Ankersmit Laboratory

for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment

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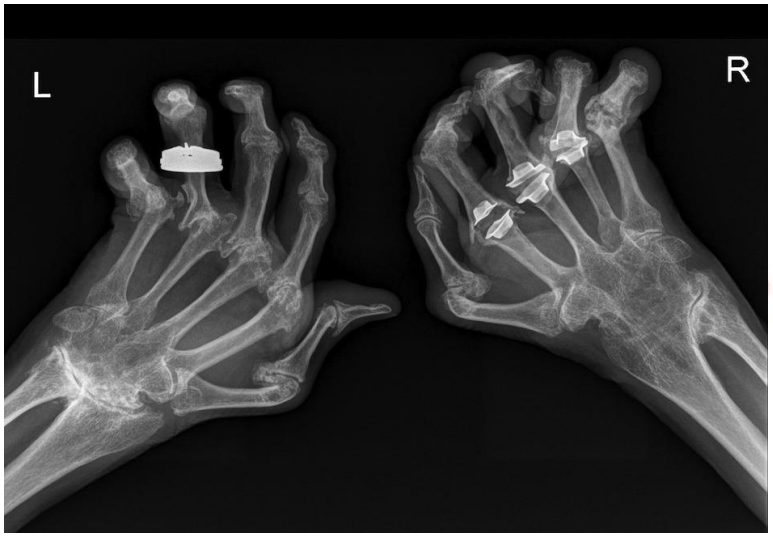
BACKGROUND

Rheumatoid arthritis (RA)

- Systemic, inflammatory, autoimmune disease
- Prevalence: 1%, ♀, 55-65 y

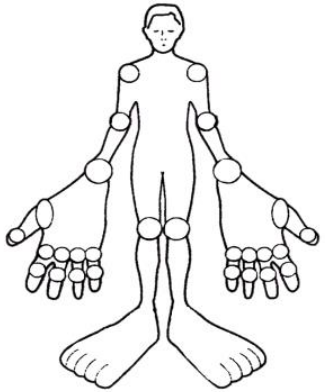


Etiology

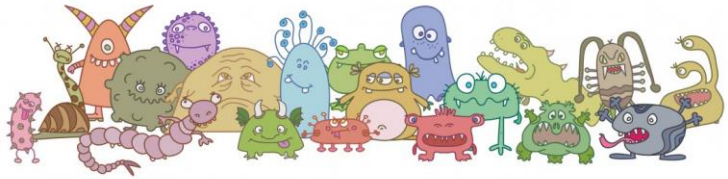
- Genetic and environmental factors
- Peridontitis
- T cell activation, IL-1, TNF-alpha, IL-6
- RF, ACPA, synovitis

Therapy

- cDMARDs, bDMARDs, NSAIDs, glucocorticoids, OP → "Treat
- SDAI, CDAI, DAS28



microbiome



- Microorganisms- gut, oral
- “additional organ”
- 100x more genes than human host
- Stable in individual but heterogeneous!
- Stress, smoking, diet, birthmode, ……..
- Influences metabolic and immune homeostasis

Aim

- Assess oral and gut microbiome in RA patients vs. HC

- Diagnostic?
- Change after treatment?
- Prognostic?

METHODS

Sample collection

Fecal samples:

- Frozen, extracted

Dental samples:

- Dental plaques scraped from dental surfaces
- Lysis with proteinkinase K
- DNA extraction

Saliva samples

- Posterior pharynx
- Lysed, extracted

patients

RA patients at Peking Union Medical Hospital, 18-65 years

- Exclusion: chronic serious infection, any current infection, cancer, pregnant or lactating women

Healthy controls: 18-65y, normal liver and kidney function, normal routine blood test, ESR, glucose, blood lipids, blood pressure

- Exclusion: chronic serious infection, any current infection, cancer, pregnant or lactating women, any autoimmune disease

fecal samples

- 77 treatment naïve RA patients
- 80 unrelated healthy controls
- 17 treatment naïve RA patients
- 17 healthy relatives
- 21 DMARD treated RA patients

=212

Oral samples

Dental:

