

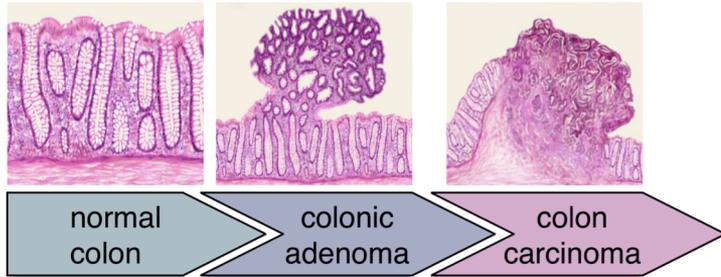
Potential of fecal microbiota for early-stage detection of colorectal cancer

Georg Zeller

EMBL



Colorectal cancer (CRC) and the microbiome



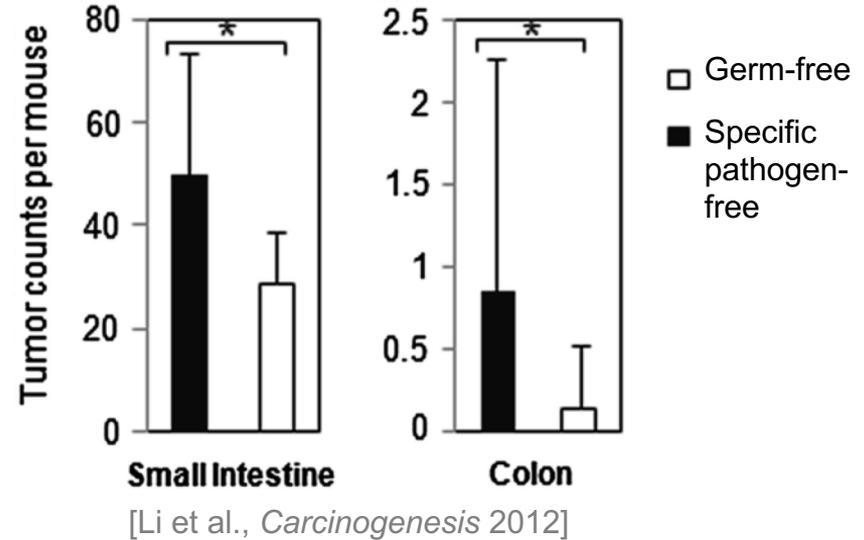
- Early hypotheses about link between CRC and the microbiome

[Sacksteder, *J. Natl. Cancer Inst.* 1976]

