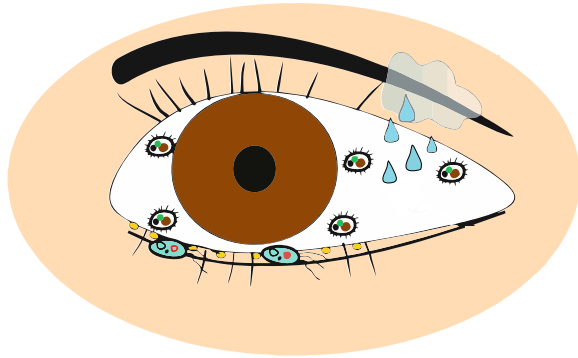


The Ocular Surface Microbiome in Dry Eye Disease



Dr. Denise C. Zysset-Burri

Molecular Diagnostics 2024
Symposium, March, 07-08, 2024

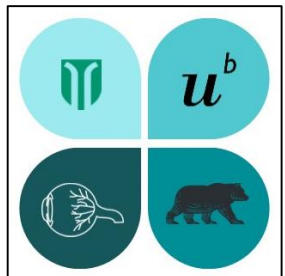
INSELSPITAL
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BERN UNIVERSITY HOSPITAL

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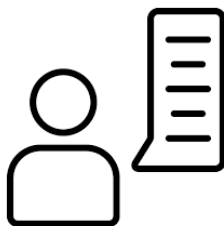
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Department of Ophthalmology, Inselspital, Bern, Switzerland
Microbiome Research Group

www.augenheilkunde.insel.ch/de/lehre-und-forschung/researchlaboratories/microbiome



Outline



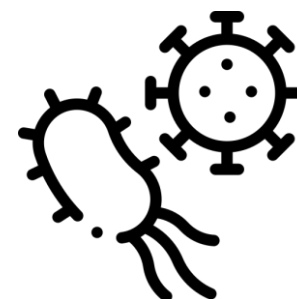
1. Introduction: The human microbiome

- Terminology and facts
- Homeostasis vs. dysbiosis
- Gut – eye axis
- The gut microbiome in eye diseases



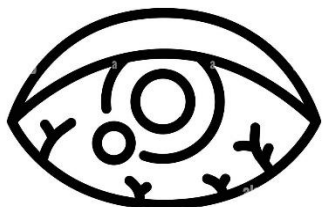
2. The ocular surface microbiome in health

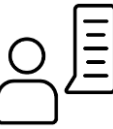
- Is there an ocular surface microbiome?
- Sampling and characterization
- The human tear proteome
- Low-abundant microbiome (opportunities and pitfalls)



3. The ocular surface microbiome in disease: Dry eye disease

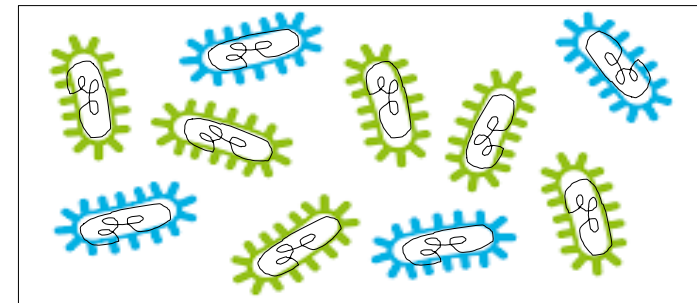
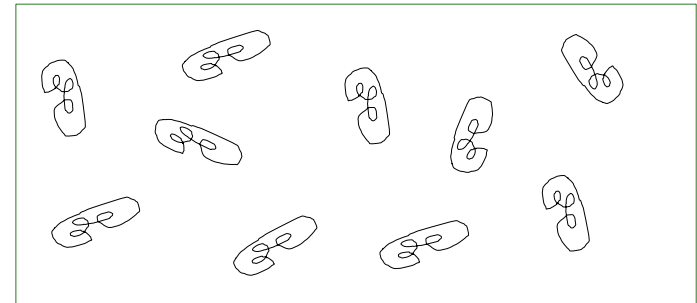
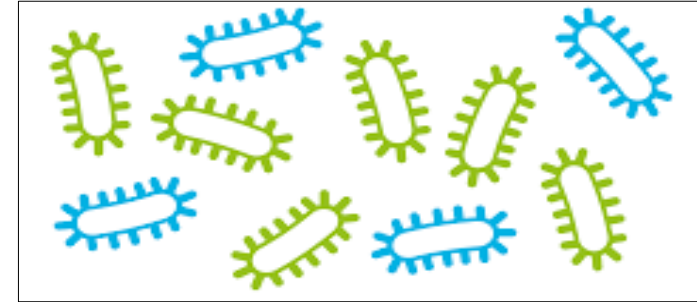
- Epidemiology / diagnosis / definition
- Study design and results
- Conclusions and further directions





The human microbiome

- **Microbiota** = all the microorganisms found in a given environment
- **Metagenome** = all the genetic material present in an environmental sample
- **Microbiome** = all the microorganisms and their genomes (i.e. microbiota + metagenome)



