

The human gut microbiome in health and disease



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Aiming at a functional understanding of
biological systems

How to study microbes: Until recently via growing them
...but 99% cannot be easily cultured, so only a few were studied individually

Petri dishes with agarose (nutrient cocktail)...invented 1887!



From toilet air



From the hand of an 8 year old

Towards structure and function of a microbiome



Molecular approaches to access a microbiome

	economics		knowledge		
	Output	Costs*	Species	Function	Variation
16S profiling	3k OTUs	● ● ●	● ●	●	x
Metagenomics	250 species/ 3 Mio genes	●	● ● ●	● ● ●	● ● ●
Metatranscriptom.	150 species/ 0.5 Mio genes	●	● ●	● ● ●	●
Metaproteomics	10k proteins	●	●	● ●	x
Metabolomics	800 metabol.	●	x	● ●	x

*per sample per data output (example human stool)

● ● ● Good/cheap ● Expensive

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II. Basics still to be discovered: Origin and temporal variation

- Birth, family and local environment

III. From associations to diagnostics

- Colon cancer, confounding factors

IV. Impact on chemical and microbial therapy

- Medication and faecal microbiota transplantation



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The Milky Way harbours ca. 100 Billion (10^{11}) stars...

We have ca. 40 Trillion (4×10^{13}), i.e. ca 400x more, microbial cells in our gut, more than human cells

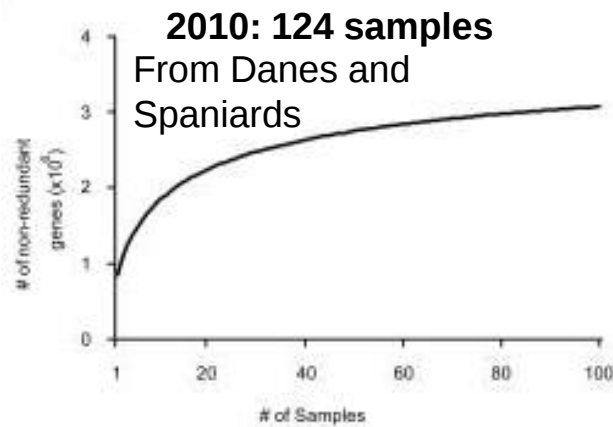
Which and how many microbes are in the gut?

In 2010 already hope for diagnostics, but not even basics were known

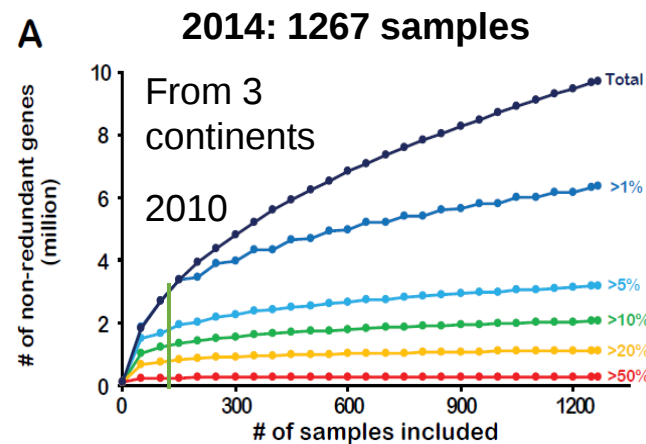
Mostly bacteria, >1000 species per person, exact number unclear

More bacterial than human cells, biomass together up to ca 1.5kg (brain 1.3kg)

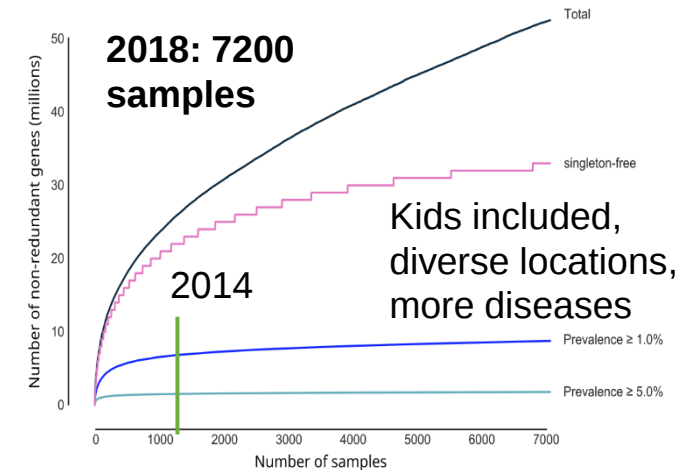
With metagenomics we see ca 250 species/pers: in 2010 together 3.3 Mio genes



3.3 Mio genes, 4Gb per sample
Qin et al, Nature 464(2010)59



10 Mio genes, 5GB per sample
Li et al, Nat.Biotech. 32(2014)834



56Mio genes, 10GB per sample
Coelho et al., in revision

Some mispredicted genes aside, each of us carries a lot of unique genes (or rare species)!

How different are our gut microbes?

Lots of biological variation in taxonomic composition, yet there is structure in the data

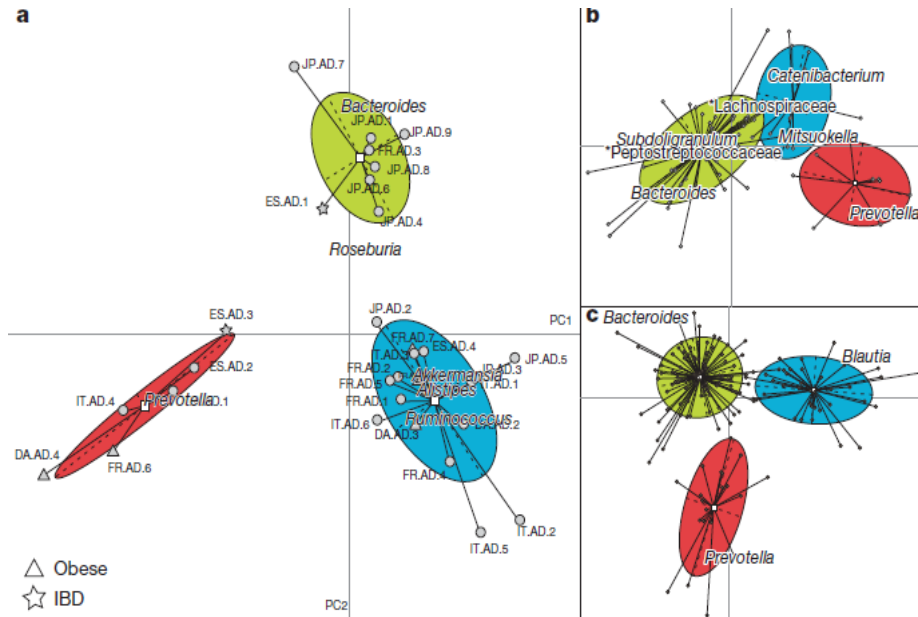
ARTICLE

Nature 473(2011)174

doi:10.1038/nature09944

1 Enterotypes of the human gut microbiome

Manimozhiyan Arumugam^{1*}, Jeroen Raes^{1,2*}, Eric Pelletier^{3,4,5}, Denis Le Paslier^{3,4,5}, Takuji Yamada¹, Daniel R. Mende¹, Gabriel R. Fernandes^{1,6}, Julien Tap^{1,7}, Thomas Bruls^{3,4,5}, Jean-Michel Batto⁷, Marcelo Bertalan⁸, Natalia Borruel⁹, Francesc Casellas⁹, Leyden Fernandez¹⁰, Laurent Gautier⁸, Torben Hansen^{11,12}, Masahira Hattori¹³, Tetsuya Hayashi¹⁴, Michiel Kleerebezem¹⁵, Ken Kurokawa¹⁶, Marion Leclerc⁷, Florence Levenez⁷, Chaysavanh Manichanh⁹, H. Bjorn Nielsen⁸, Trine Nielsen¹¹, Nicolas Pons⁷, Julie Poulain³, Junjie Qin¹⁷, Thomas Sicheritz-Ponten^{8,18}, Sebastian Tims¹⁵, David Torrents^{10,19}, Edgardo Ugarte³, Erwin G. Zoetendal¹⁵, Jun Wang^{17,20}, Francisco Guarner⁹, Oluf Pedersen^{11,21,22,23}, Willem M. de Vos^{15,24}, Søren Brunak⁸, Joel Doré⁷, MetaHIT Consortium†, Jean Weissenbach^{3,4,5}, S. Dusko Ehrlich⁷ & Peer Bork^{1,25}

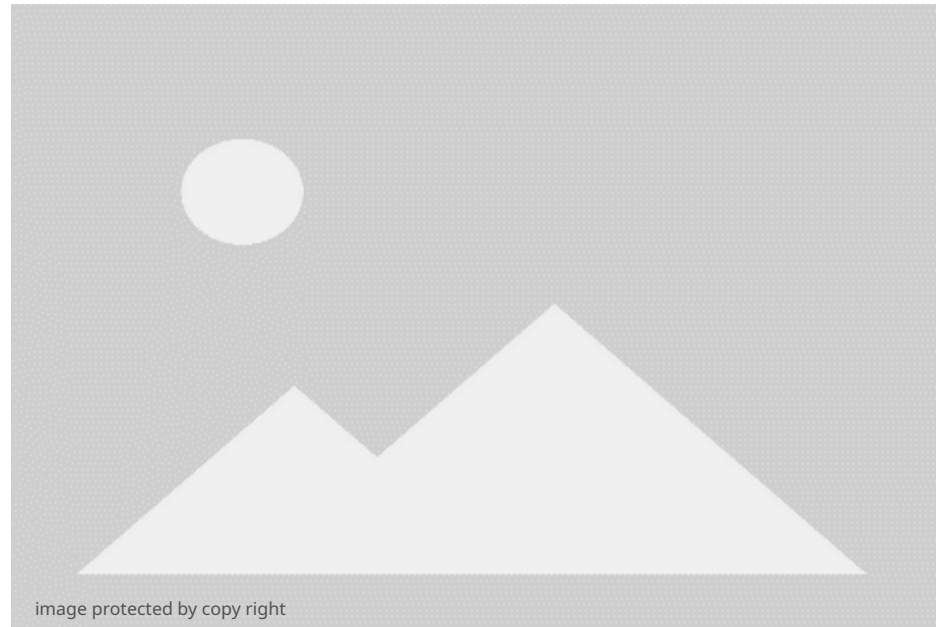


3 distinct community types at genus level...