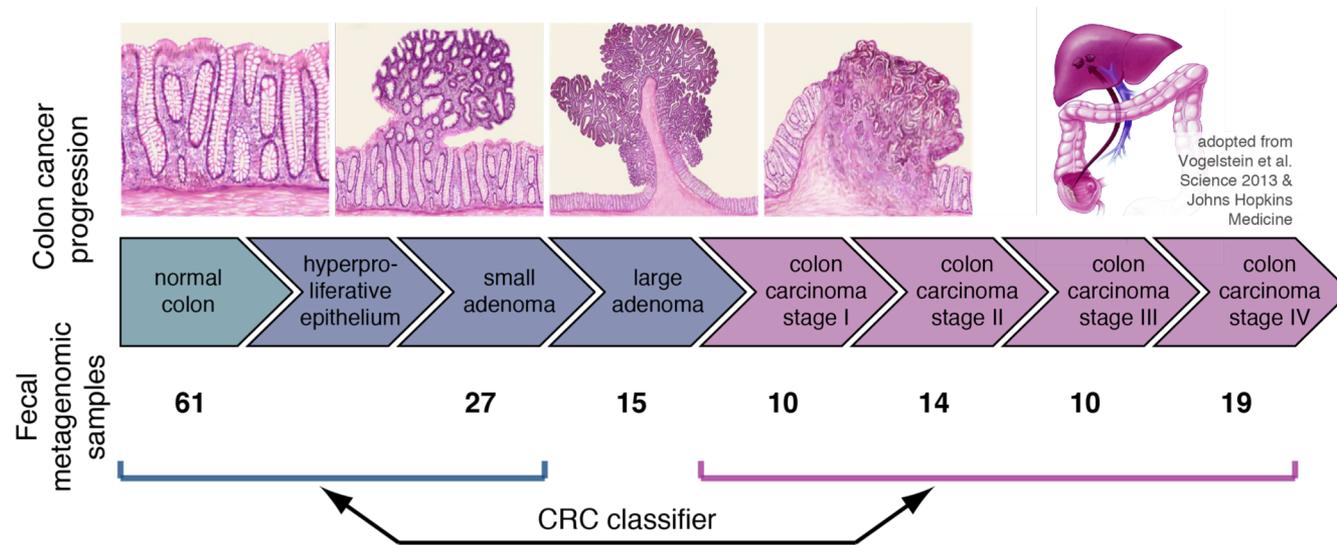
- *Fusobacteria* found in tumor tissue

[Kostic et al., *Genome Res* 2011],

[Castellari et al., *Genome Res* 2011]



Microbiota-based detection of colorectal cancer



Julien Tap



Anita Voigt

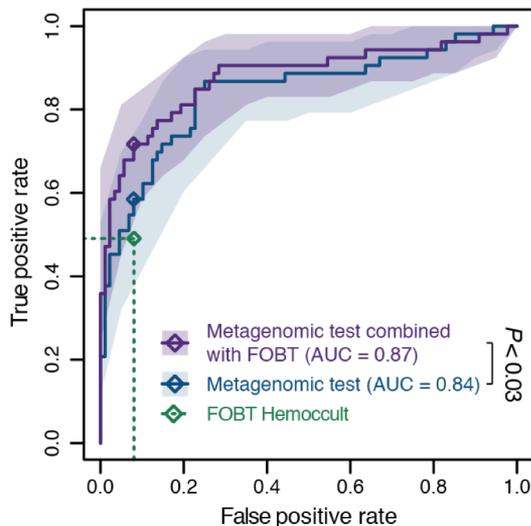
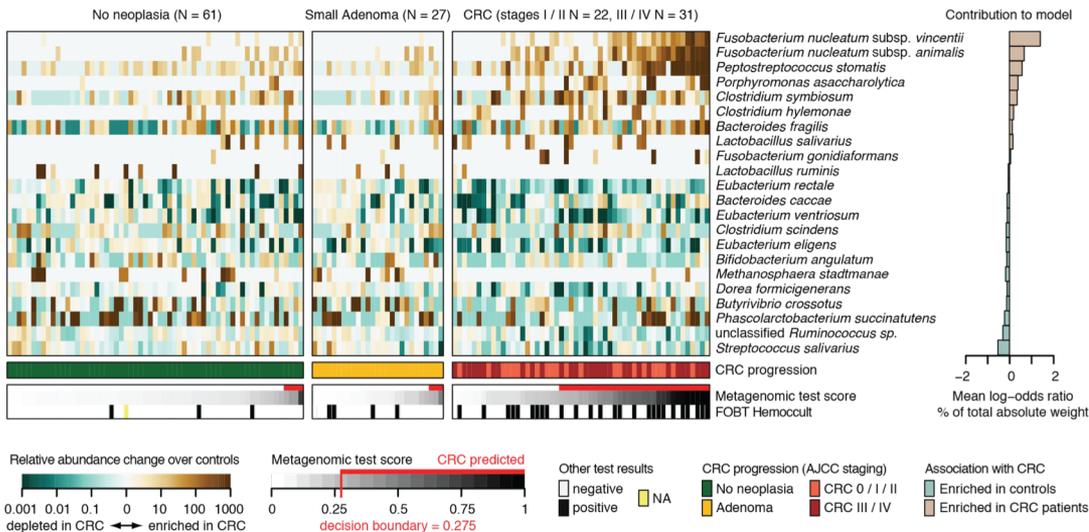


Peer Bork

- Mortality reduction achieved through early detection (survival rate >80% for early stages, <10% for metastatic stages)
- Better non-invasive screening tests needed
- **Goal:** Explore feasibility of CRC detection from fecal metagenomics