The human microbiome

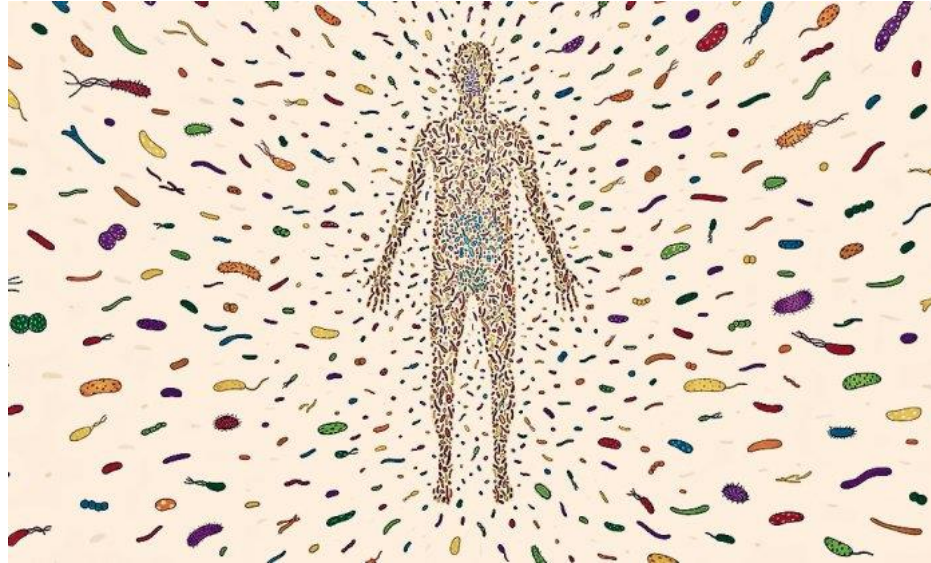
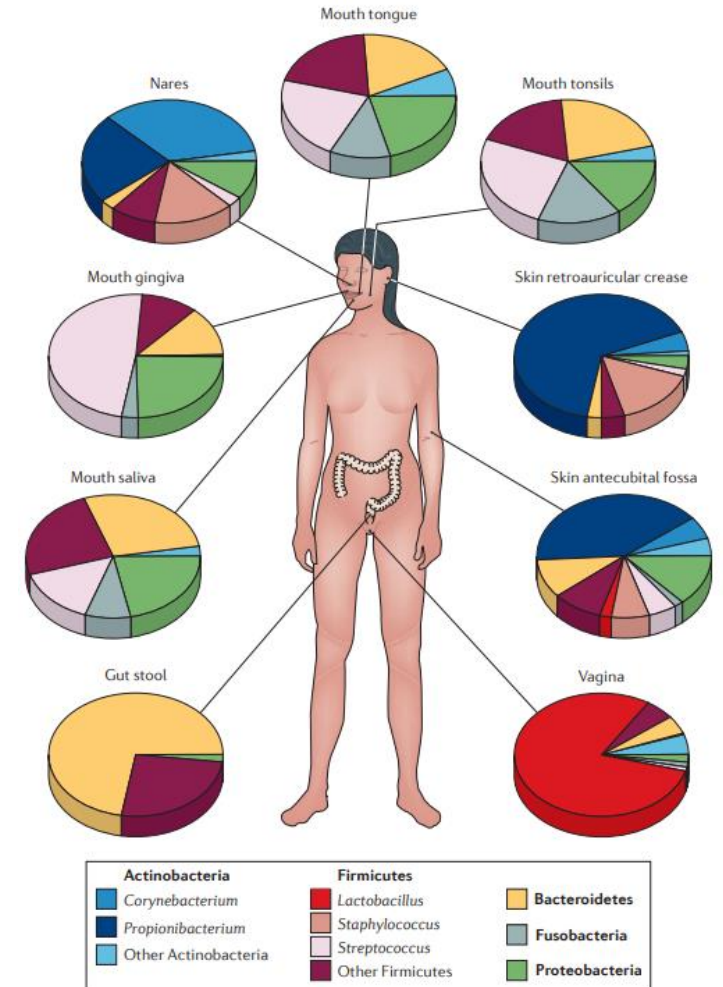
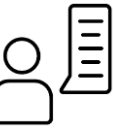


Image: www.amnh.org

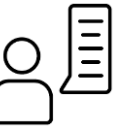
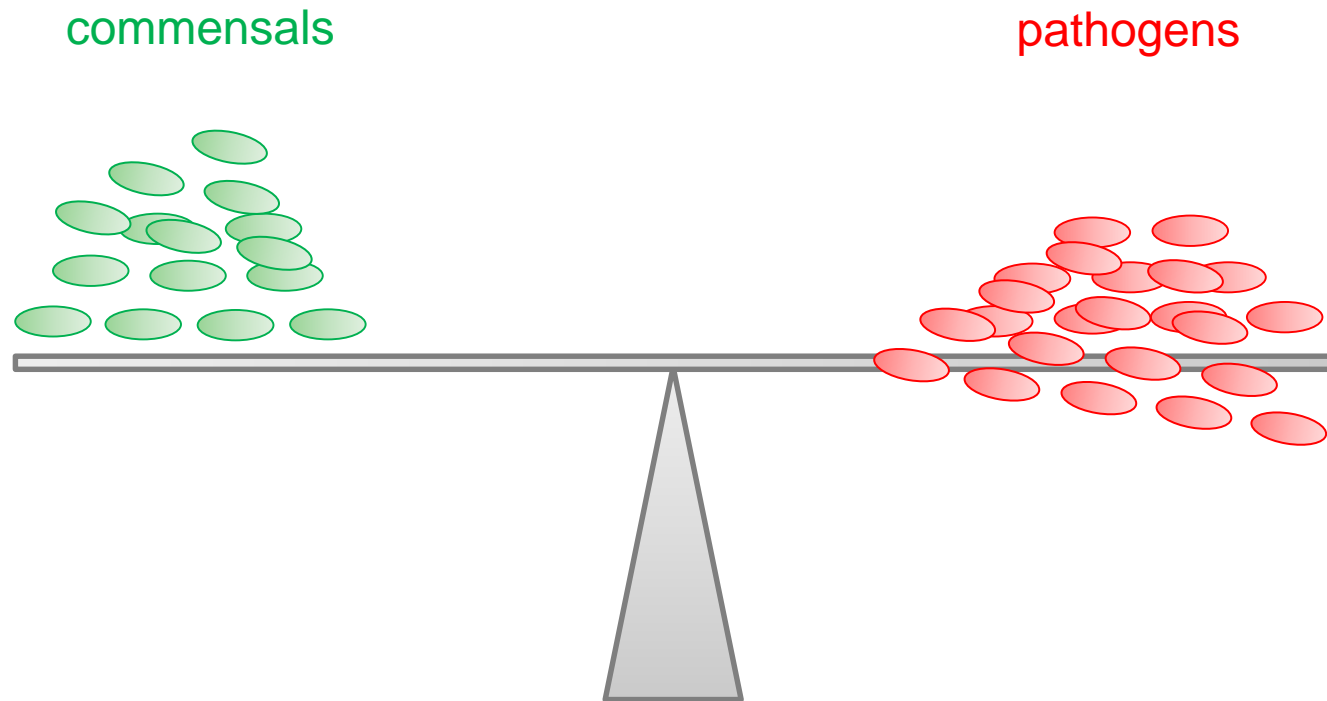
- ~ 39 trillion bacteria
- functions: digestion of food, defence against pathogenic microbes, stimulation/regulation of the immune system
- characteristic composition in each niche
- dependent on demographic parameters
- role in health and disease