Enterotypes in the landscape of gut microbial community composition

Paul I. Costea¹, Falk Hildebrand^{1,2,3}, Manimozhiyan Arumugam⁴, Fredrik Bäckhed^{5,6}, Martin J. Blaser⁷, Frederic D. Bushman⁸, Willem M. de Vos^{9,10}, S. Dusko Ehrlich^{11,12}, Claire M. Fraser¹³, Masahira Hattori¹⁴, Curtis Huttenhower¹⁵, Ian B. Jeffery¹⁶, Dan Knights^{17,18}, James D. Lewis¹⁹, Ruth E. Ley²⁰, Howard Ochman²¹, Paul W. O'Toole¹⁶, Christopher Quince²², David A. Relman^{23,24,25}, Fergus Shanahan¹⁶, Shinichi Sunagawa^{1,26}, Jun Wang^{5,27,28,29,30}, George M. Weinstock³¹, Gary D. Wu³², Georg Zeller¹, Liping Zhao³³, Jeroen Raes^{2,3,34*}, Rob Knight^{35,36,37,38*} and Peer Bork^{1,39,40*}

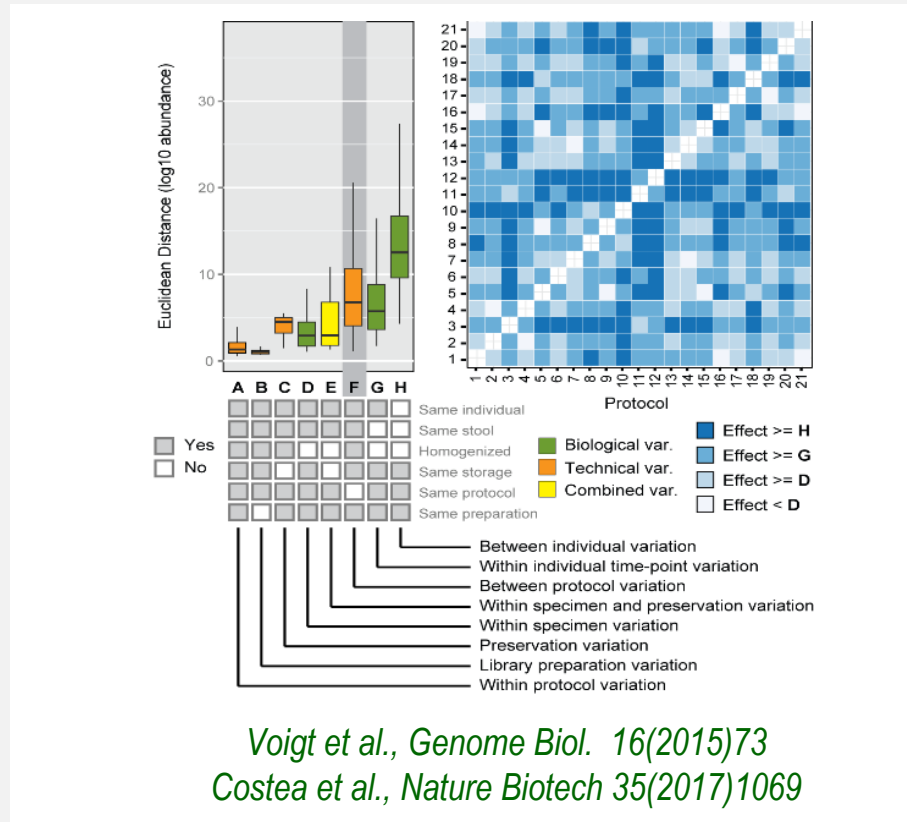
Nature Microbiol. 3(2018)8



...now in context of a complex composition landscape

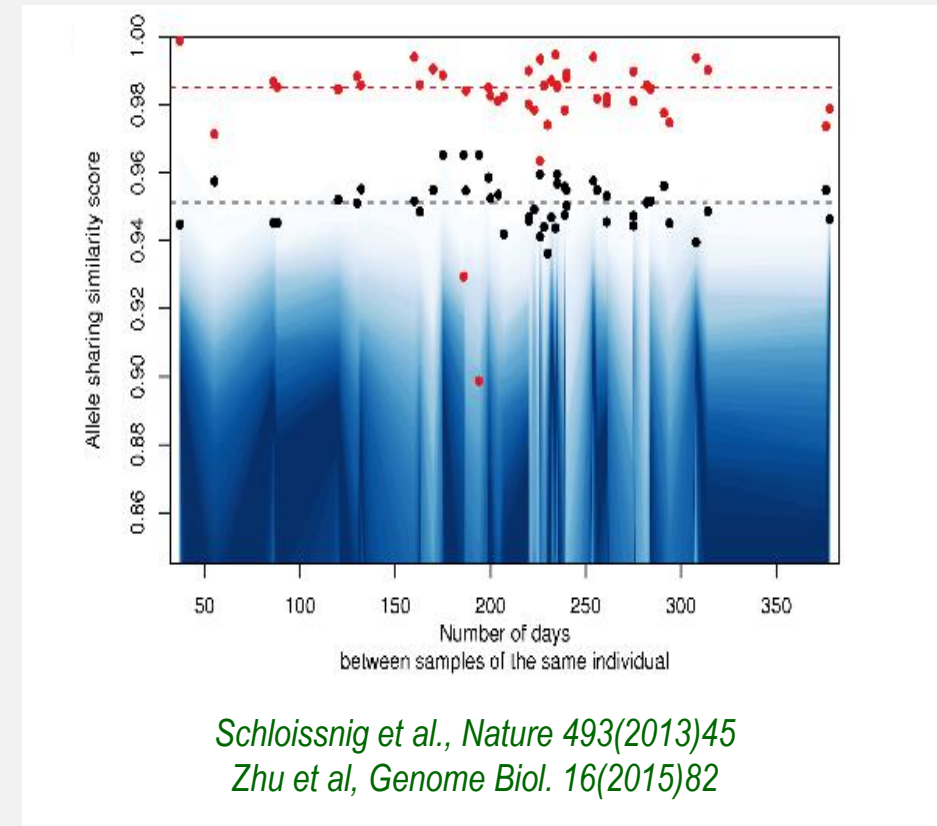
There are still limitations, e.g. little comparability or resolution

Lots of technical variation as standards are still emerging



Different protocols but also same protocol in
in different labs vary considerably

Taxonomic resolutions towards strain populations



Two individuals differ in conspecific strains,
even monozygotic twins have individual strains

Numerous 16S profiling and metagenomic association studies (MWAS), but difficult to compare

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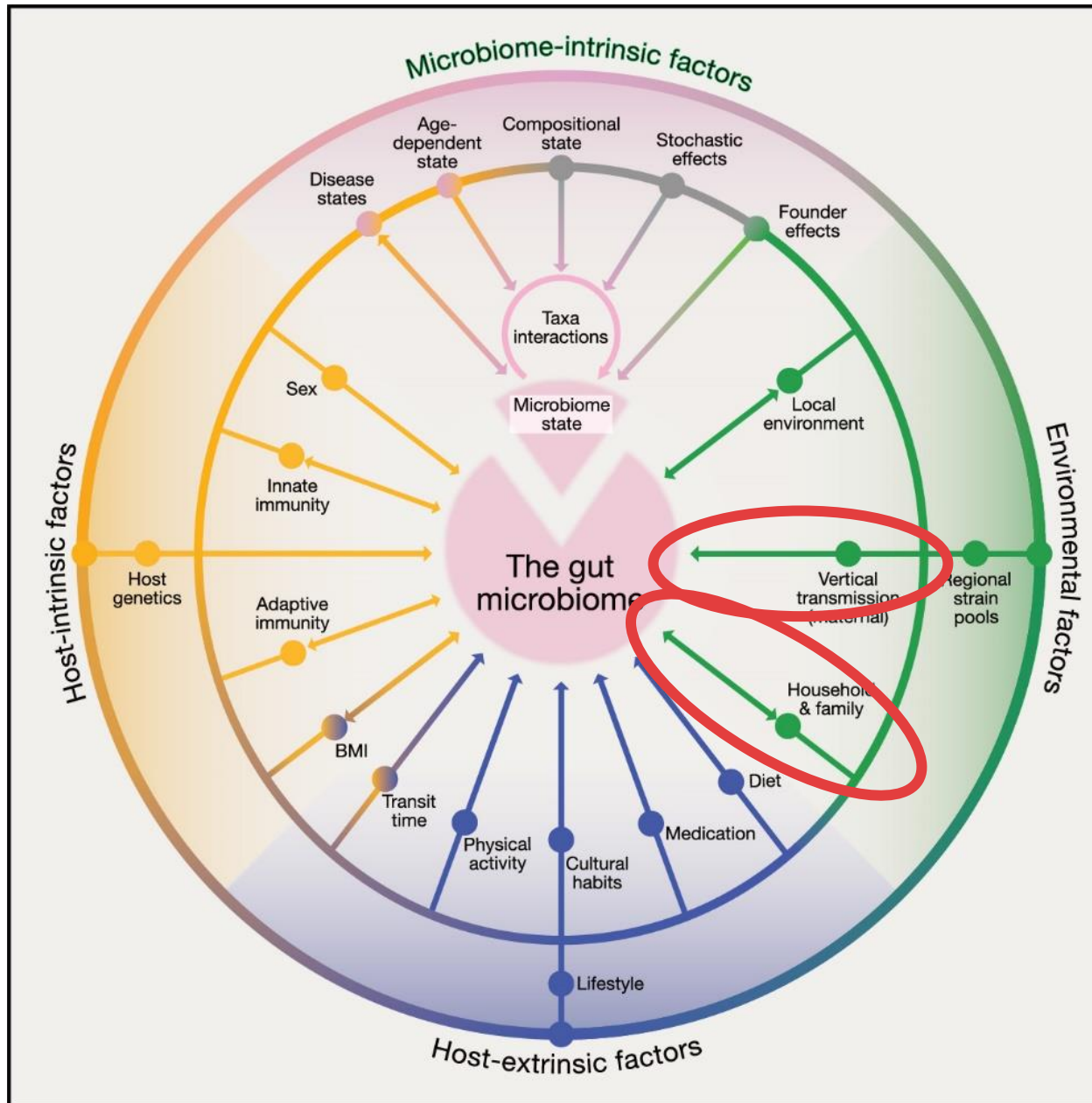
IV. Impact on chemical and microbial therapy

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The human gut microbiome variation and associated factors

Effect
size of
host
genetics
<2%!