[Zeller*, Tap*, Voigt* et al., *Mol. Syst. Biol.* 2014]

A metagenomic classification model for CRC

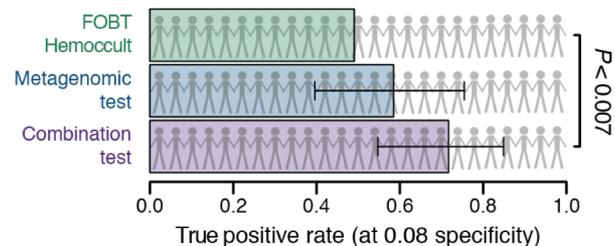


LASSO model based on species profiling of fecal metagenomes.

[Zeller*, Tap*, Voigt* et al., *Mol. Syst. Biol.* 2014]

Combination with standard clinical test (FOBT) increases detection rate by >45% over FOBT alone.

[Sobhani, Bork, Zeller et al., European Patent EP2955232A1, 2017]



Meta-analysis of microbiome case-control studies

How **consistent** are the CRC-microbiome associations in the face of **technical variability**?

Can we arrive at a “**common truth**” by pooling data?

How well do **metagenomic signatures generalize** across studies?

Meta-analysis of CRC metagenomics studies



Jakob Wirbel



Mani Arumugam



Peer Bork

Raw data from
shotgun
metagenomic
studies



Consistent
taxonomic
profiling with
mOTUs



Univariate testing



Machine learning
with **SIAMCAT**



Ece Kartal



Alessio Milanese

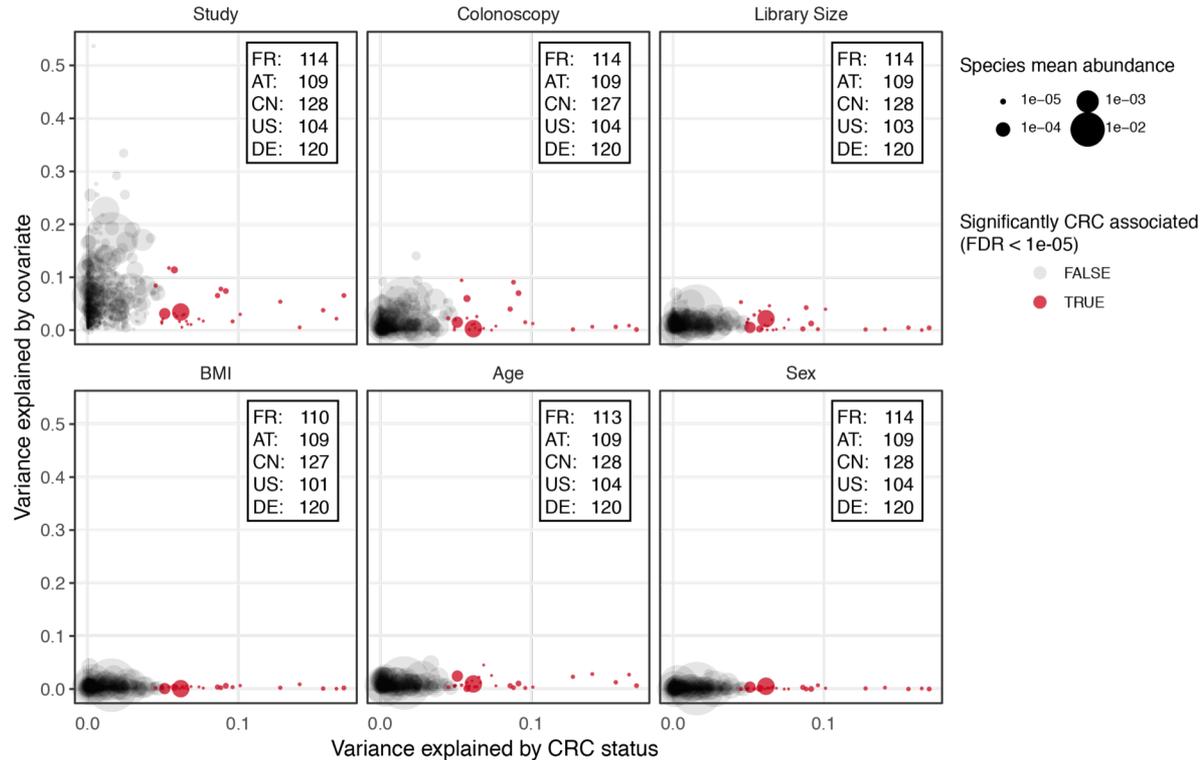
[Milanese et al.,
Nat. Commun., accepted]