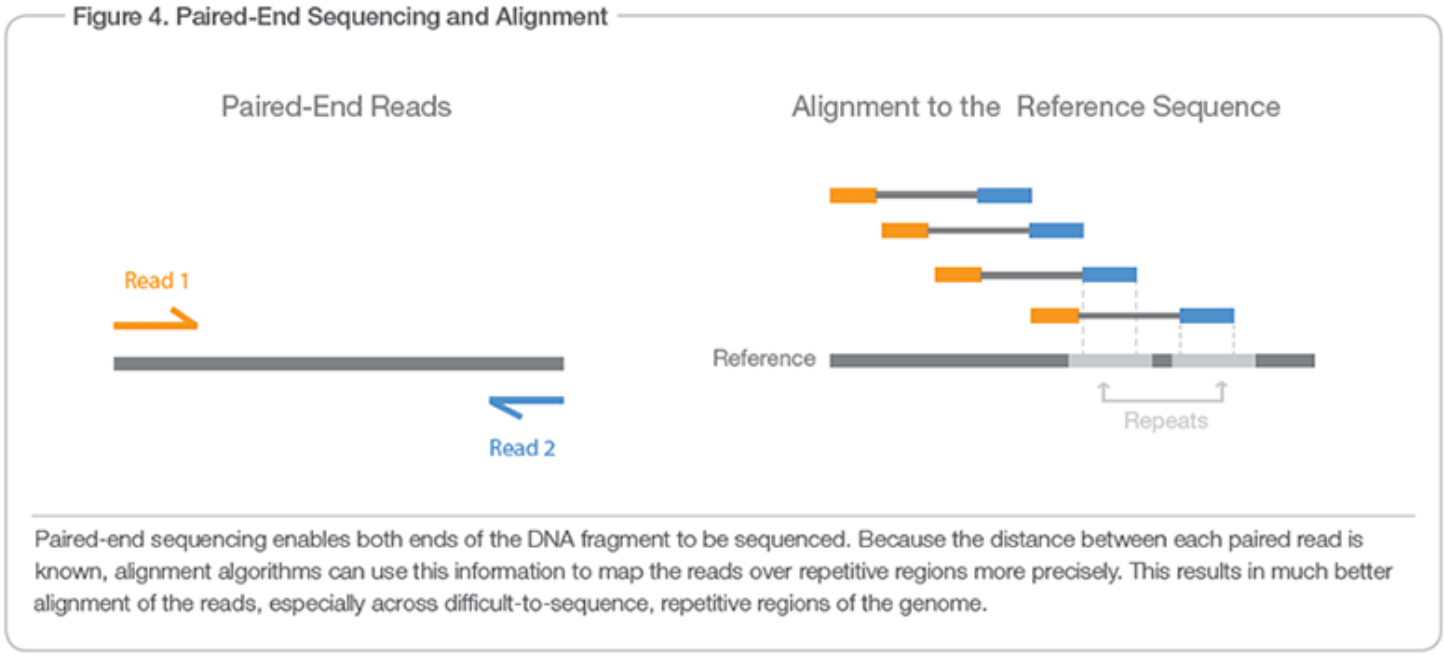
- 54 treatment naïve RA patients
- 51 controls

Saliva:

- 51 treatment naïve RA patients
- 47 controls

Metagenomic sequencing

- DNA broken up randomly
- Paired-end metagonomic sequencing (Illumina platform)



Gene catalog construction

- Gene prediction with GeneMark v2.7d
- Integrated data into an existing gut microbial reference-gene catalog
- Redundant genes removed
- 212 Fecal samples: → 3 800 011 genes
- 203 oral samples: → 3 234 997 genes

RESULTS

Gut microbiome

- Gut microbial diversity and richness- similar
- Molecular mimicry of RA-associated antigens

RA vs. HC: different gut microbiome

- 117 219 genes different in RA vs. HC (Wilcoxon rank sum)
- → clustered into Metagenomic linkage groups (MLG) according to correlated abundance variation
- 88 MLGs with at least 100 genes each

- RA gut enriched in Gram positive bacteria and depleted in Gram negative bacteria

Correlation with clinical indices

Positive (RA)

- IgA (C. asparagiforme, Bacterioides sp.)
- IgG (Lactobacillus sp.)
- Platelet count (E. faecalis)

Negative (HC)

- IgA, IgG (Con-7851, B. bifidum)
- Anti-CCP, RF (Haemophilus sp., Strep. Austr., …..)

RA vs. HC: different oral microbiome

- Dental: 371 990 gene markers different
- Salivary: 258 055 gene markers different

- → 171 dental MLGs, 142 salivary MLGs

Correlation with clinical indices

Negative (HC)

- CRP, anti-CCP (Aggregatibacter sp, Haemophilus spp., Neisseria spp,…)