Rothschild et al..
Nature (2018)



Association studies do not reveal causality...does not matter for diagnostics though

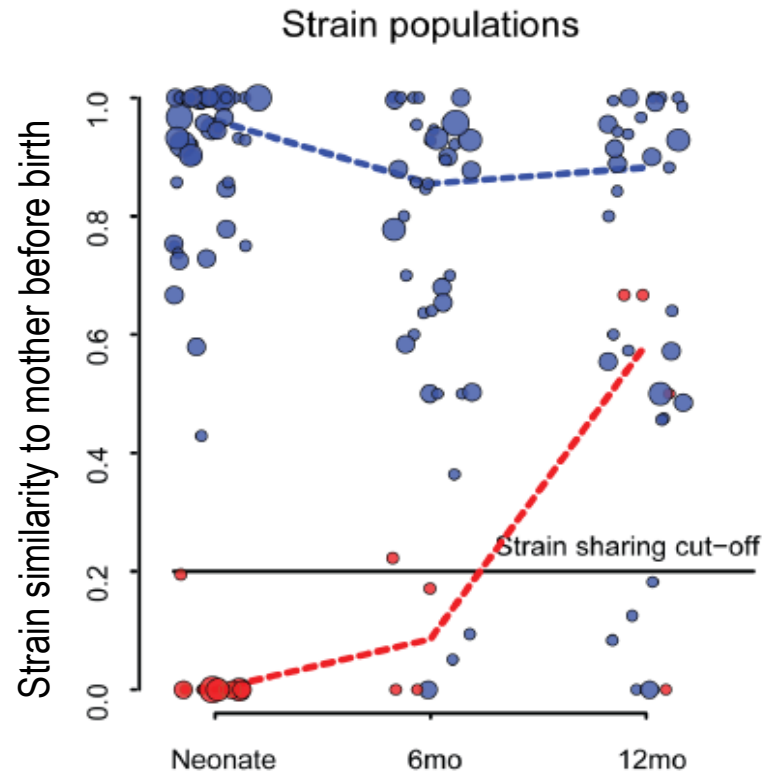
Transmission from mother?

Schmidt, Raes[#] and Bork[#], *Cell* 172(2018)1298

Where do our bugs come from and do we keep them ?

At birth? All from the mother and father has no impact (microbe-wise)?

Baby strains in comparison to their mothers



● From mother ● From environment

Cohort: 400 Families from 5 countries:

Most species and strains come from mother via the birth channel

Ferretti et al., Cell Host Mic 24(2018)133

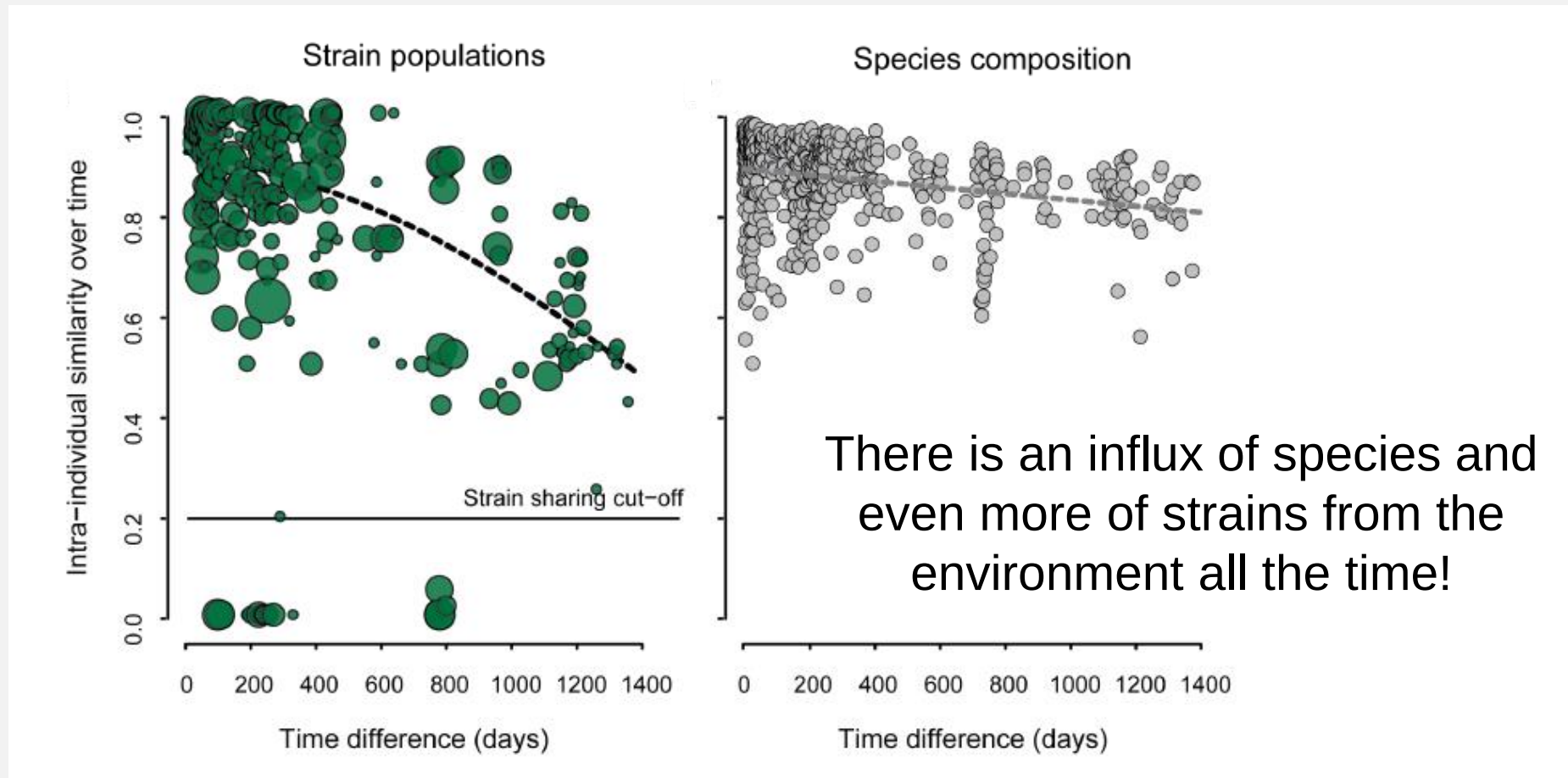
At Caesarean birth bugs come from environment

Also after birth some transmission from mother to baby (more than from father)

Bacterial strains from mother are a kind of protection, as “proven” in mother

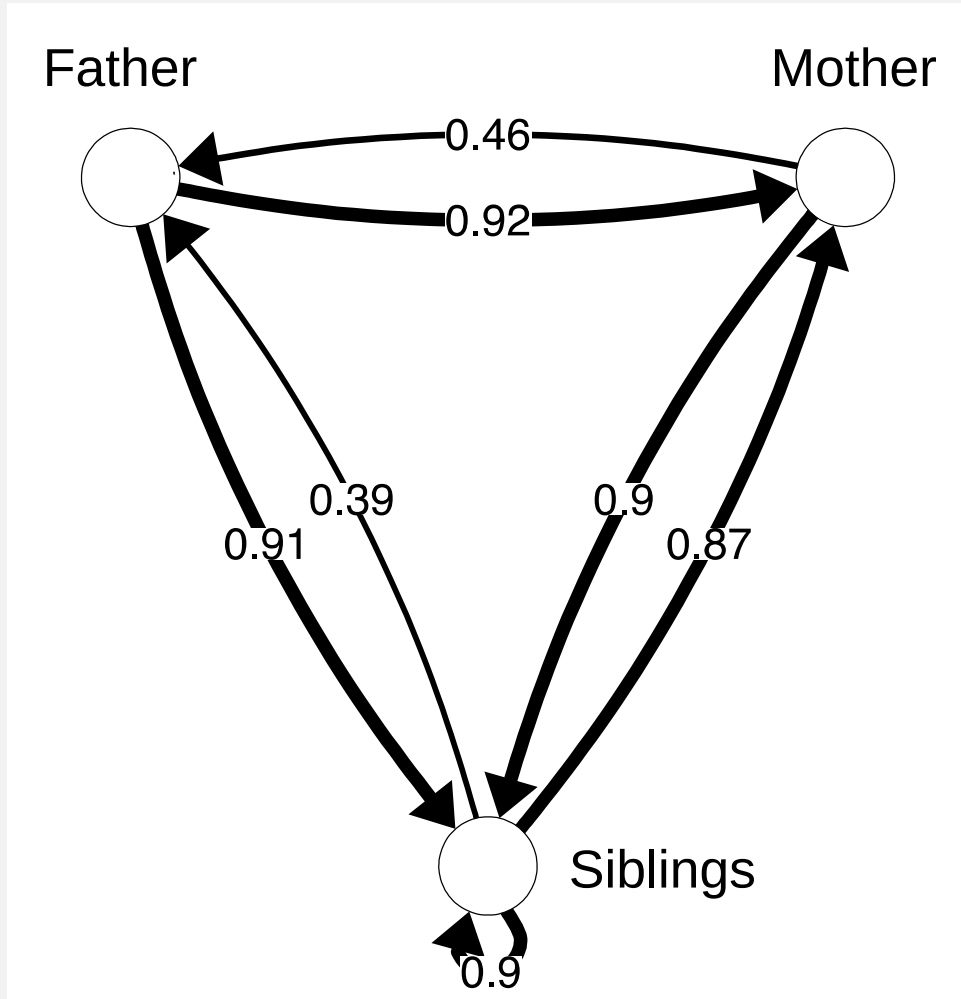


Strain pools change faster than species composition over time



Environment impacts conspecific strain composition, even species can change

Transmission of gut microbial strains between family members



Longitudinal sampling of family members allows to infer directionality of transfer (first in one person, than in another)

Fathers spread most strains to family members !