Konrad Zych

<https://bioconductor.org/packages/SIAMCAT>

Heterogeneity and confounding in multi-study microbiome-disease associations

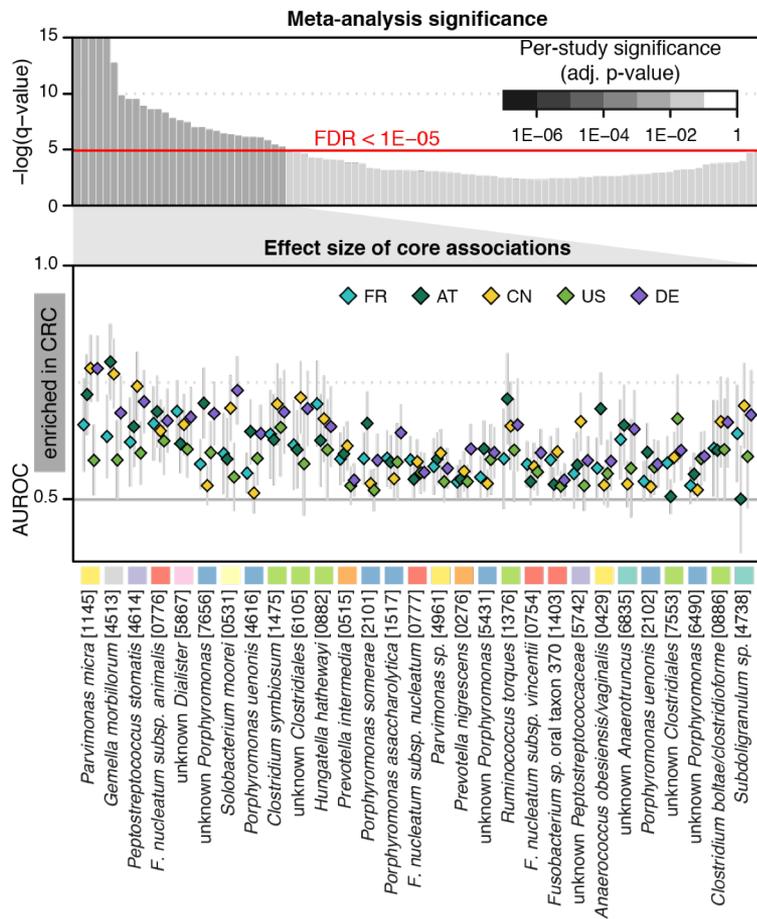


Confounder detection using ANOVA:

Most gut microbial species vary more by study than by disease.

biological confounders (age, colonoscopy) are weaker.