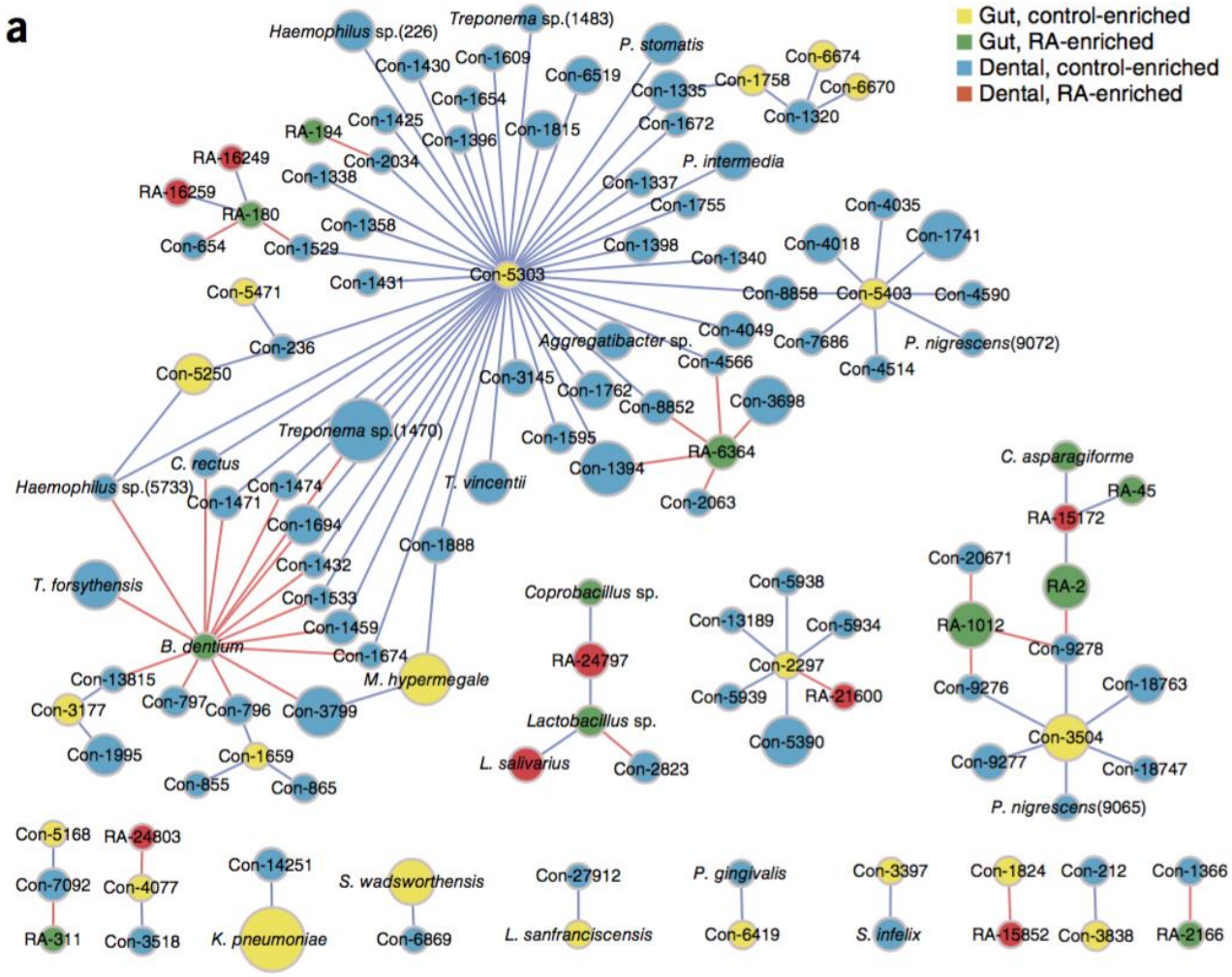
RA

- Anti-CCP
- CRP
- RF

Gut vs. oral

- covariation of bacteria at different body sites

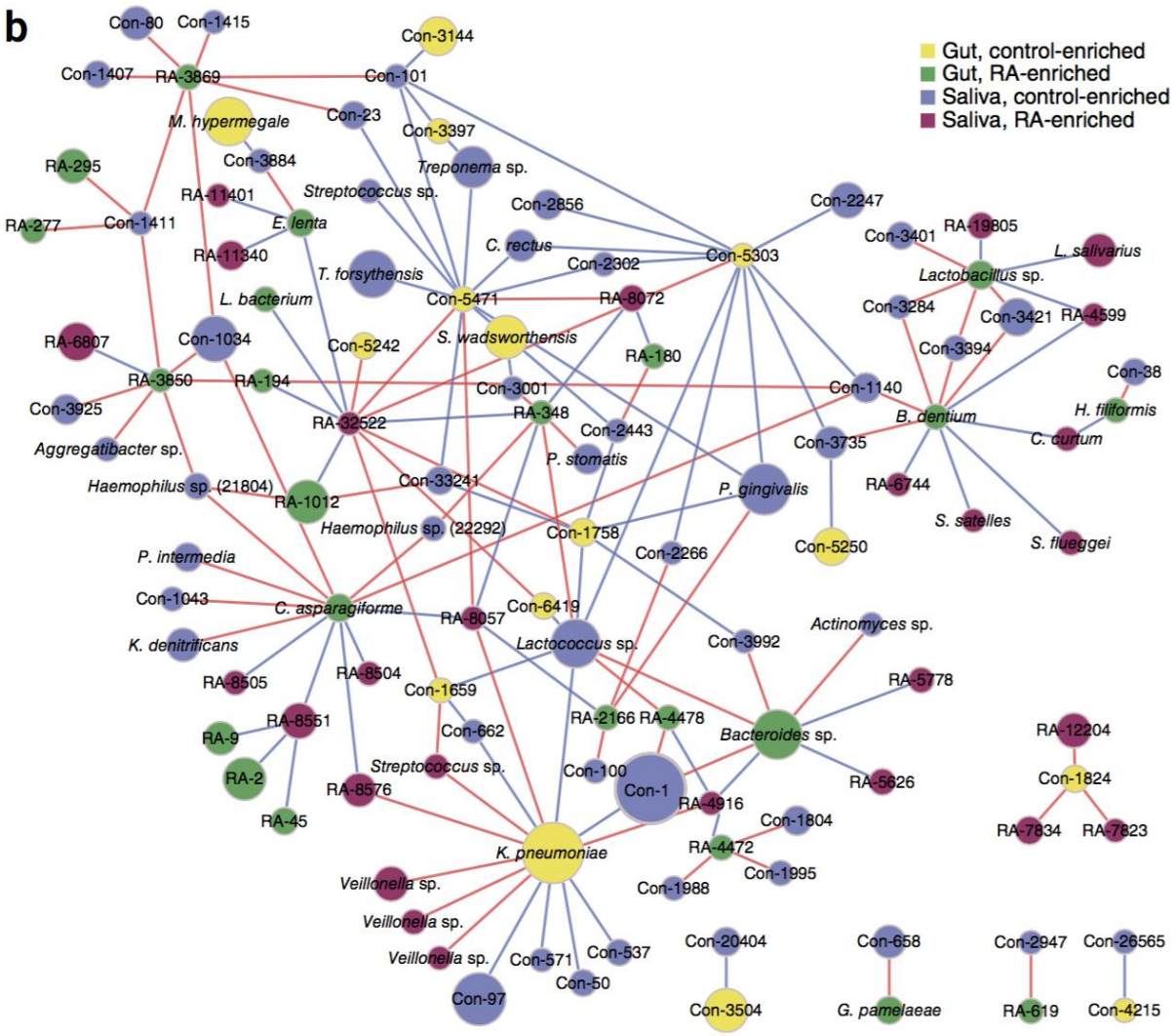
Gut vs. oral



a- correlation MLPs gut and dental
Blue → Spearman's correlation coefficient > 0.4, $P < 0.05$; red → Spearman's correlation coefficient < −0.4, $P < 0.05$

Gut vs. oral

b- correlation MLPs gut and salivary
Blue → Spearman's correlation coefficient > 0.4, $P < 0.05$; red → Spearman's correlation coefficient < −0.4, $P < 0.05$



Diagnostic?

- random forest calculation based on MLGs
- Suggest using 8 (of 88) fecal MLGs
- 6 dental MLGs
- 2 salivary MLGs

- Classification based on 2 sides -> no subject misclassified except for 1 relative HC
- Both treatment naïve and DMARD treated RA patients