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Medical relevance of the gut microbiome

- **Diagnosis of diseases:** First microbiome-based tests soon applied
- **Personalized medication** after gut microbiome assessment:
choice of drug (response, resistance awareness), dose, drug combinations, side effect assessment
- **Microbial therapies:** (1) Fecal microbiota transplantation, little understood; needs improvements to be widely applicable, (2) probiotics, (3) prebiotics or combinations of (2) and (3) (synbiotics)

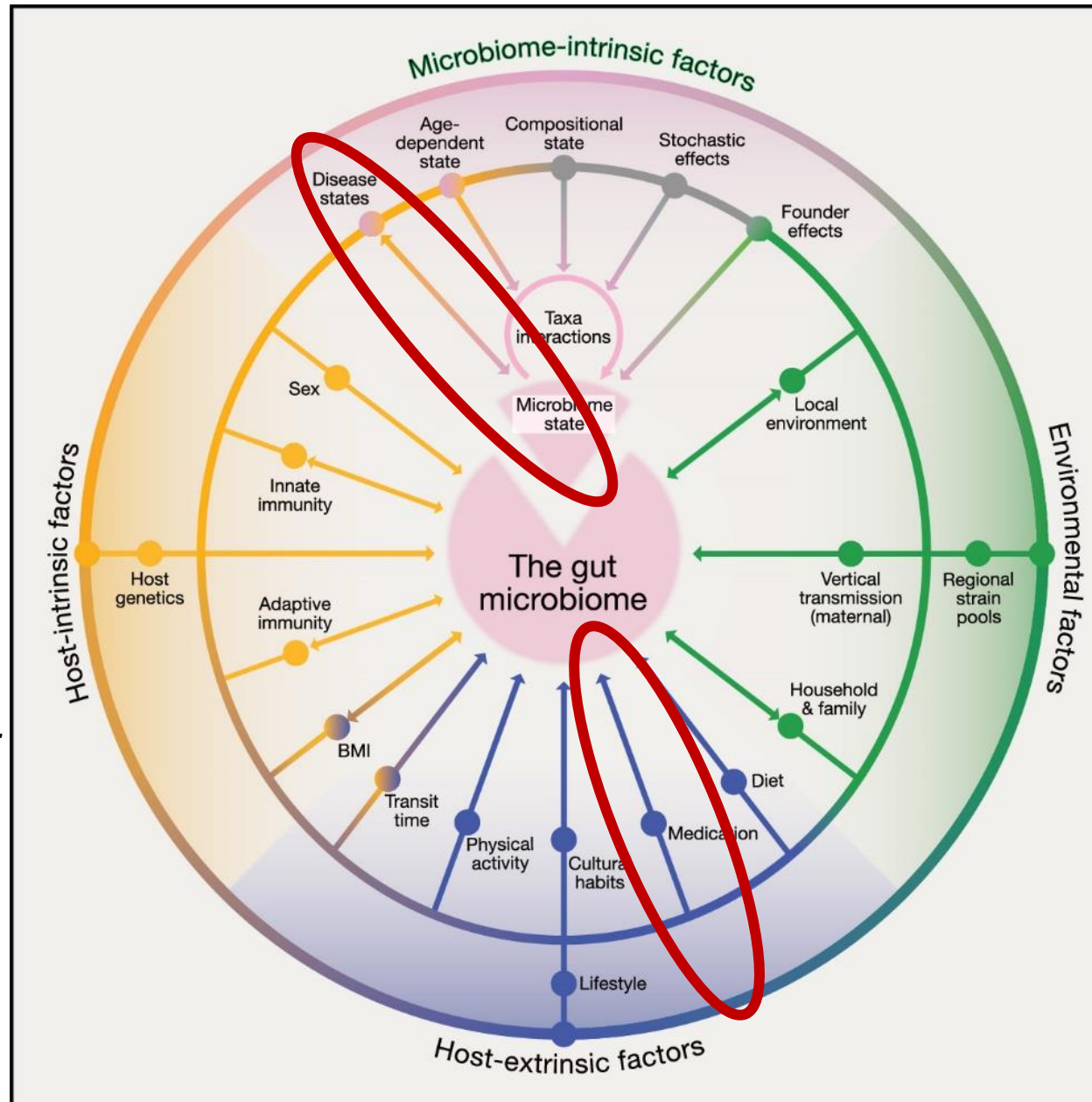
Still many issues due to limited knowledge, e.g.

- Due to large individual variations, we still don't know what a “healthy” microbiome is and in how many flavors it can come
- Gut bugs can be “good” or “bad” depending on environment

Microbial biomarkers need to take co-variation into account

Effect
size of
host
genetics
<2%!

*Rothschild et al..
Nature (2018)*

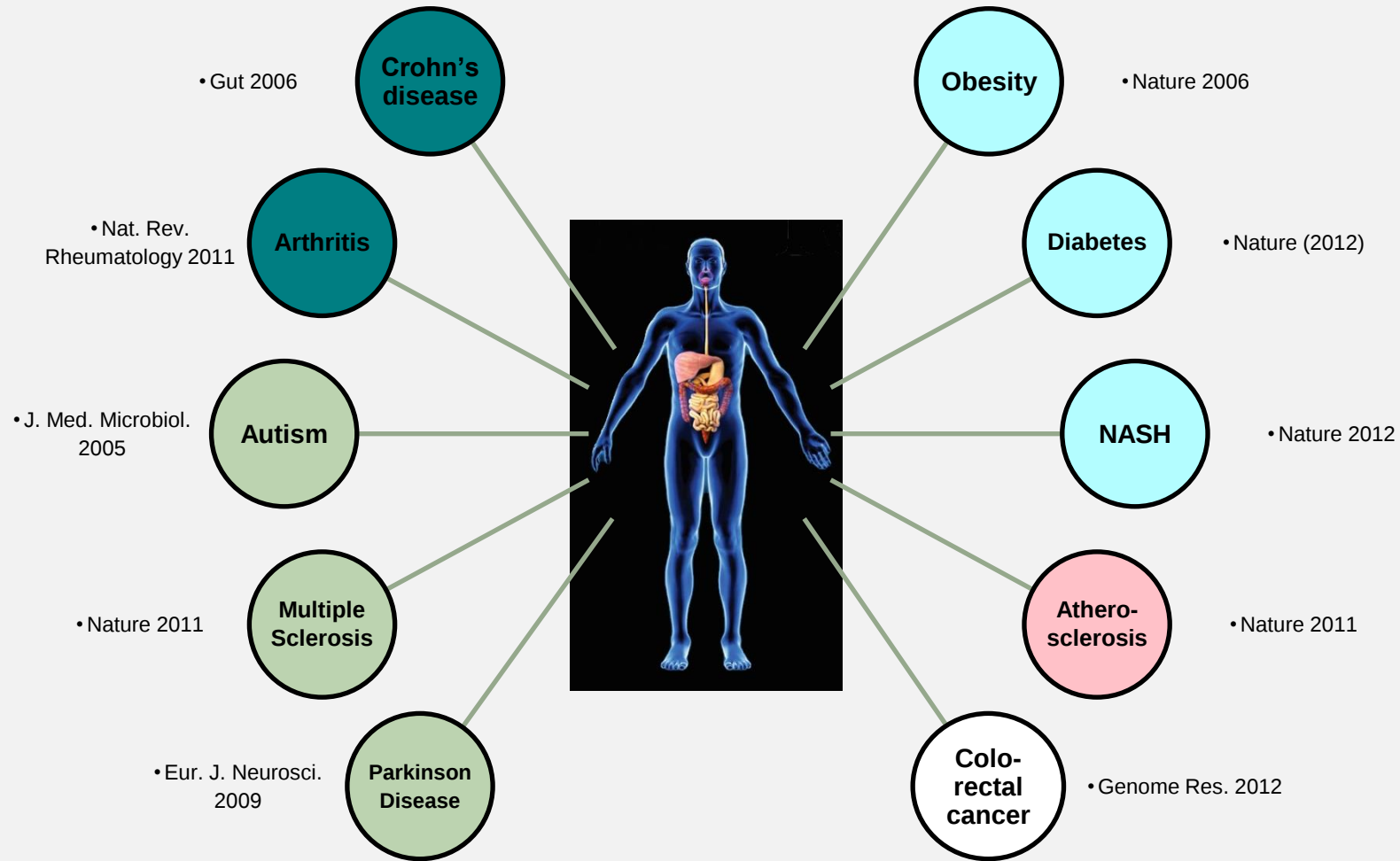


Biomarkers for diagnosis
have to be sensitive and
(disease-) specific

Medication has effect on
microbiome and *vice versa*

Schmidt, Raes[#] and Bork[#], Cell 172(2018)1298

Metagenome-wide association studies (MWAS) link gut microbiome to a multitude of diseases