Meta-analysis finds a polymicrobial signature for CRC

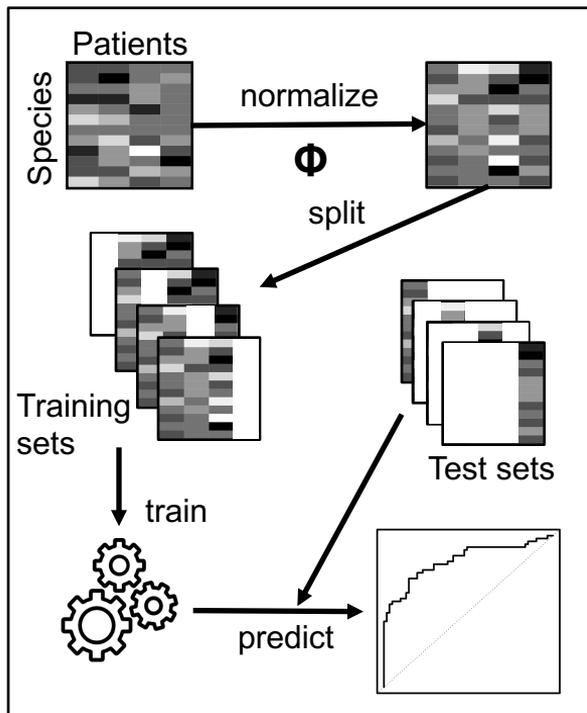


- Globally robust, highly significant gut microbial species associations
- Yet, heterogeneity across studies noticeable
- No single dominant species, limited value of individual species as biomarker

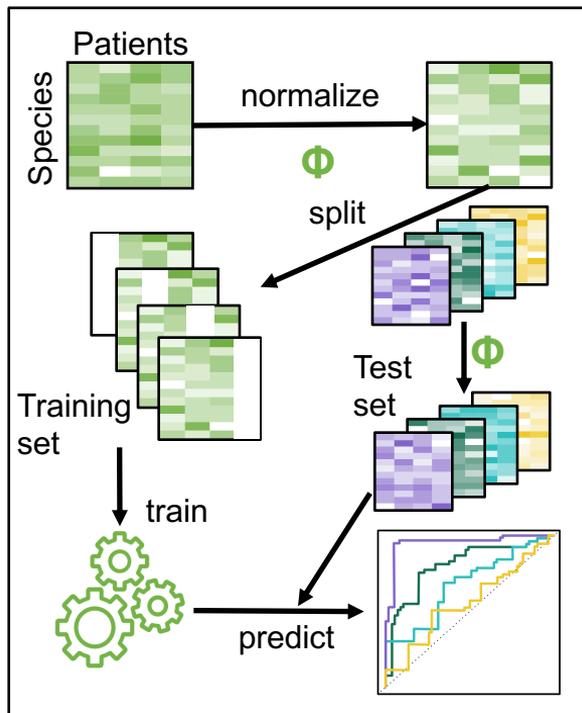
[Wirbel et al. *Nat. Med.*, accepted]

Machine learning approach

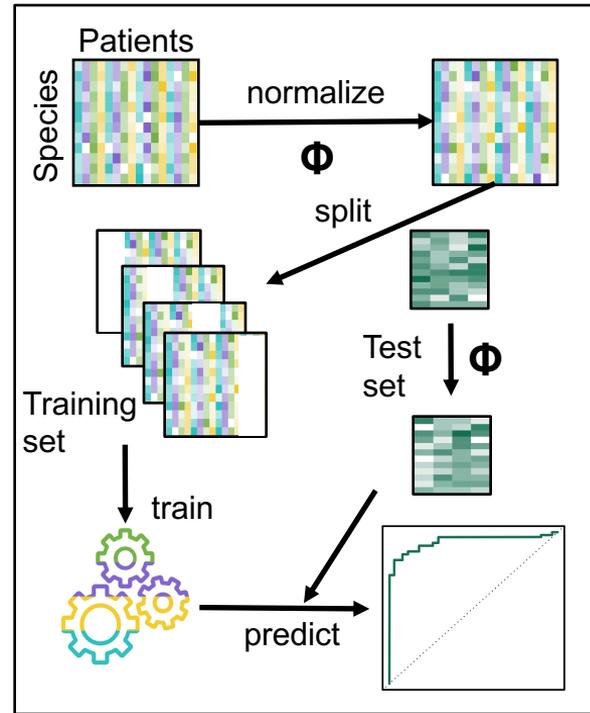
Cross-validation



Study-to-study transfer



Leave-one-study-out (LOSO)