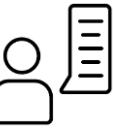


Lasken R. S. and McLean J. S.: Recent advances in DNA sequencing of microbial species from single cells. *Nature Reviews Genetics*, 2014

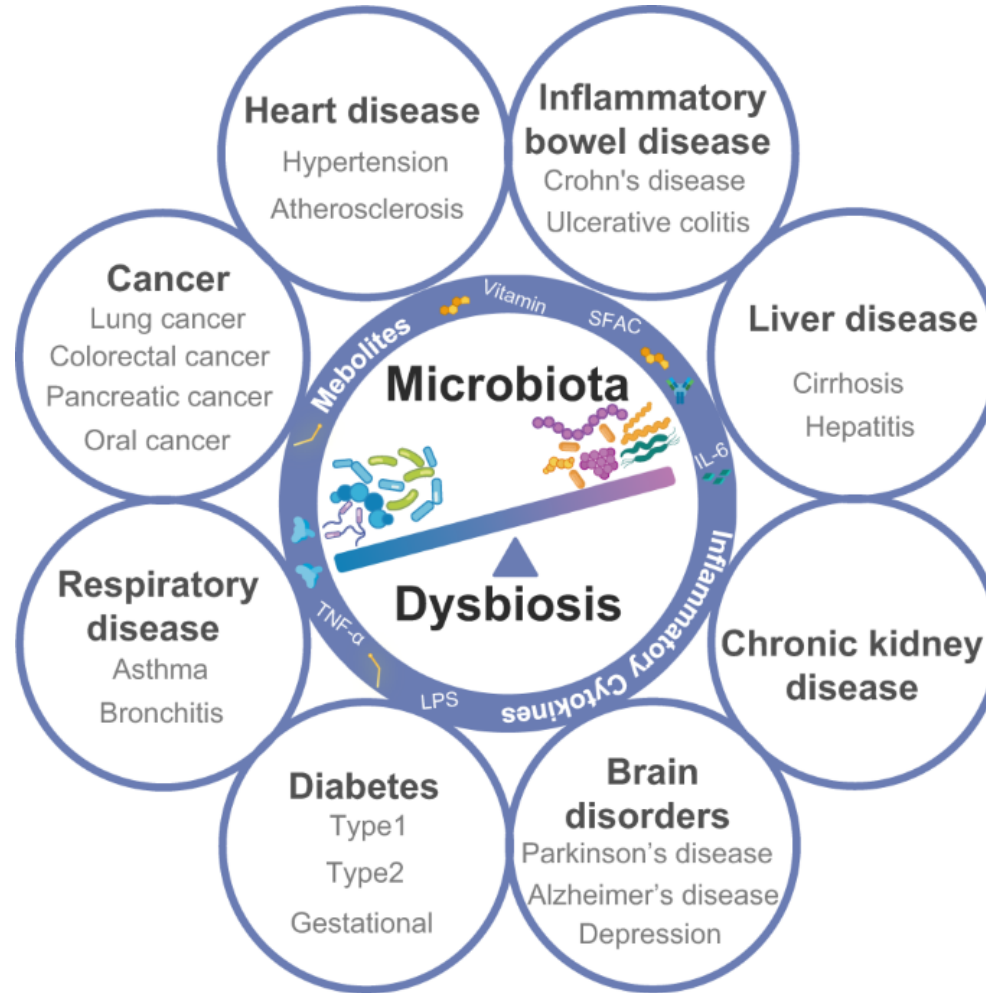


Homeostasis vs. dysbiosis



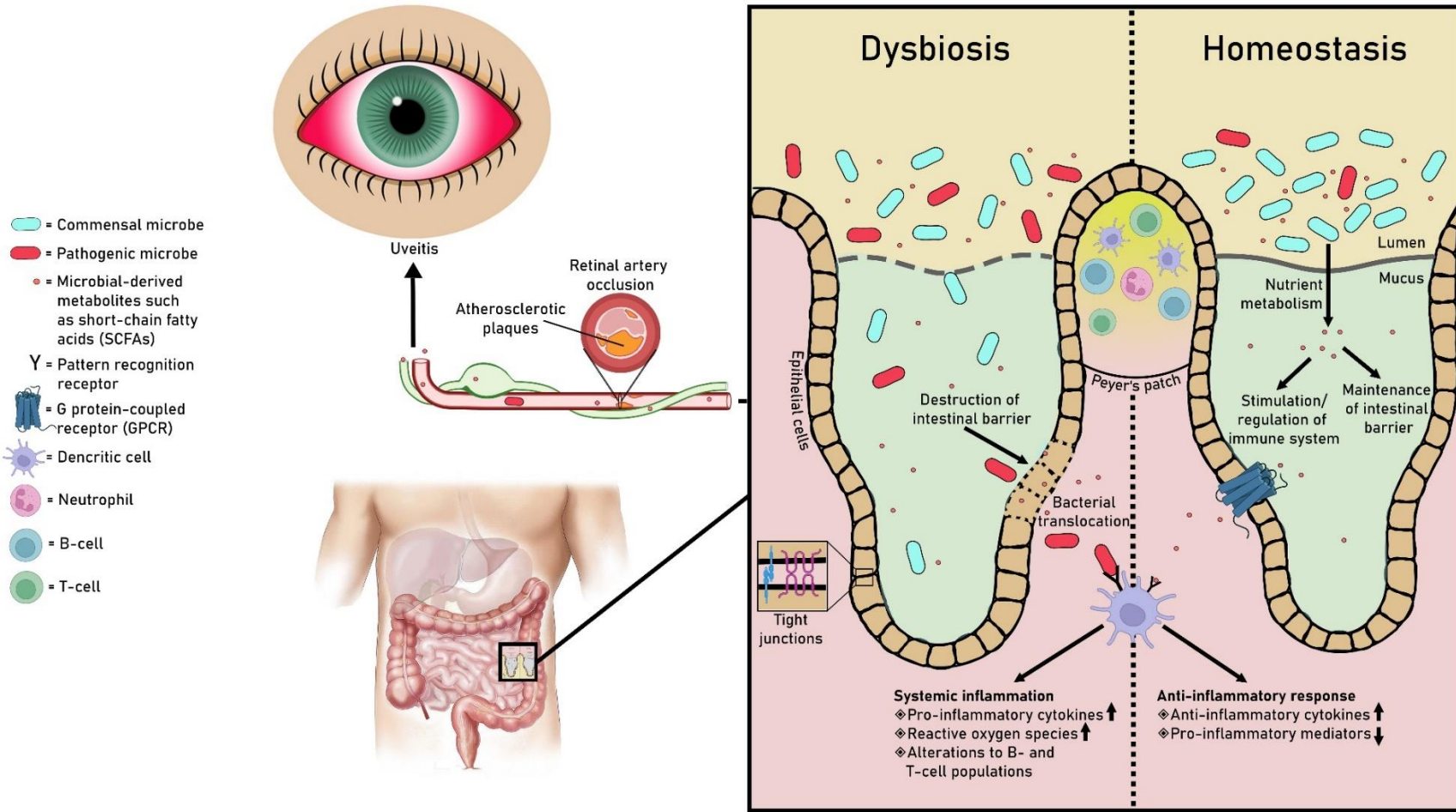


The role of the microbiome in disease

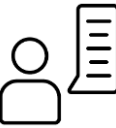


Hou K. et al.: Microbiota in health and diseases. Signal Transduction and Targeted Therapy, 2022

