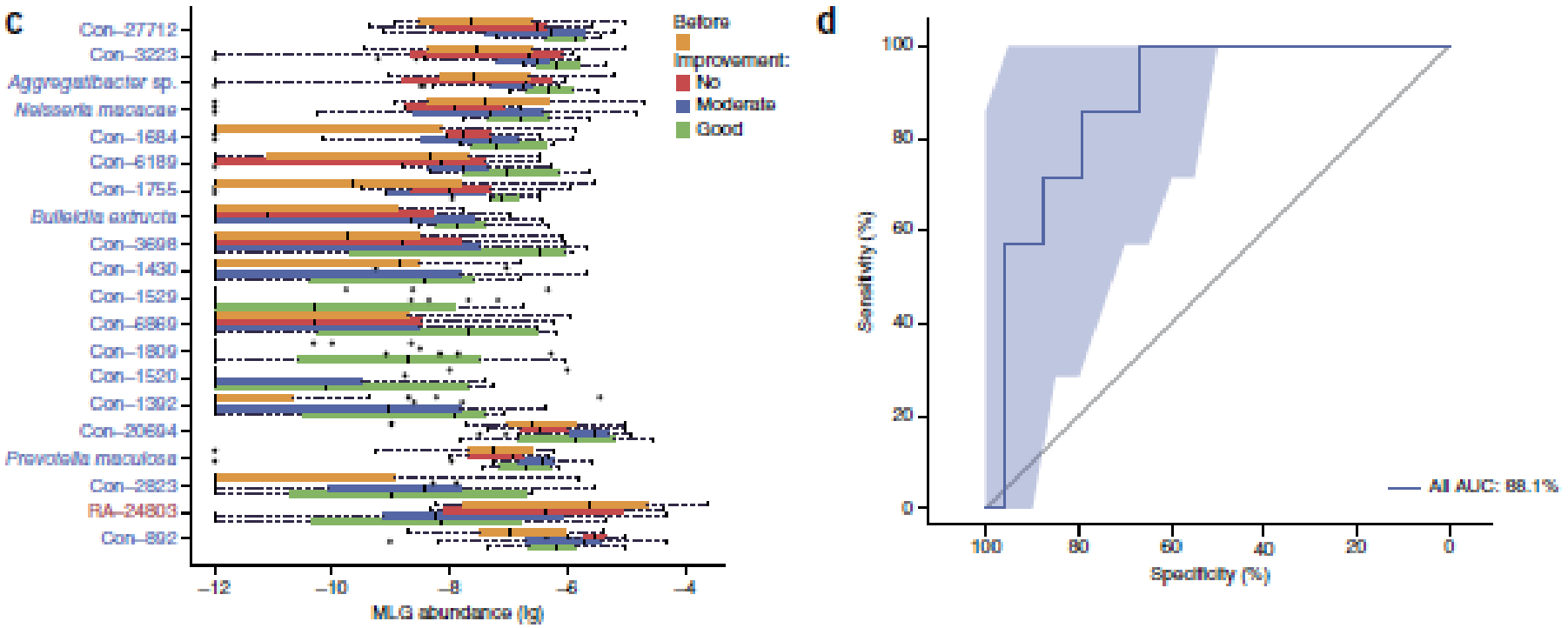
Gut, saliva and dental microbial markers are associated with rheumatoid arthritis



Zhang et al. Nature Medicine 21, 895-905, 2015

Enrichment of dental control MLGs by DMARD treatment

ROC prediction based on before treatment



Zhang et al. Nature Medicine 21, 895-905, 2015

Take home messages

- Even without knowledge of causality, microbial markers have great potential in relation to diagnosing of patients
- Few robust gut microbial markers are associated with colorectal cancer and rheumatoid arthritis in different populations
- Potential for improved early diagnosis based on microbiome analyses
- Potential for stratification of patients prior to treatment
- Much larger sample sizes needed to validate our findings

Status and concluding remarks

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Thank you for listening