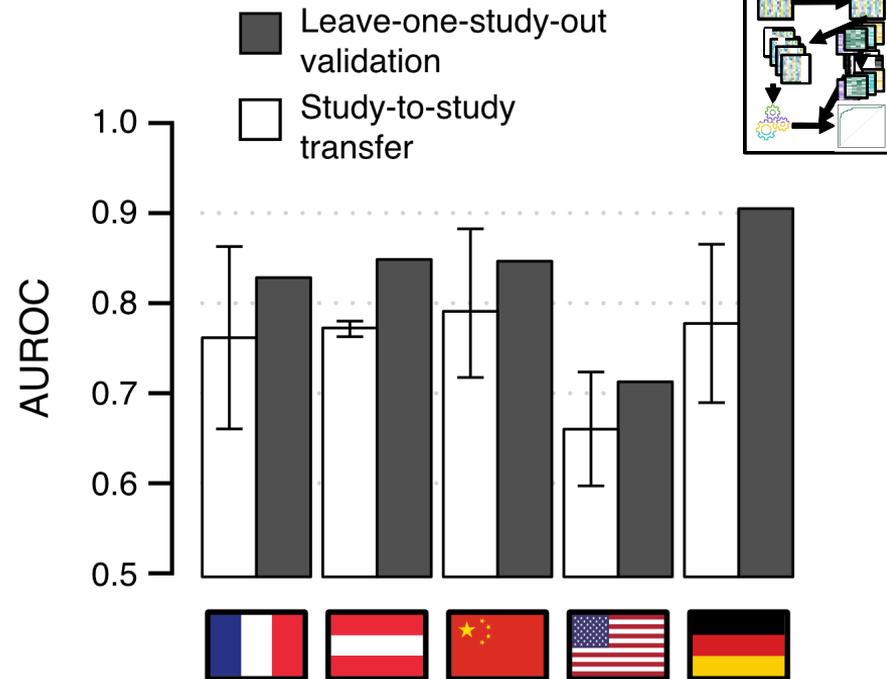


See also [Pasolli et al., *PLoS Comput. Biol.* 2016]

Models transfer well across datasets



- Classifiers generalize well across studies.
- Pooling data across studies improves CRC detection accuracy.

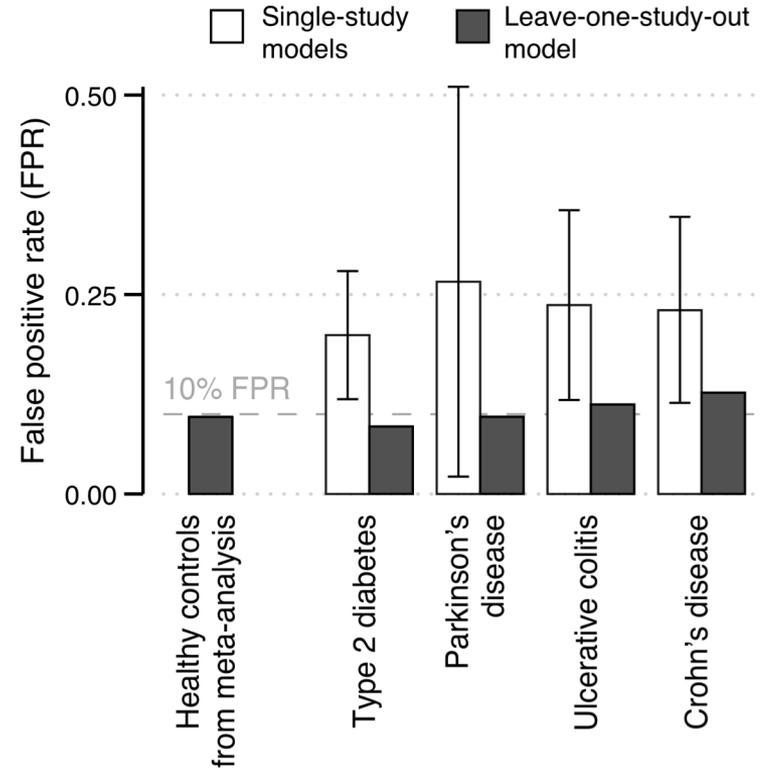


[Wirbel et al. *Nat. Med.*, accepted]

How disease-specific are the CRC models?