Gut – eye axis



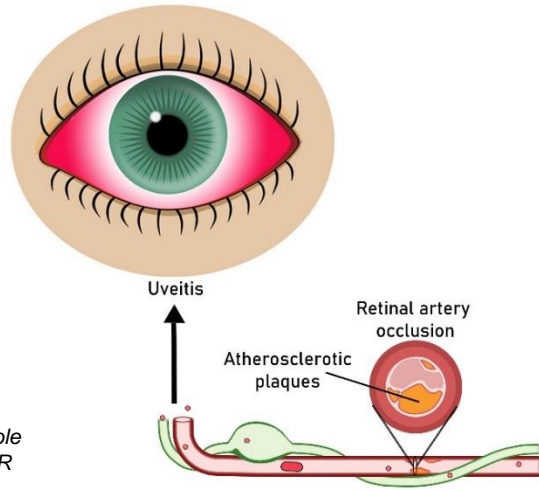
- complex interactions with the immune system
- microbial translocation
- regulation of anti- and pro-inflammatory cytokines
- systemic inflammation
- transport to distant sites



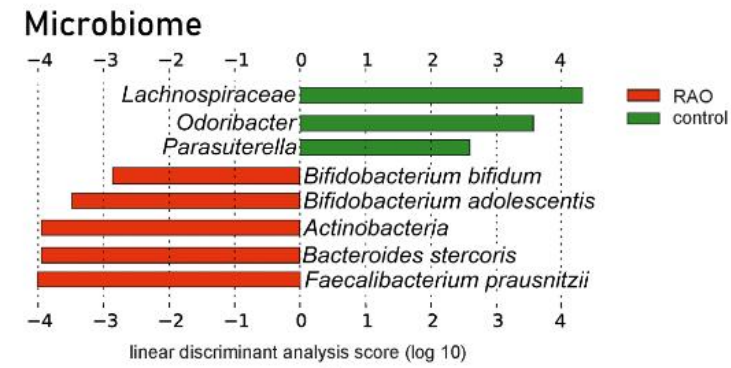
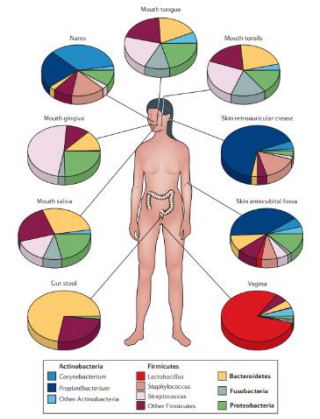
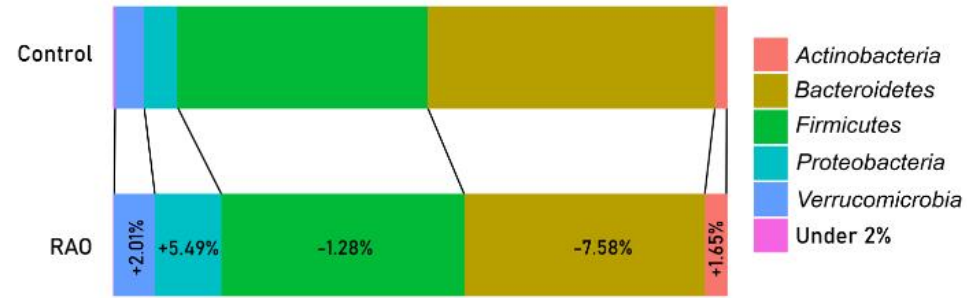
Adapted from Zysset-Burri D. C. et al.: The role of the gut microbiome in eye diseases. PRER (2022)