- EXCEPTION: dental samples from RA with low disease activity

Microbiom as diagnostic tool

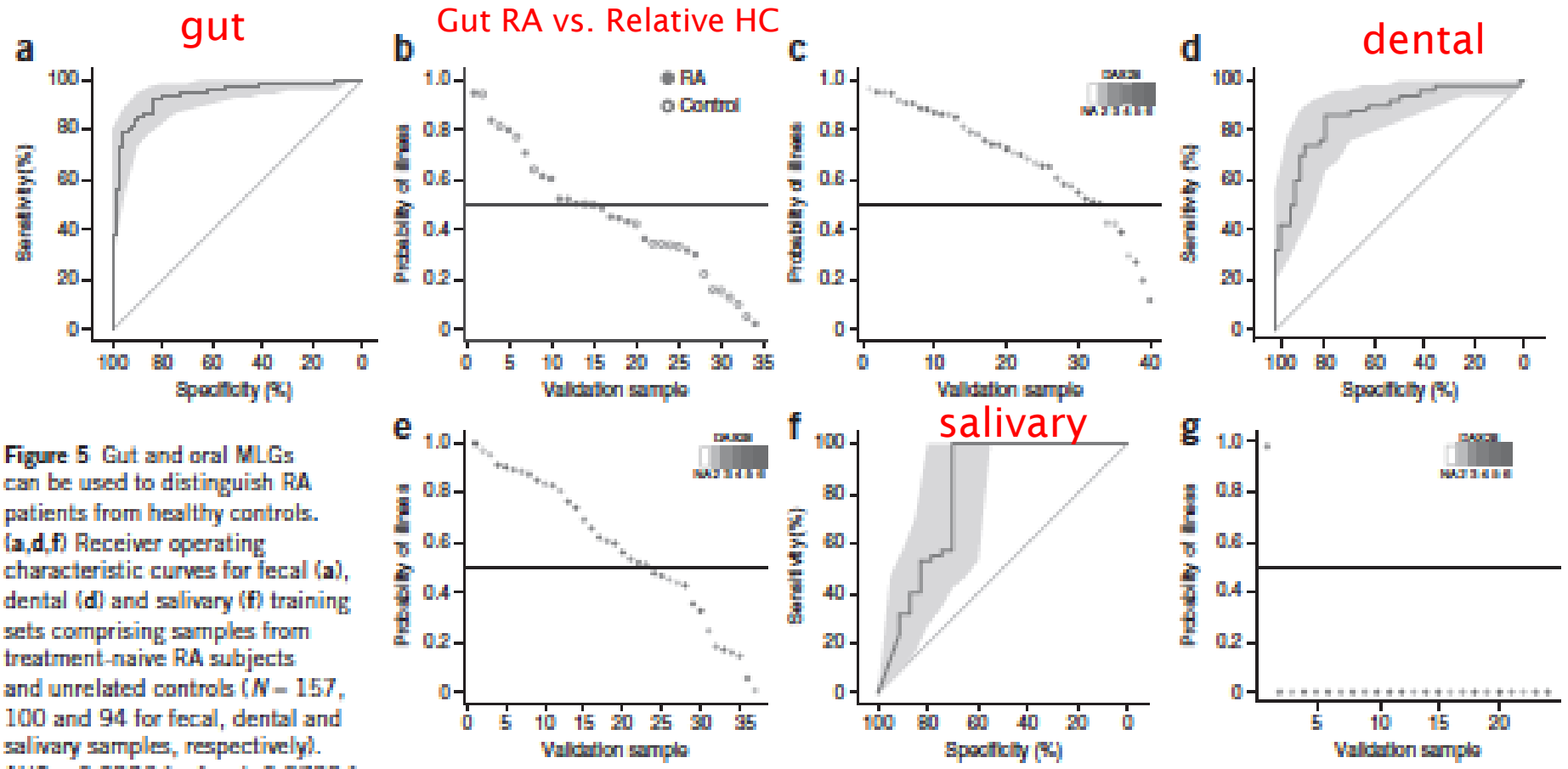
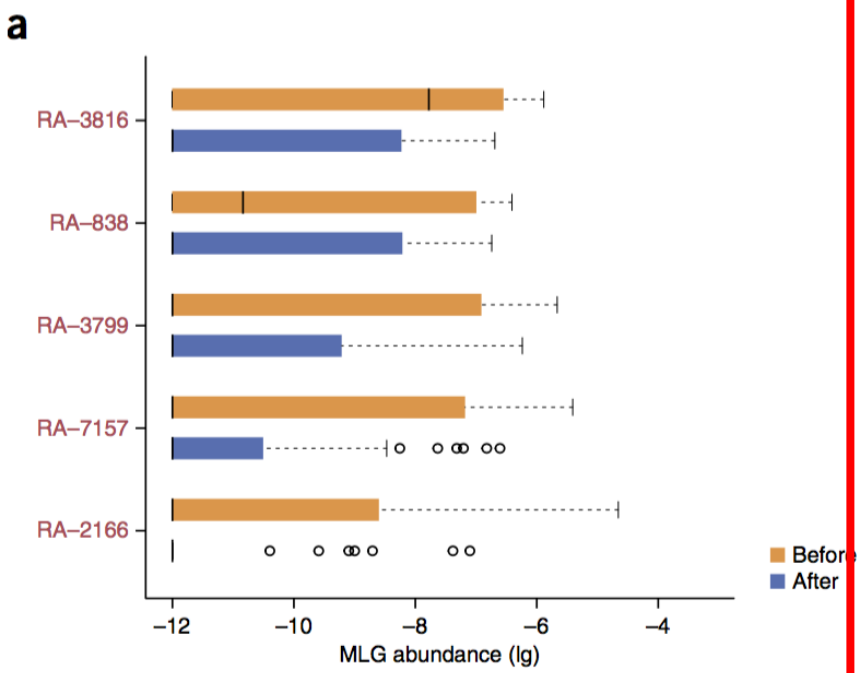