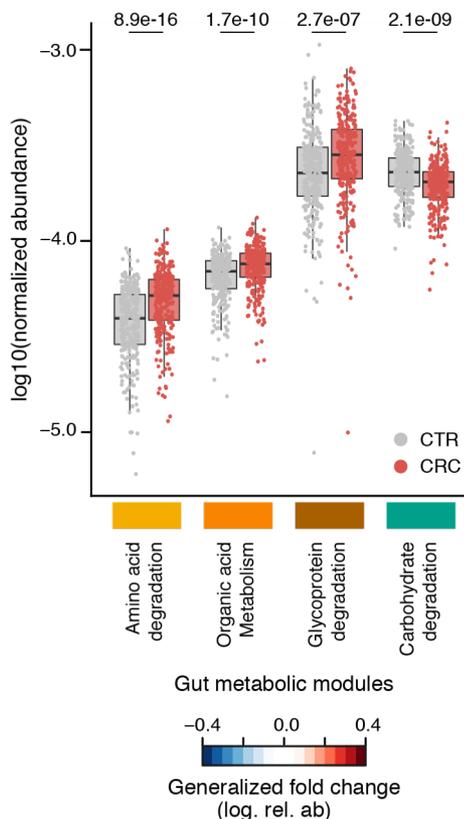
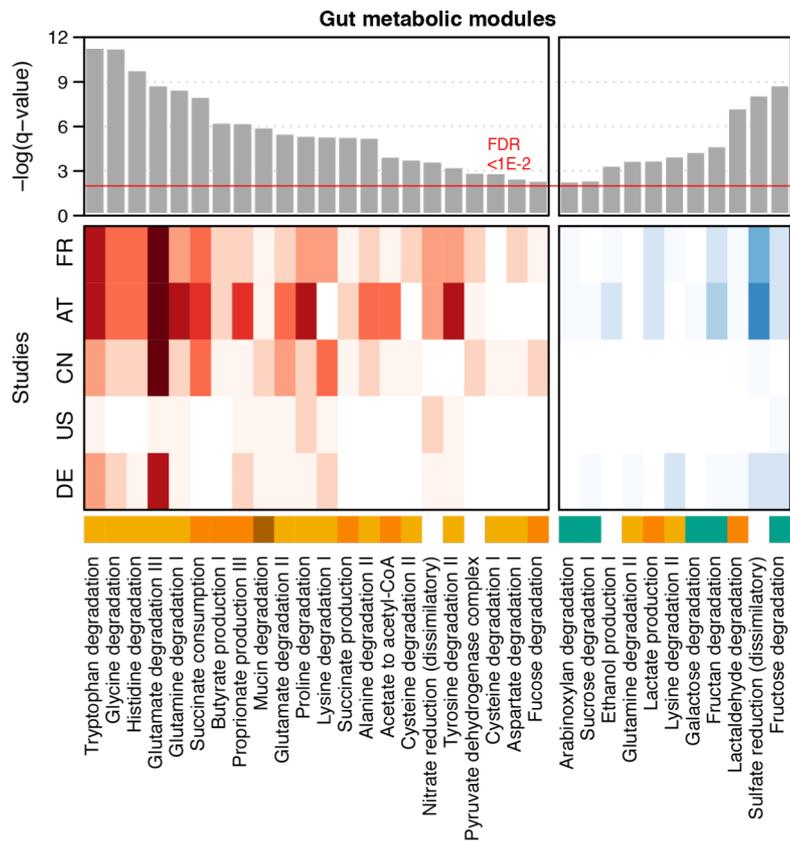
[Duvall et al., *Nat Commun* 2017]:

“... Thus, many associations found in case–control studies are likely not disease-specific but rather part of a non-specific, shared response to health and disease...”