The gut microbiome in RAO

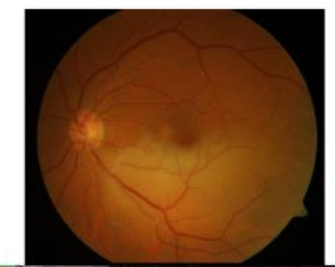
- RAO, **retinal artery occlusion**
- Obstruction of retinal blood flow -> lack of oxygen delivery -> severe vision loss in ischemic area
- Ophthalmic emergency
- No evidence based treatment with visual benefit
- **TMAO**, trimethylamine-N-oxide: gut derived, pro-atherogenic metabolite, upregulated in patients and correlated with specific microbes -> *risk factor and therapeutic target!*



Adapted from Zysset-Burri D. C. et al.: The role of the gut microbiome in eye diseases. PRER (2022)



Diet
 Converts dietary choline, phosphatidylcholin, betaine and L-carnitine into TMA, which is oxidized into TMAO
 Promotes formation of foam cells from macrophages, altering cholesterol transport



- Environment**
- ◆ Obesity
 - ◆ Atherosclerosis
 - ◆ Hypertension
 - ◆ Diabetes
 - ◆ Stress
 - ◆ Alcohol abuse
 - ◆ Smoking
 - ◆ Unhealthy dietary patterns

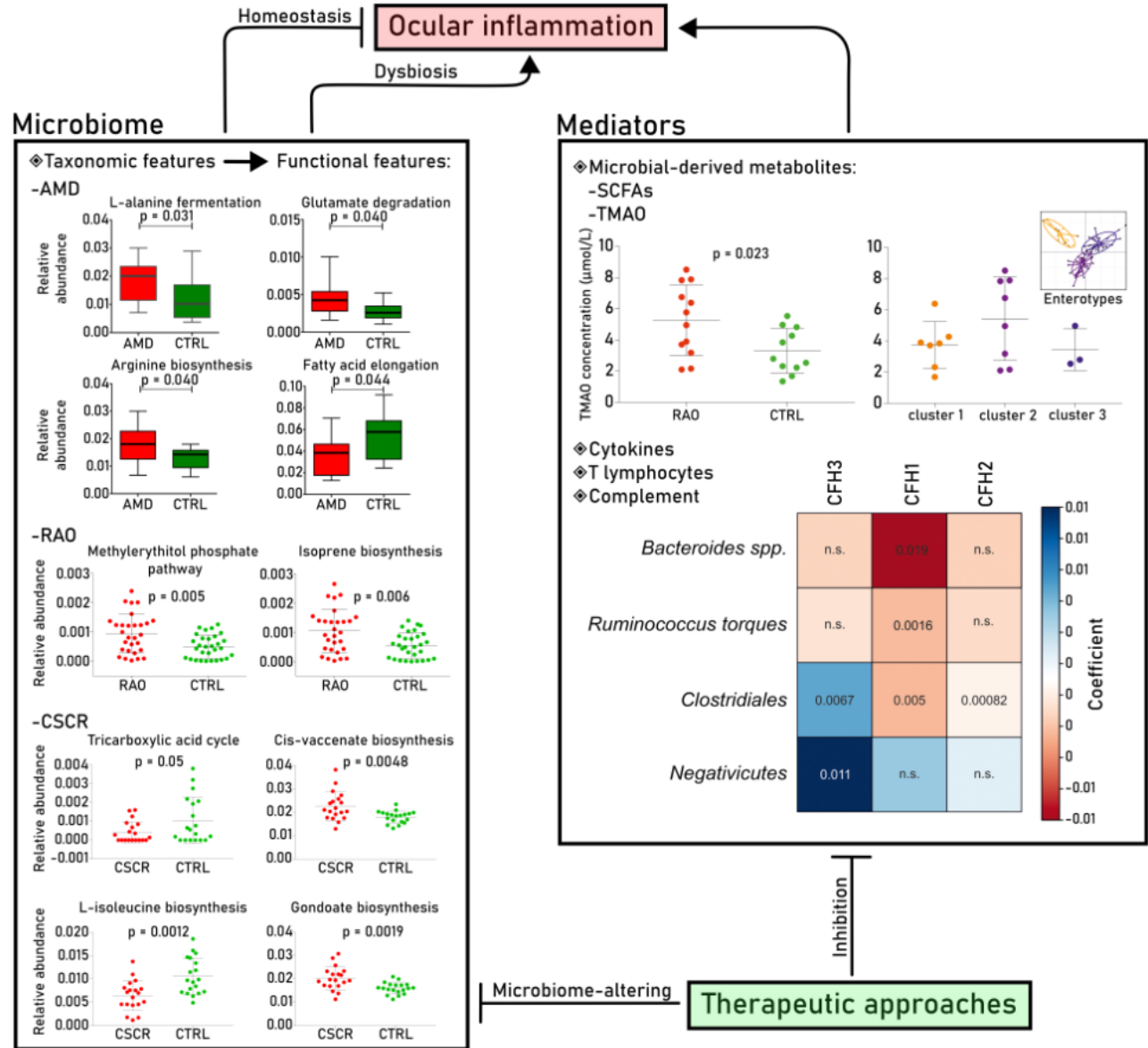


Zysset-Burri D. C. et al.: The role of the gut microbiome in eye diseases. PRER (2022)