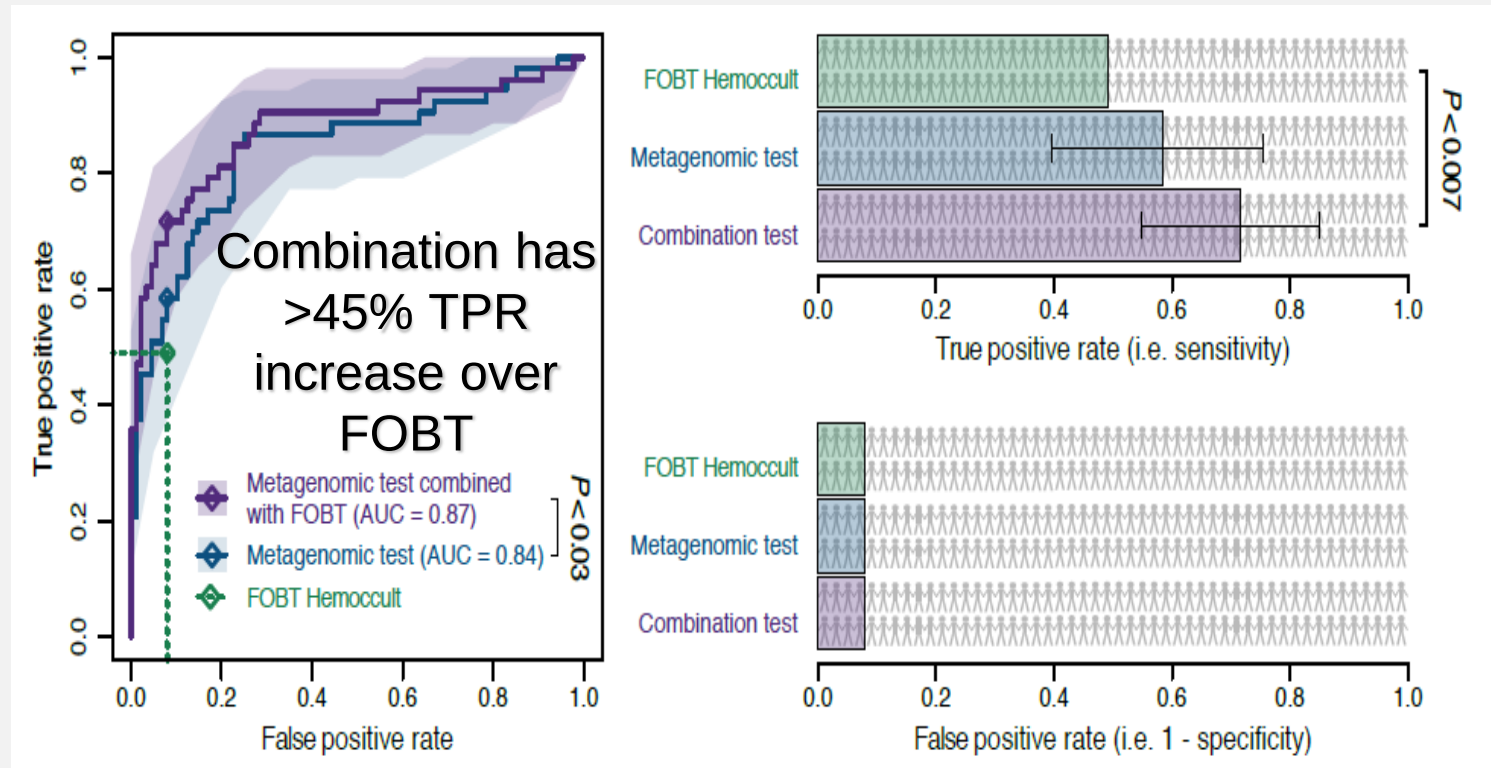
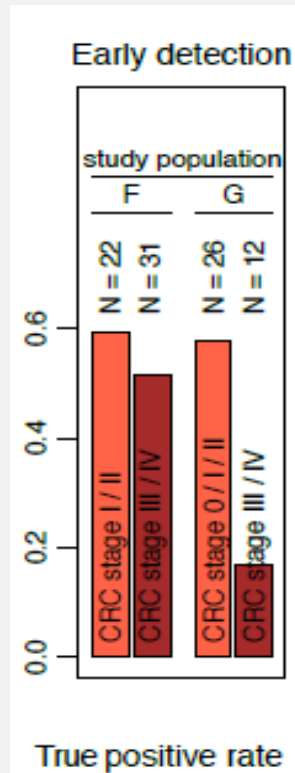
Yet, we still don't know what „healthy“ is!

Indication areas

-  Inflammatory diseases
-  Neurological disorders
-  Metabolic diseases
-  Cardiovascular diseases
-  Cancer

Colon cancer: Early stage detection and complementarity to Fecal occult blood test (FOBT)

French cohort (N=156) with external validation on a German cohort reveals 20+ marker species



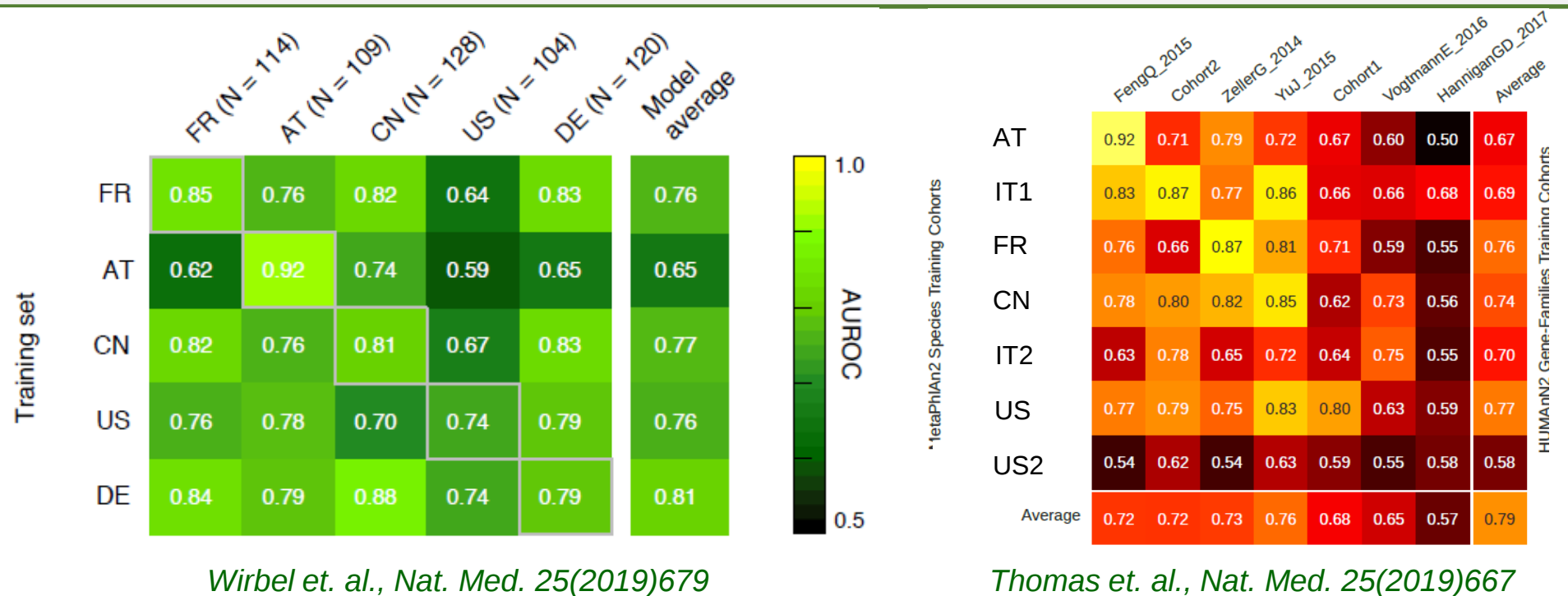
Marker species detect stage I/II, not adenomas though

FOBT and microbial signals complementary, a combination thus enhances prediction accuracy...

Marker species stand out across country and study biases

Consistency despite different processing and analysis

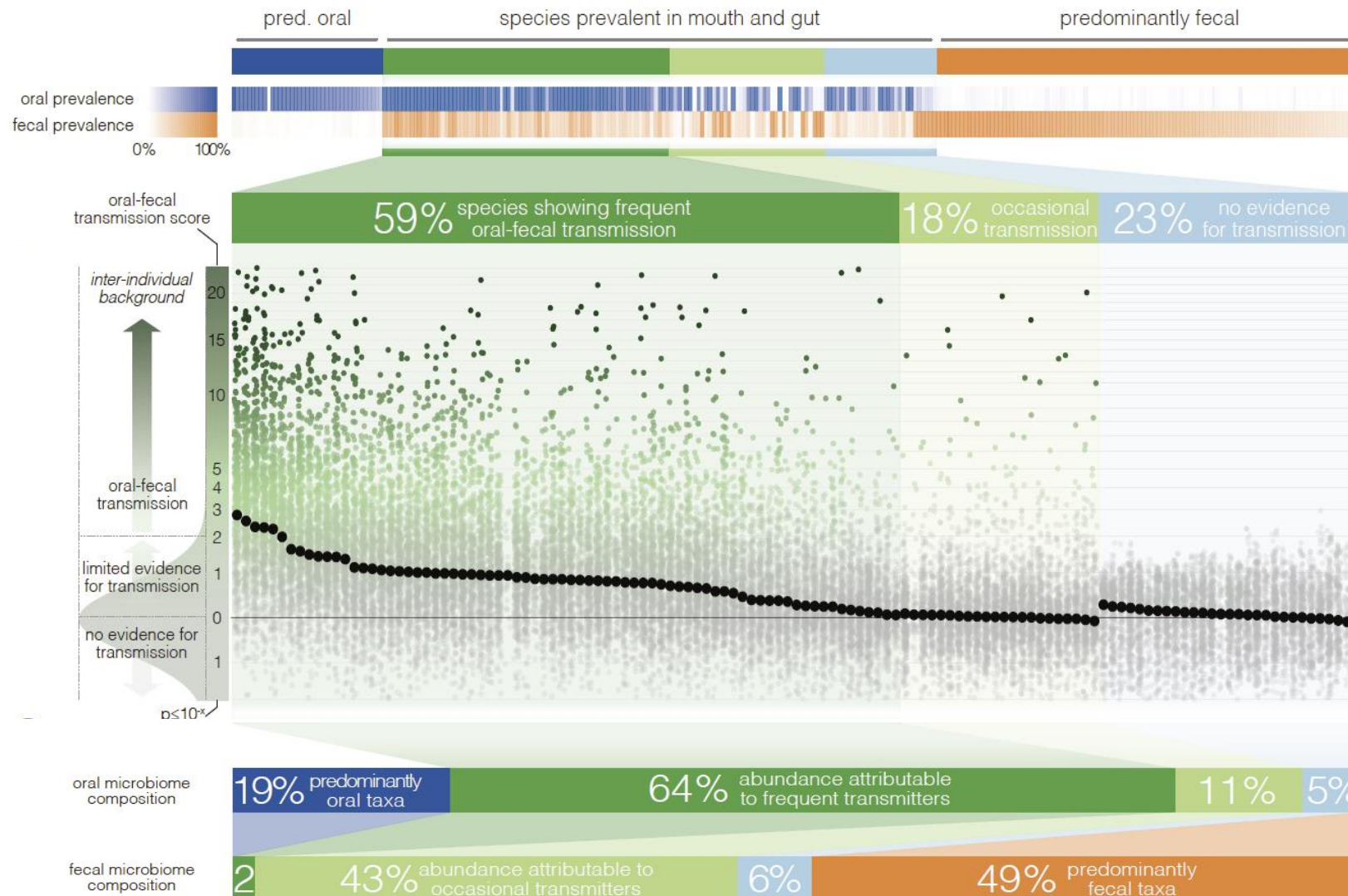
Several cohorts in 2 studies with consistent signal, additionally validated by 3 external cohorts



Two different species-calling approaches (mOTU and metaphlan), different stats etc

Combination of cohorts gains statistical power

Many oral species are transmitted to gut in healthy individuals



SNV-based identification of transmission:

Out of 310 species from 470 healthy individuals, **77% show evidence of transmission**

45% of fecal species are at least occasionally replaced by oral strains

*Schmidt, Hayward et. al.,
eLife (2019) e42693*

Many CRC marker species are oral, transmitted and from patient

In CRC patients, transmission is increased over healthy, even more so for marker species

CRC patients compared to healthy



...all great, but metagenomics 500 EURO, FOBT 5 Euro...