[Wirbel et al. *Nat. Med.*, accepted]

Functional metagenome analysis



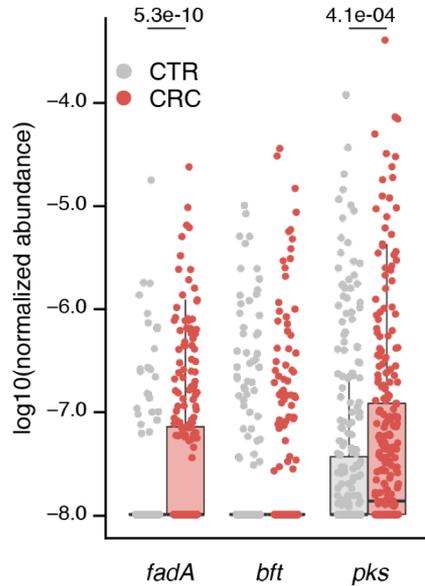
Metagenomic enrichments consistent with dietary risk factors:

- **Red / processed meat** – amino acid degradation
- **Dietary fiber** – carbohydrate degradation

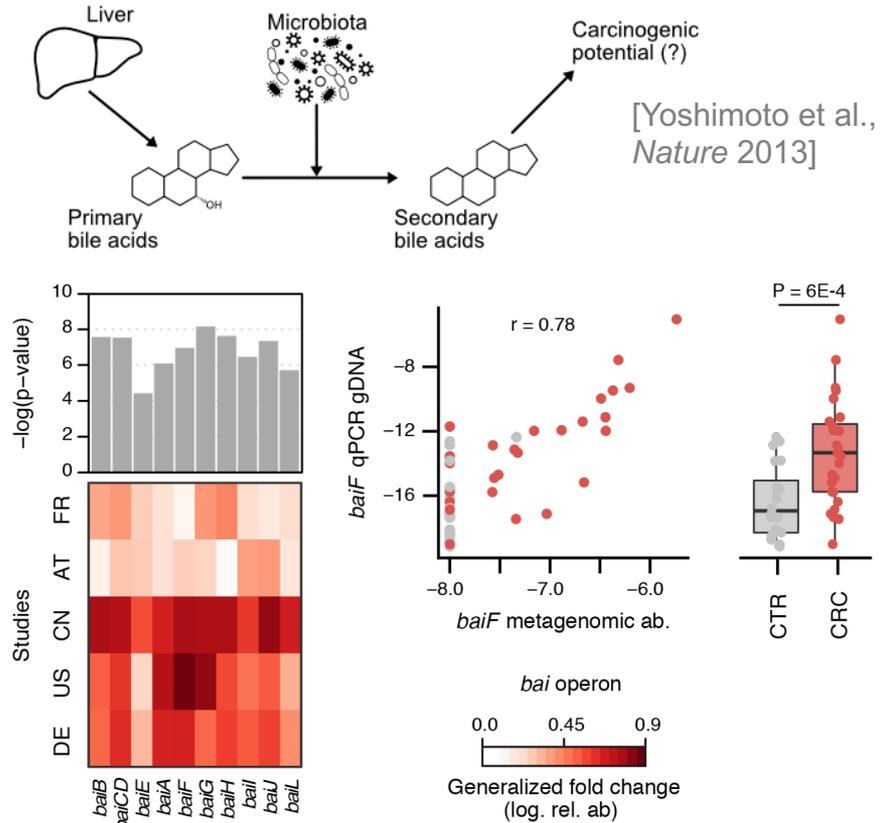
Mechanistic hypotheses from functional metagenomics analysis

CRC-enriched virulence factors:

- *Fusobacterium nucleatum* adhesin *FadA*
- *Bacteroides fragilis* enterotoxin *BFT*
- *E. coli* colibactin (encoded on *pks* island)



Significant enrichment of *bai*-encoded secondary bile acid conversion pathway



Summary and acknowledgments

- Globally robust, highly significant gut microbial associations with CRC
- Classifiers can be transferred with an AUC of ~0.8 across studies
- Classifiers *specifically* detect CRC
- Strong enrichment of genes encoding virulence factors and secondary bile acid production in CRC metagenomes

THANK YOU!



Jakob Wirbel, Alessio Milanese,
Jonas Fleck, Julien Tap, Anita Voigt,
Ece Kartal, Peer Bork



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Petra Schrotz-King



Nicola Segata



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