Therapeutic approaches

- ... to inhibit ocular inflammation
- **(I) Microbiome:**
Microbiome-altering approaches: antibiotics, probiotics, fecal transplantation
 - > to maintain / restore *homeostasis*
 - > to prevent *dysbiosis*
- **(II) Mediators:**
Inhibition of: microbial-derived metabolites (e.g. TMAO), cytokines, T lymphocytes, complement system

AMD, age-related macular degeneration
 CSCR, central serous chorioretinopathy
 RAO, retinal artery occlusion
 SCFAs, short chain fatty acids
 TMAO, trimethylamine-N-oxide



Zysset-Burri D. C. et al.: The role of the gut microbiome in eye diseases. PRER (2022)

Is there an ocular surface microbiome?

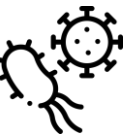
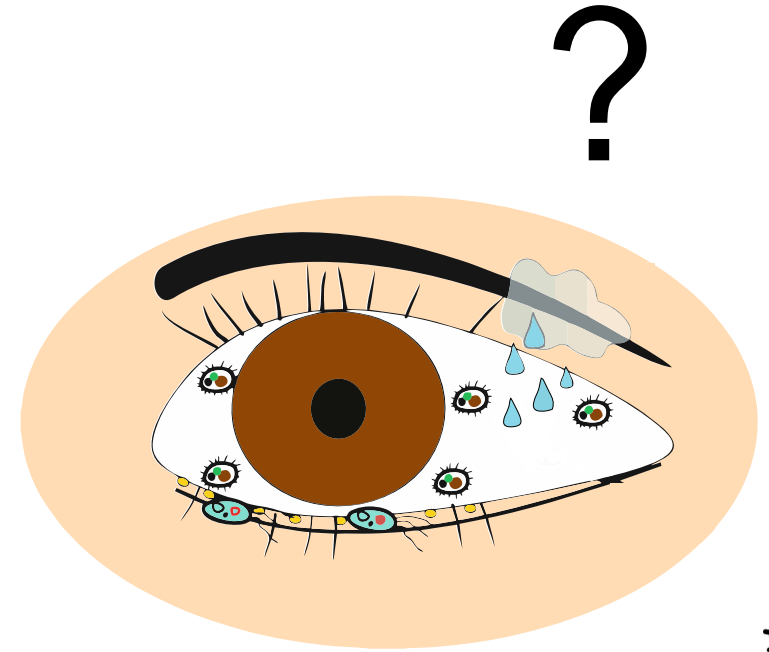
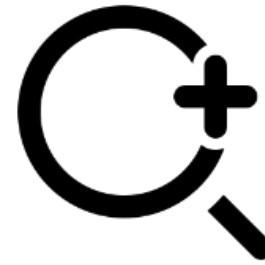
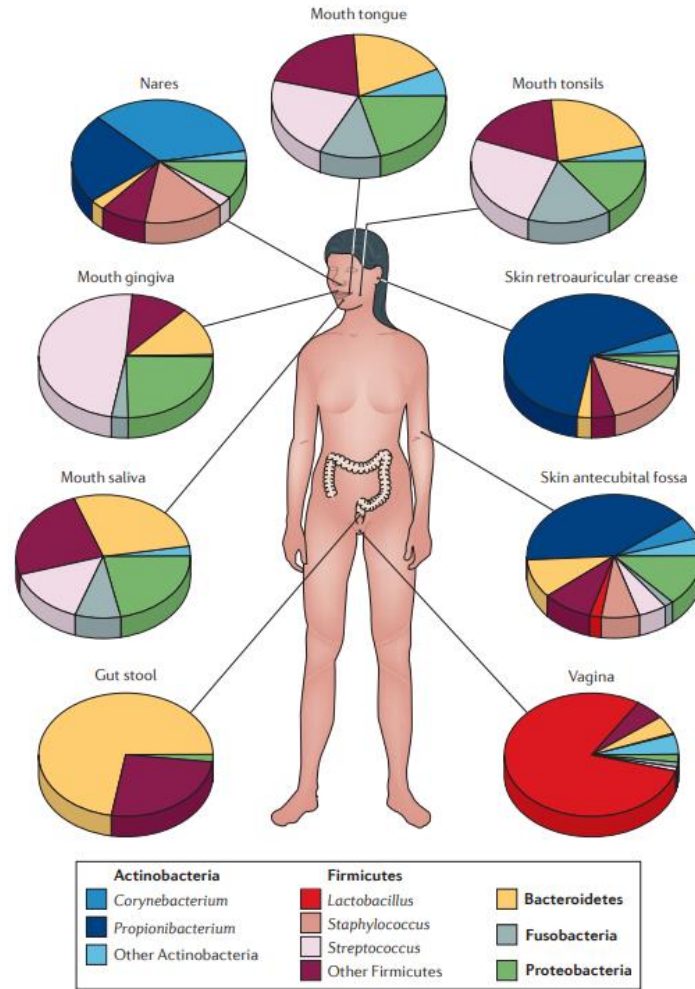
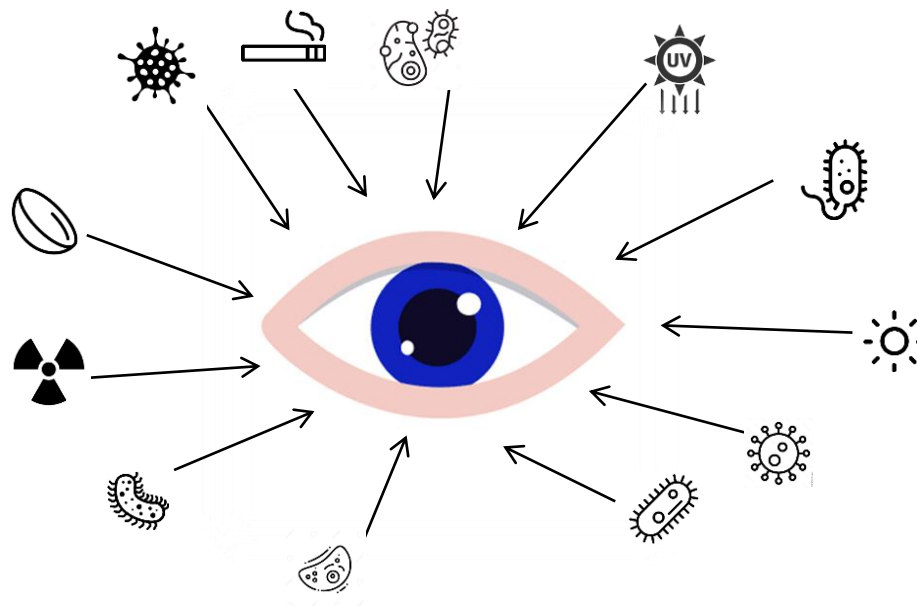