*Schmidt, Hayward et. al.,
eLife (2019) e42693*

Associations can be unspecific, confounded or indirect

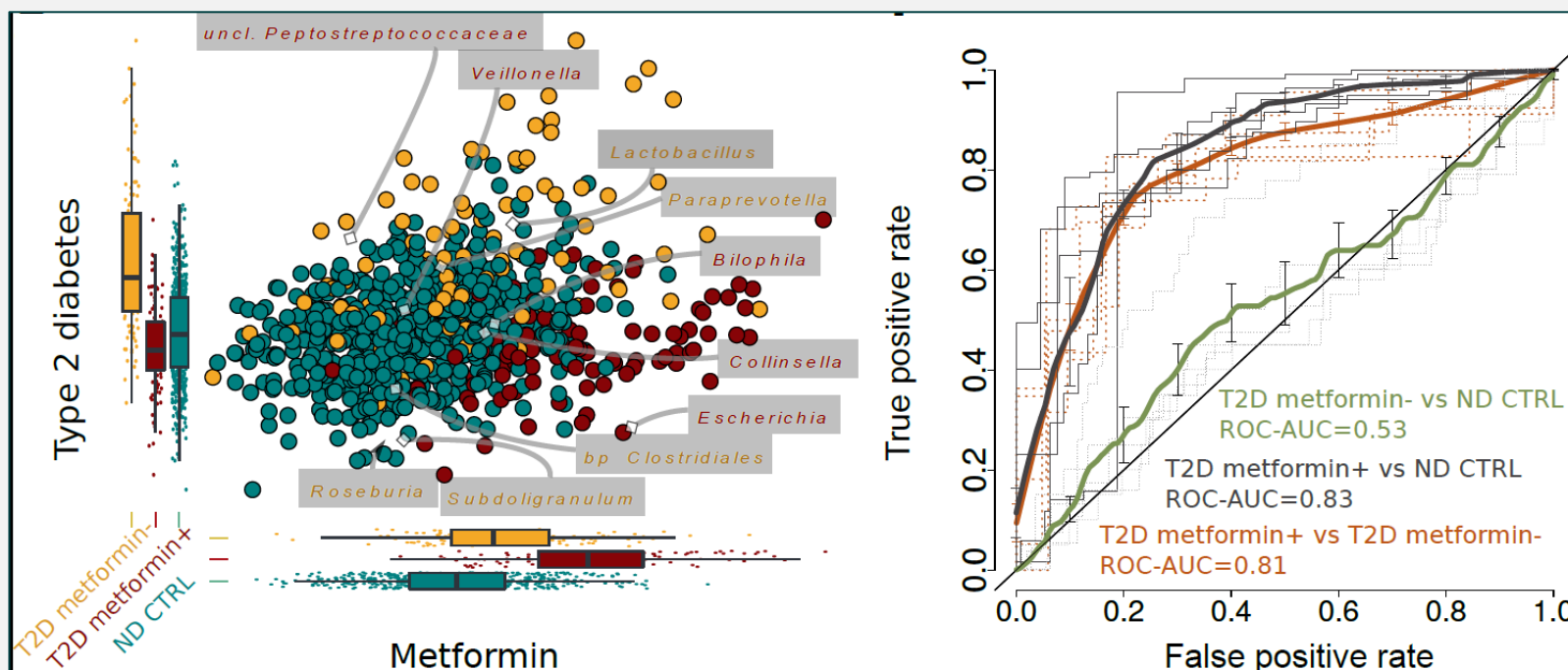
(What if all CRC patients have inflammation and we developed an unspecific inflammation test?)

“The gut microbiome is associated with type 2 diabetes”

Qin et al., Nature 2012, AUC 0.81 (Chinese cohort)

Karlsson et al., Nature 2013 AUC 0.83 (Swedish cohort)

BUT the drug metformin is a major confounder that needs to be disentangled



*Forslund, Hildebrand et al.,
Nature 228(2015)262*

(with EU Metahit consortium)

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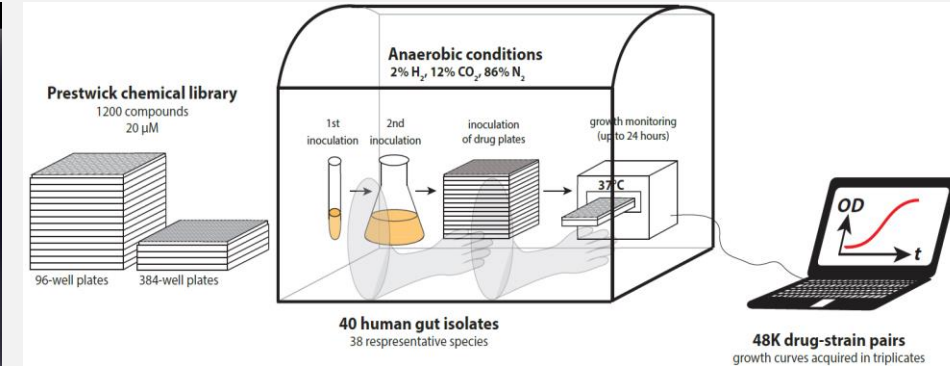
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(1) Therapy by medicinal drugs

In vitro drug-bug screen for direct interactions

1200 medicinal drugs vs 40 representative gut strains

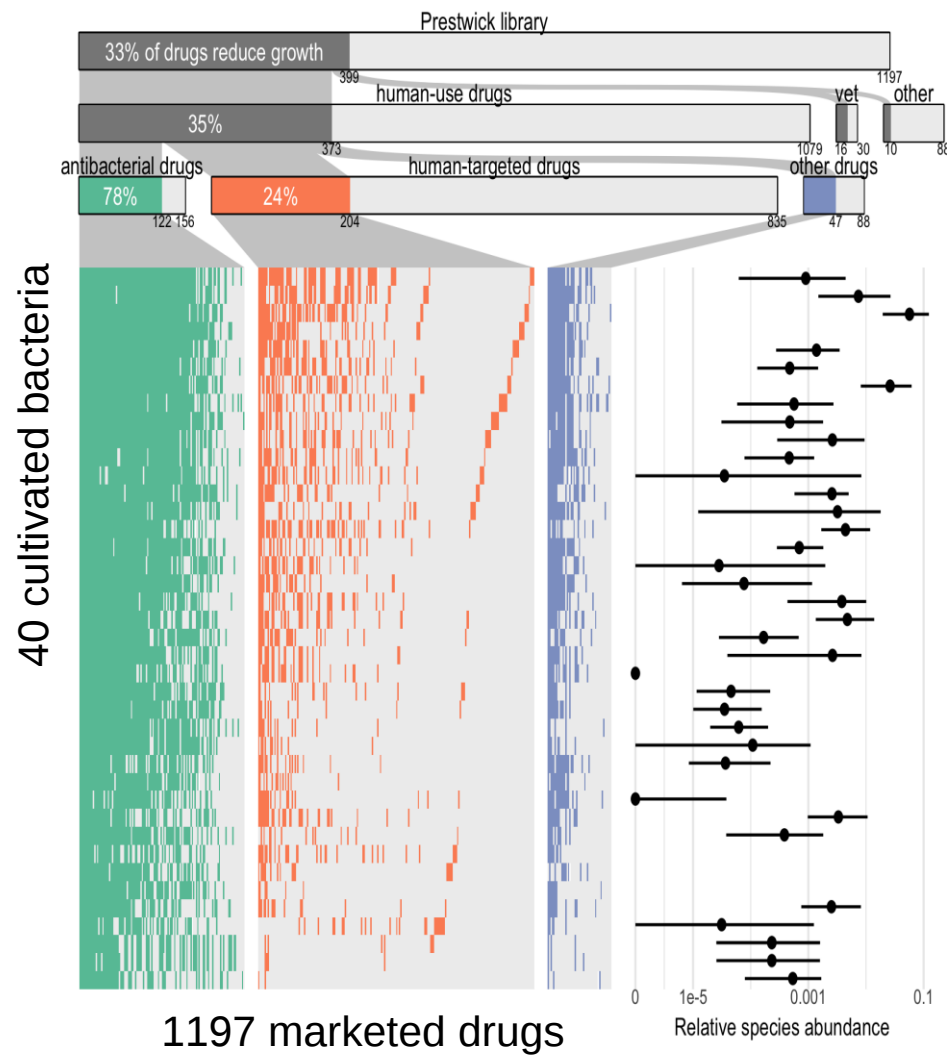


Automation of work flow at EMBL,
robotics also in anaerobic chamber;
1200 drugs vs 1 bug in 1 day