Figure 5 Gut and oral MLGs can be used to distinguish RA patients from healthy controls. (**a**,**d**,**f**) Receiver operating characteristic curves for fecal (**a**), dental (**d**) and salivary (**f**) training sets comprising samples from treatment-naive RA subjects and unrelated controls ($N = 157$, 100 and 94 for fecal, dental and salivary samples, respectively). AUC = 0.9396 for fecal, 0.8702 for dental and 0.8135 for salivary samples. The 95% confidence intervals (CIs) are shown as shaded areas. (**b**) Classification of fecal samples from 17 controls and 17 RA subjects, either consanguineous or nonconsanguineous relatives. Open circles, controls; filled circles, RA subjects. (**c**,**e**,**g**) Classification of fecal (**c**), dental (**e**) and salivary (**g**) samples from DMARD-treated RA patients ($N = 40$, 37 and 24 for fecal, dental and salivary samples, respectively), shaded on a scale relative to DAS28. NA (no shading), DAS28 not available. The classification results for all samples are listed in **Supplementary Table 1**. Diagonal lines in graphs mark an AUC of 0.5 (i.e., random classification). Horizontal lines mark the probability cutoff (0.5).

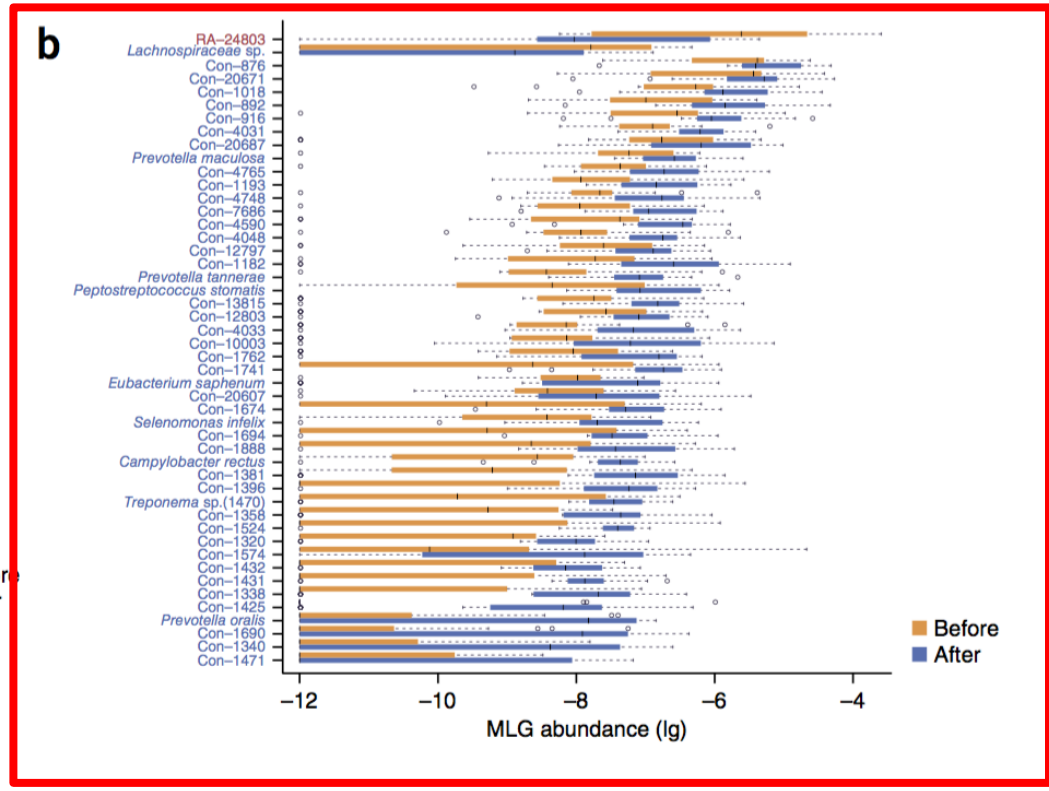
Influence of DMARD treatment

- Samples before and 3 months after DMARD start
- HC MLGs increased, especially in patients with better improvement
- =MLGs associated with CRP, anti-CCP, RF