Image: adapted from Zysset-Burri et al.: Understanding the interactions between the ocular surface microbiome and the tear proteome. Invest Ophthalmol Vis Sci, 2021

The beginning



- **Sterile**

- 1930's: first evidence of bacteria in the conjunctival sac; **transient** colonization
- 1984: **permanent** colonization of commensal bacteria: *Staphylococcus species*, *Corynebacterium species*, *Propionibacterium acnes* cultured from lid margins



The Bacterial Flora of the Normal Conjunctiva with Comparative Nasal Culture Study

Robert A. Keilty, M.D.

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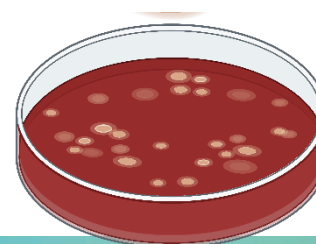
[https://doi.org/10.1016/S0002-9394\(30\)92437-3](https://doi.org/10.1016/S0002-9394(30)92437-3)

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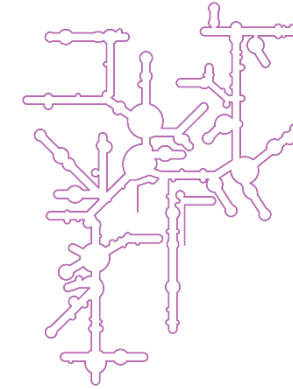
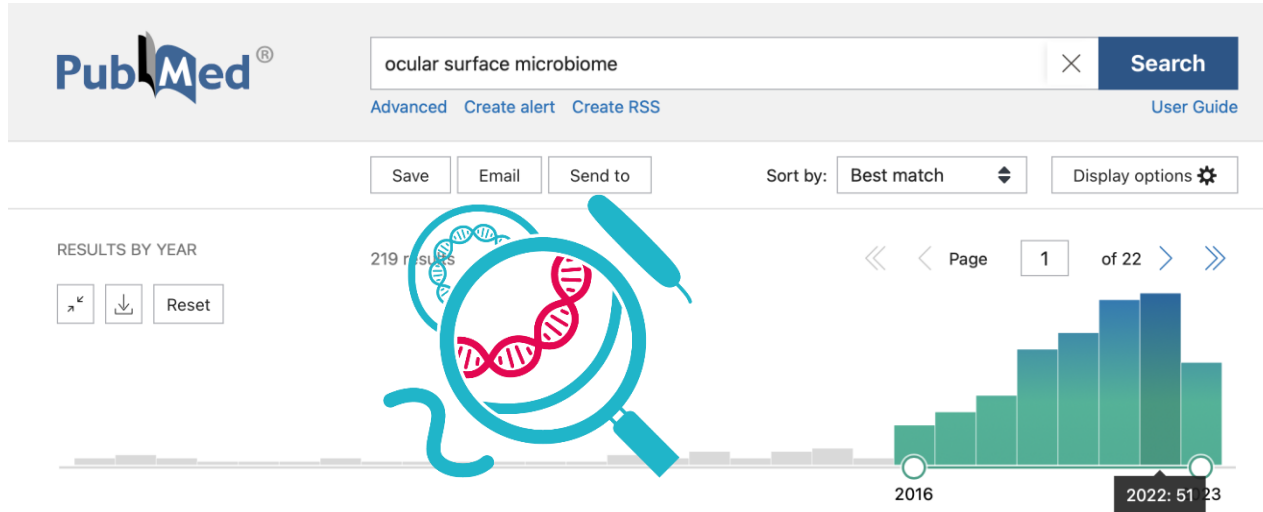
Using swabs cultured on blood-agar plates, fifty-seven percent of normal conjunctival sacs were found to carry one or more organisms on one or both sides, with no clinical evidence of inflammatory reaction of the conjunctiva, while forty-three percent were absolutely sterile. The organisms were of low virulence but of the suppurating variety, the hemolytic staphylococcus characteristic of the nasal flora predominating. Comparison with the nasal flora of the same series indicated that the source of infection was from the nose by way of the lacrimonasal duct. From the pathological laboratories of the Diagnostic Center, United States Veterans' Bureau.