Movie by EICAT

With Typas, Patil and Zeller groups at EMBL

Many “human targeted” therapeutic drugs change our gut microbiome



- Antibacterial
- Human-targeted
- Antifungal and antiviral

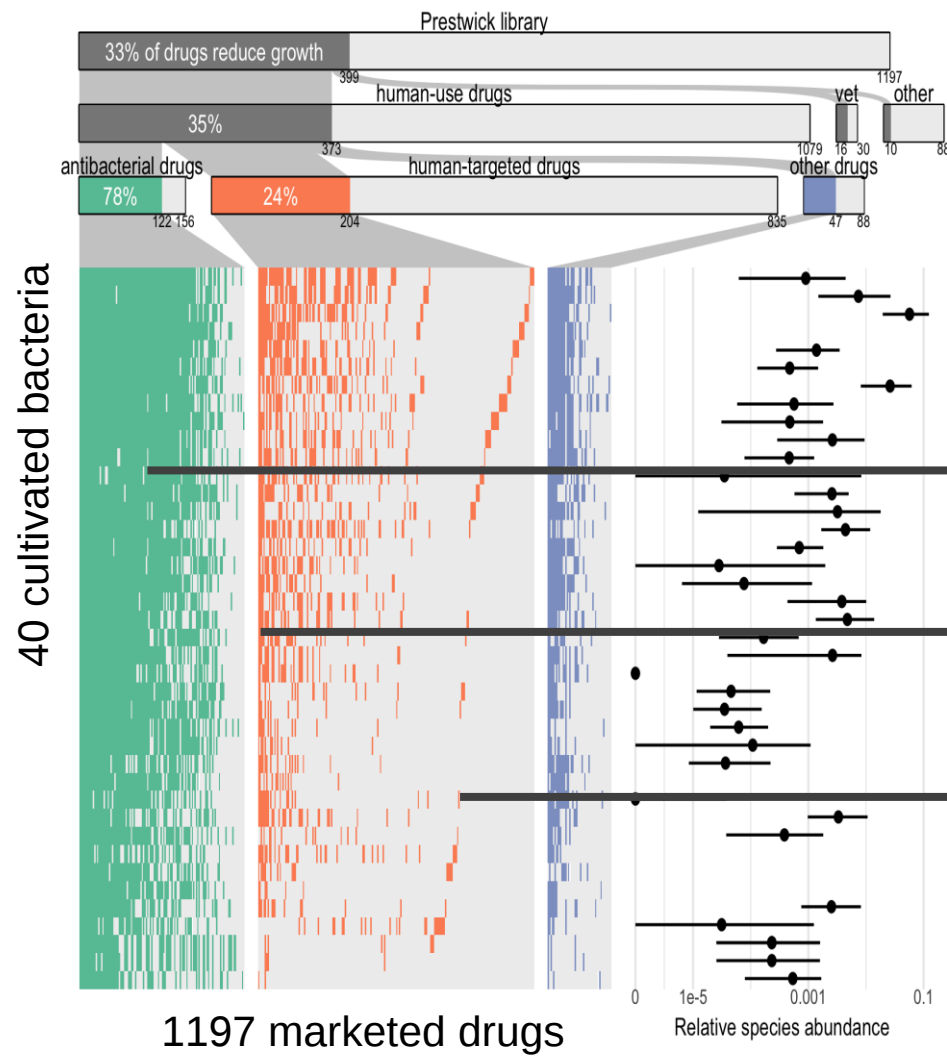
>24% of the human-targeted drugs deplete at least one gut bacterium

This fraction is probably much higher (low doses, only 40 of 1000+ tested)

Some changes part of drug action, most lead to unwanted side effects

*With Typas, Patil and Zeller groups at EMBL
Maier, Pruteanu, Kuhn et al., Nature 555(2018)623*

Many “human targeted” therapeutic drugs change our gut microbiome



- Antibacterial
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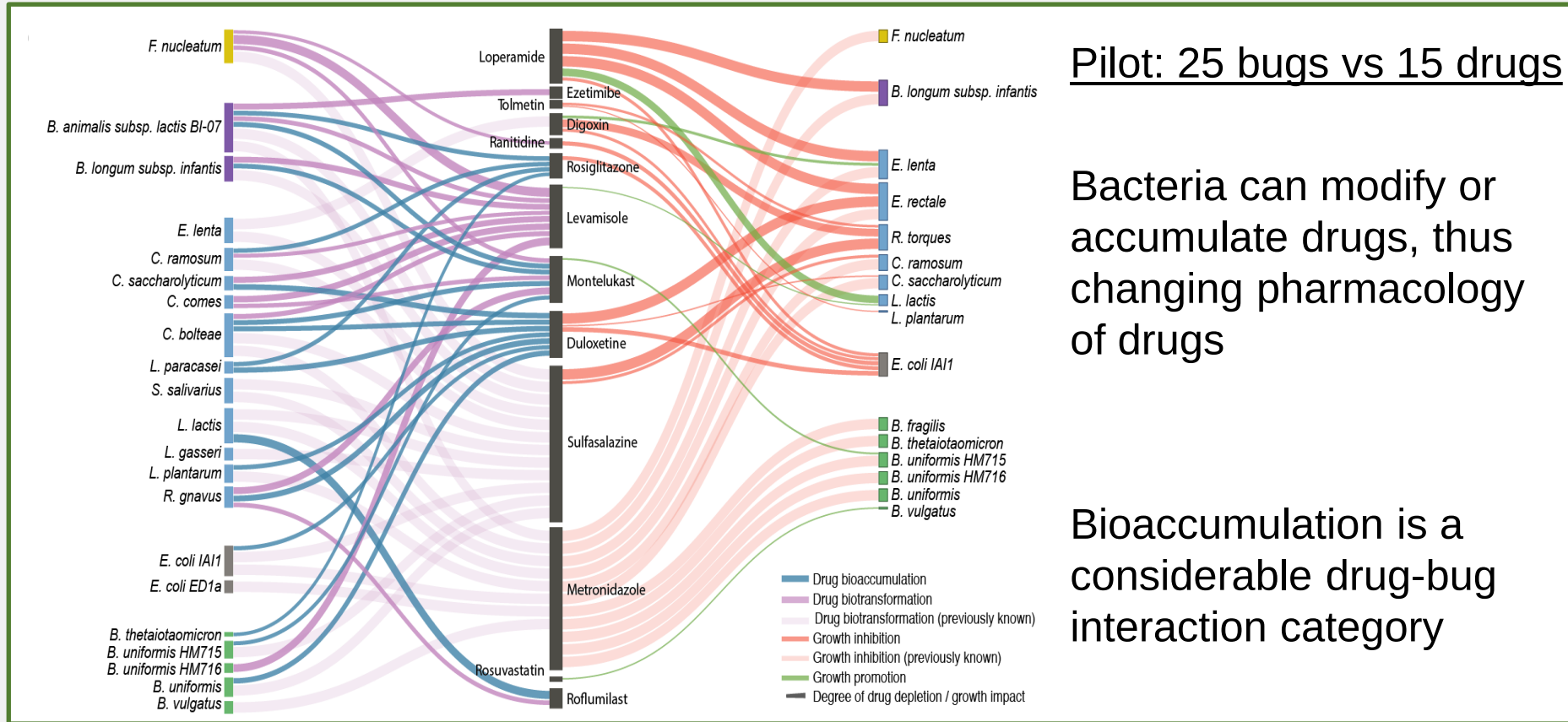
Drugs as baseline for new antibiotics?

- Antibiotics are designed against pathogens, not against commensals
- Some human-targeted drugs act like broad-spectrum antibiotics
- Some drugs only inhibit one specific species

With Typas, Patil and Zeller groups at EMBL
Maier, Pruteanu, Kuhn et al., Nature 555(2018)623

Quantifying the impact of bug on drugs (in addition to drugs on bugs)

Gut bacteria interact with drugs and food (e.g. biotransformation)



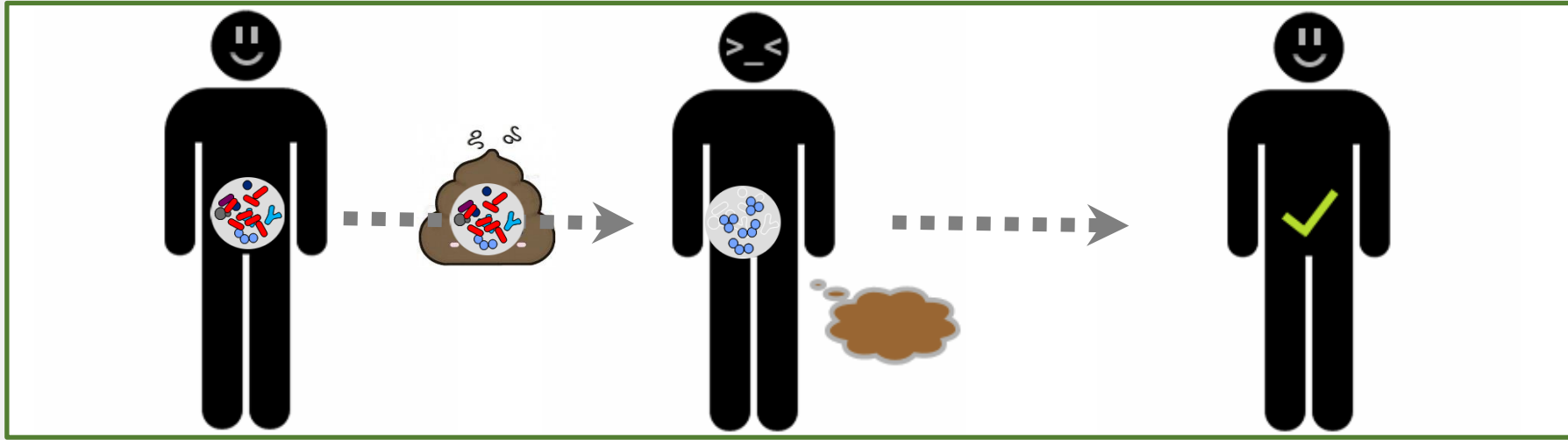
Emerging individual drug-bug and drug-chemical interaction networks

Goal: individual microbiome status guides medication

With Patil and Typas groups (EMBL); Brochado et al., Nature 2018, Klünemann et al., Nature, in revision

(1) Microbial therapy:

Tracing strain transfer in Fecal Microbiota Transplantation (FMT)



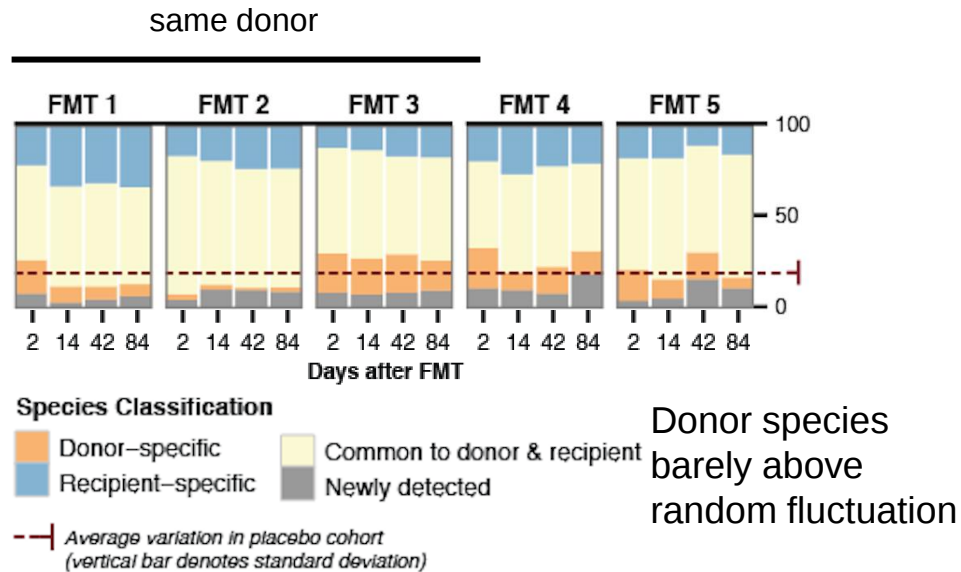
Analysis usually at species/OTU level, but most species are shared