Influence of DMARD treatment



gut



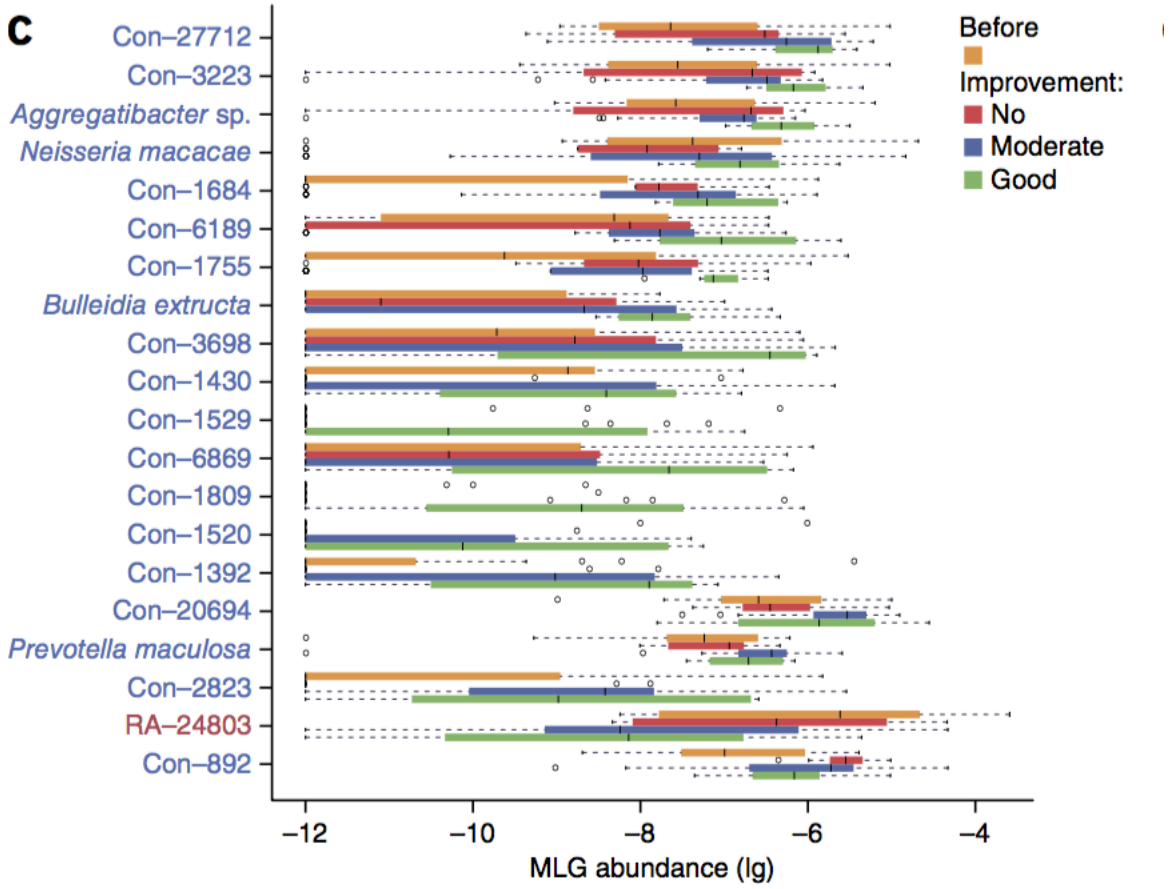
dental

More dental and salivary MLGs significant changes than gut MLG

Influence of DMARD treatment

Change of dental MLGs depending on treatment outcome

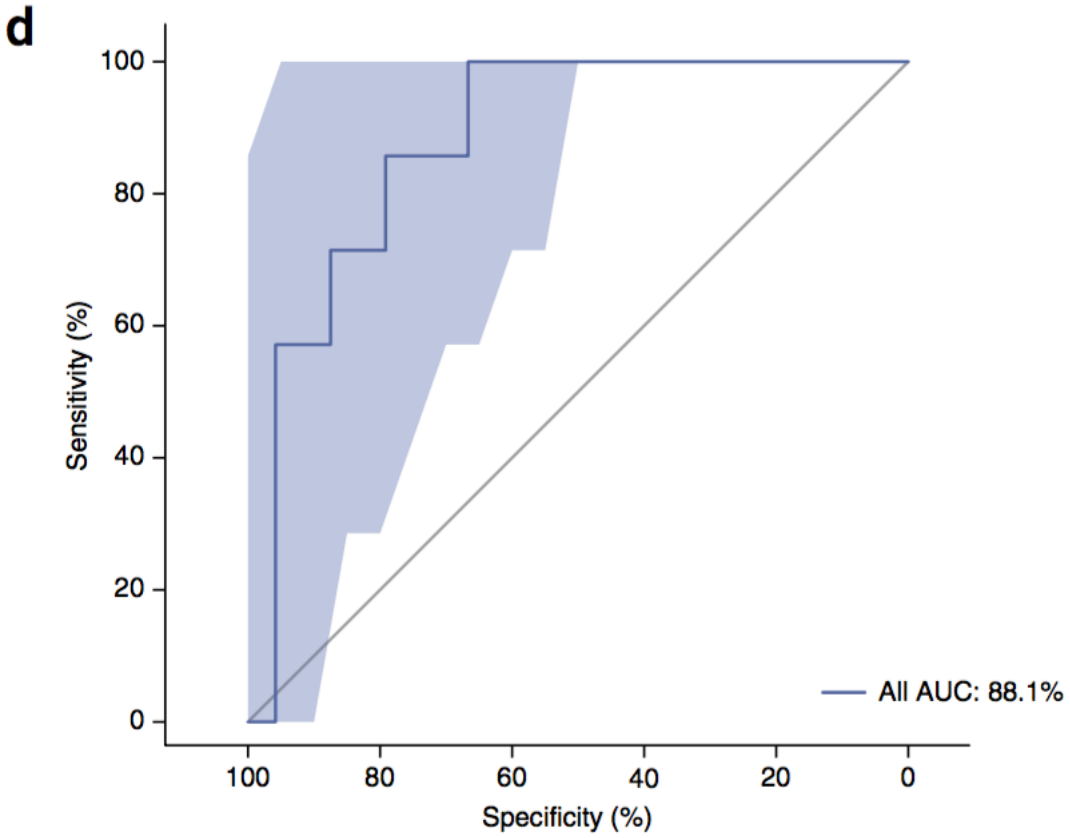
Bigger difference in patients with better improvement!