- Transfer of stool from a healthy donor to patient
 - Usually following antibiotics treatment or bowel lavage
- Positive effects reported in GI and non-GI diseases
 - Over 90% success in treating *Clostridium difficile* infection
 - Not so straight forward in other diseases
- Mechanism is still unknown

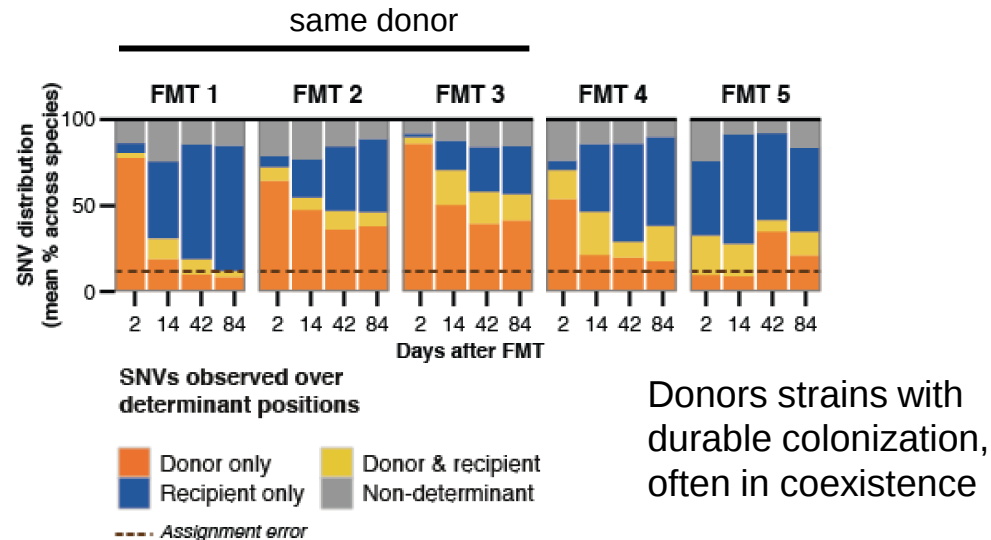
Strain replacement after FMT for metabolic syndrome

The need of donor-recipient microbiome “compatibility”

Species



Strains



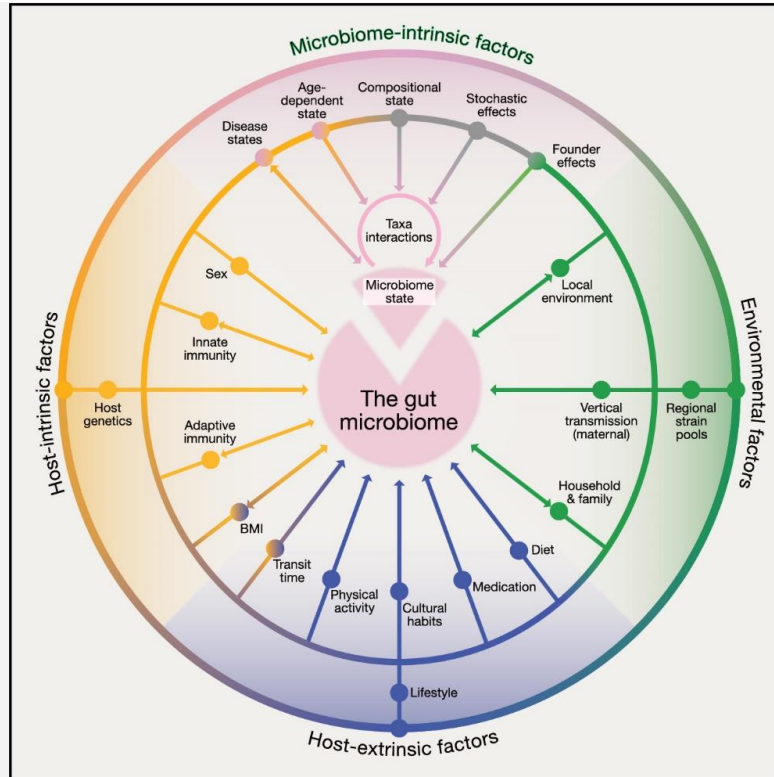
1. There is no “superdonor” – 1 donor has different outcomes
2. Donor strains can colonise and persist over at least 3 months
3. New donor strains colonise better than donor species, perhaps by hiding from the immune system
4. Extensive donor and recipient strain coexistence

Strain replacement implies personalized treatment options, e.g. by replacing multidrug resistance. Thus, microbiome census first, then treatment

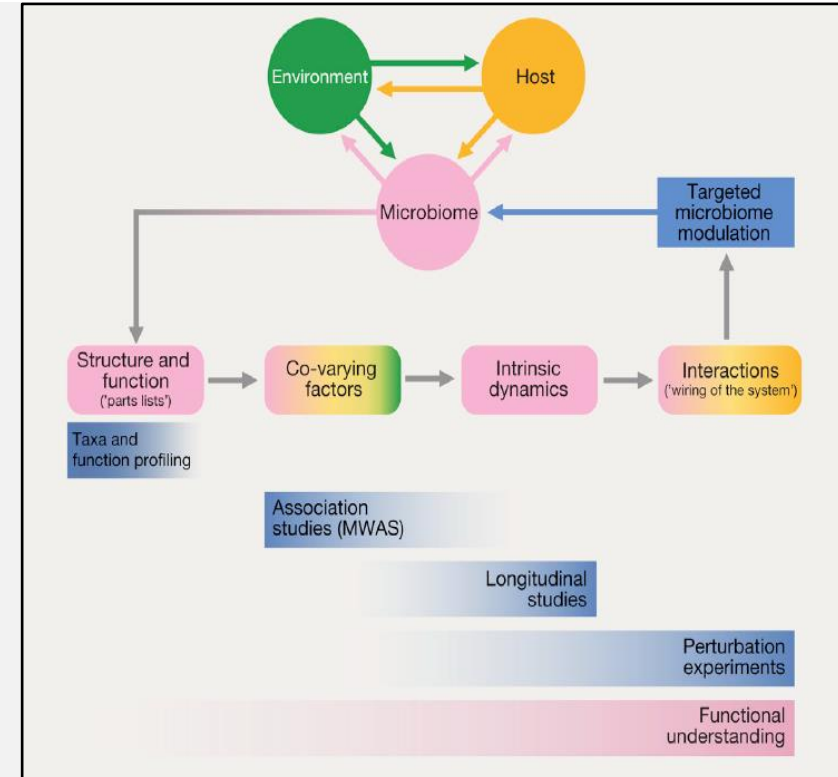
With W. de Vos, M. Nieuwdorp

Li et al., Science 352(2016)586

The human gut microbiome and its clinical relevance



Metagenome-wide association studies (MWAS) still reveal basics and are sufficient for diagnostics



MWAS need to be coupled with *in vitro* microbiomics for mechanistic insights enabling individual modulation



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Deciphering function and evolution of biological systems

The main focus of this **Computational Biology** group is to predict function and to gain insights into evolution by comparative analysis of complex molecular data. The group currently works on three different scales:

- **genes and proteins,**
- **protein networks and cellular processes, and**
- **phenotypes and environments.**



They require both tool development and applications. Some selected projects include comparative gene, genome and metagenome analysis, mapping interactions to proteins and pathways as well as the study of temporal and spatial protein network aspects. All are geared towards the bridging of genotype and phenotype through a better understanding of molecular and cellular processes.

The group is partially associated with Max Delbrück Center for Molecular Medicine (MDC), Berlin.

Selected recent publications and News

Disentangling Genetic and Environmental Effects on the Proteotypes of Individuals.

Romanov N, Kuhn M, Aebersold R, Ori A, Beck M, Bork P
Cell 177, 1-11, 2019 Apr 25, doi: 10.1016/j.cell.2019.03.015
[[Abstract](#)][[PDF](#)]

Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer.

Wirbel J, Pyl PT, Kartal E, Zych K, Kashani A, Milanese A, Fleck JS, Voigt AY, Palleja A, Ponnudurai R, Sunagawa S, Coelho LP, Schrotz-King P, Vogtmann E, Habermann N, Niméus E, Thomas AM, Manghi P, Gandini S, Serrano D, Mizutani S, Shiroma H, Shiba S, Shibata T, Yachida S, Yamada T, Waldron L, Naccarati A, Segata N, Sinha R, Ulrich CM, Brenner H, Arumugam M, Bork P, Zeller G.
Nature Medicine, Epub 2019 Apr 1; 25(4):679-689, doi: 10.1038/s41591-019-0406-6
[[Abstract](#)]

Structure and function of the global topsoil microbiome.

Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, Huerta-Cepas J, Medema MH, Maltz MR, Mundra S, Olsson PA, Pent M, Pölme S, Sunagawa S, Ryberg M, Tedersoo L, Bork P
Nature, 2018 Aug 1, 10.1038/s41586-018-0386-6
[[Abstract](#)]

Pervasive Protein Thermal Stability Variation during the Cell Cycle

Becher I, Andrés-Pons A, Romanov N, Stein F, Schramm M, Baudin F, Helm D, Kurazawa N, Mateus A, Mackmull MT, Typas A, Müller CM, Bork P, Beck M, Savitski MM
Cell, 2018 Apr 26, doi:10.1016/j.cell.2018.03.053
[[Abstract](#)]

Extensive impact of non-antibiotic drugs on human gut bacteria.

Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Dose H, Mori H, Patil KR, Bork P & Typas A.
Nature, Shared link 2018 March 19, doi:10.1038/nature25979
[[Abstract](#)]

The Human Gut Microbiome: From Association to Modulation.

Schmidt TSB, Raes J, & Bork P.
Cell, View it online 2018 March 8, Volume 172, Issue 6, p1198-1215 doi:10.1016/j.cell.2018.02.044

For details see: www.bork.embl.de



Thanks also to group alumni and collaborators

IHMC, IHMS, METAHIT (EU), METACARDIS (EU), I. Sobhani, (UPEC, F), M. von Knebel, H. Brenner, N. Ulrich (HD), N. Segata (Univ. Trento); K. Korpela (Univ. Helsinki), Sofia Forslund (now MDC), Genecore facility (EMBL), N. Typas, K. Patil, G. Zeller (EMBL) ... and many more



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EMBL