Influence of DMARD treatment

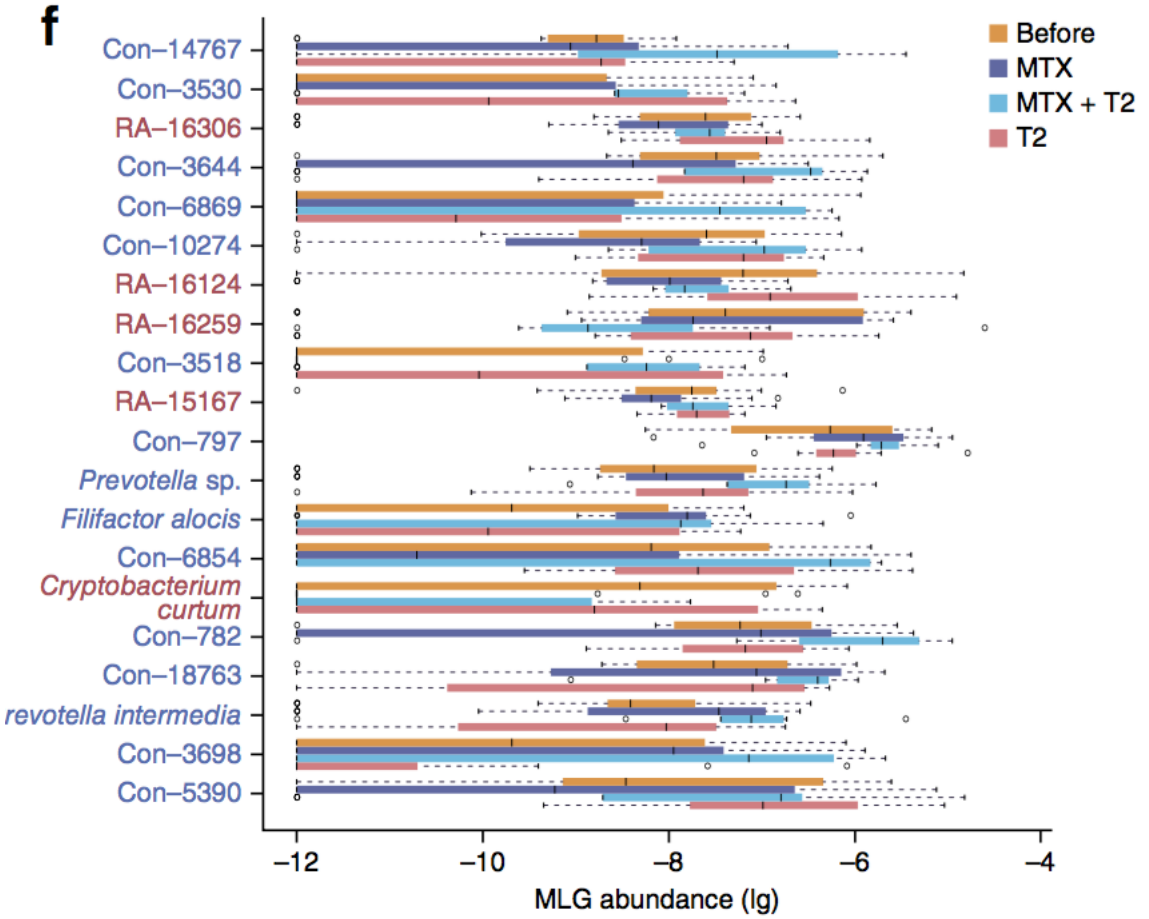
Cross-validated random forest models for dental MLPs before treatment:

prediction of improvement after DMARD treatment



Influence of DMARD treatment

Change of salivary MLGs affected by DMARD treatment



Discussion

- Alterations in RA- associated Gut and oral microbiomes
- Partly relieved by DMARD treatment
- Gut and oral MLGs correlate with each other
- Gut and oral MLGs correlate with clinical indices
- Allow classification (RA/ HC)
- Allow prediction of treatment outcome

Outlook

- Pathogenesis?
- Diagnosis?
- Prognosis?
- Treatment decision?