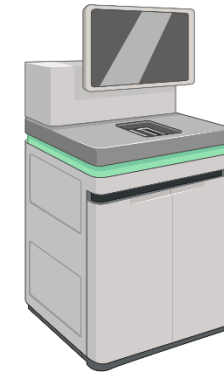
Dougherty JM, McCulley JP.: Comparative bacteriology of chronic blepharitis. Br J Ophthalmol. (1984)



Modern sequencing technologies



16s rRNA sequencing



whole-metagenome shotgun sequencing



Immunology and Microbiology

Understanding the Interactions Between the Ocular Surface Microbiome and the Tear Proteome

Denise C. Zysset-Burri,^{1,2} Irina Schlegel,¹ Joel-Benjamin Lincke,¹ Damian Jaggi,¹ Irene Keller,^{2,3} Manfred Heller,⁴ Sophie Braga Lagache,⁴ Sebastian Wolf,^{1,2} and Martin S. Zinkernagel^{1,2}

¹Department of Ophthalmology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
²Department for BioMedical Research, University of Bern, Bern, Switzerland
³Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland
⁴Proteomics and Mass Spectrometry Core Facility, Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland

Correspondence: Denise C. Zysset-Burri, University Hospital Bern, Freiburgstrasse 4, CH-3010 Bern, Switzerland; denise.zysset@insel.ch

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 Accepted: July 14, 2021
 Published: August 9, 2021

Citation: Zysset-Burri Denise C, Schlegel I, Lincke Joel-Benjamin, et al. Understanding the Interactions Between the Ocular Surface Microbiome and the Tear Proteome. *Invest Ophthalmol Vis Sci*. 2021;62(13):4021. doi:10.1167/62.13.4021

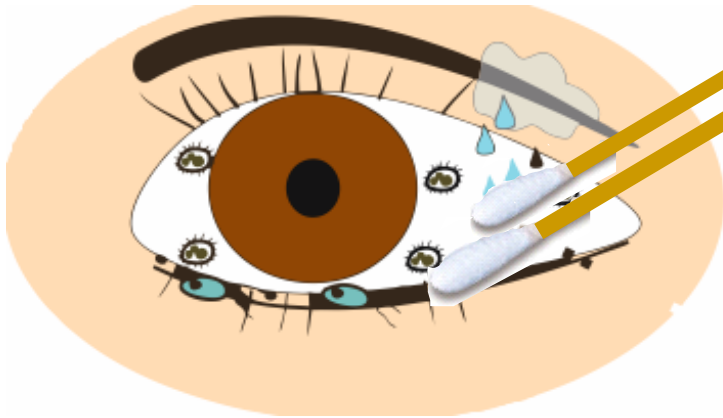
PURPOSE. The purpose of this study was to explore the interplay between the ocular surface microbiome and the tear proteome in humans in order to better understand the pathogenesis of ocular surface-associated diseases.

METHODS. Twenty eyes from 20 participants were included in the study. The ocular surface microbiome was sequenced by whole-metagenome shotgun sequencing using lid and conjunctival swabs. Furthermore, the tear proteome was identified using chromatography tandem mass spectrometry. After compositional and functional profiling of the metagenome and functional characterization of the proteome by gene ontology, association studies between the ocular microbiome and tear proteome were assessed.

RESULTS. Two hundred twenty-nine taxa were identified with *Actinobacteria* and

- n = 20 healthy eyes / 20 participants
- lid and conjunctiva swabs
- **whole-metagenome shotgun sequencing**
- associations with the tear proteome

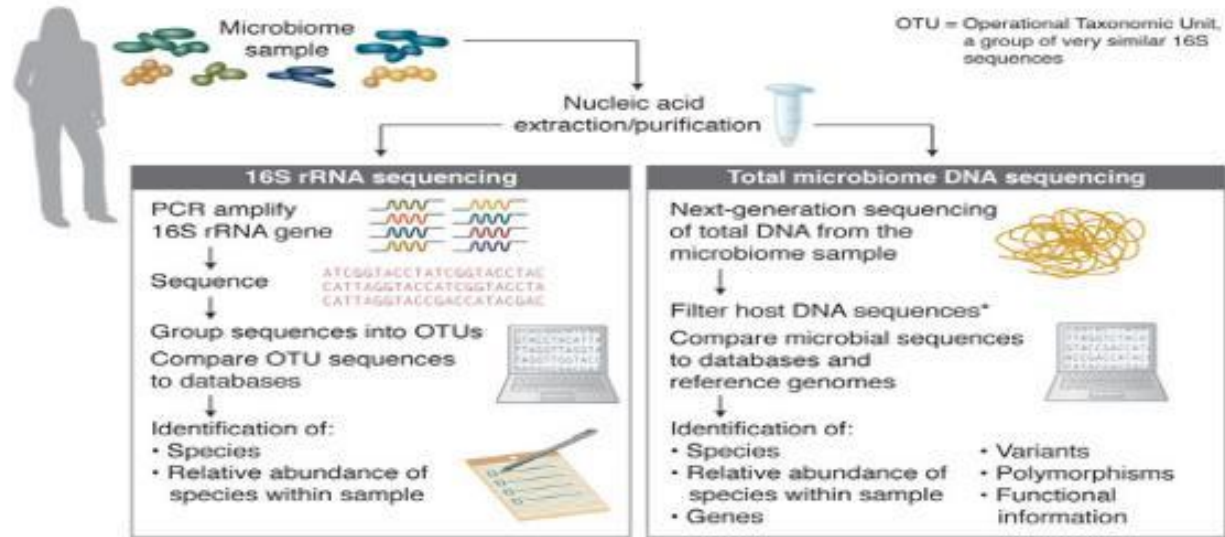
Sampling



conjunctival swab
lid swab

DNA isolation

whole-metagenome
shotgun sequencing



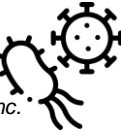
1. Taxonomic profiling: bacteria

1. Taxonomic profiling: bacteria, viruses, Archae, eukaryotes
2. Functional profiling: genes and pathways

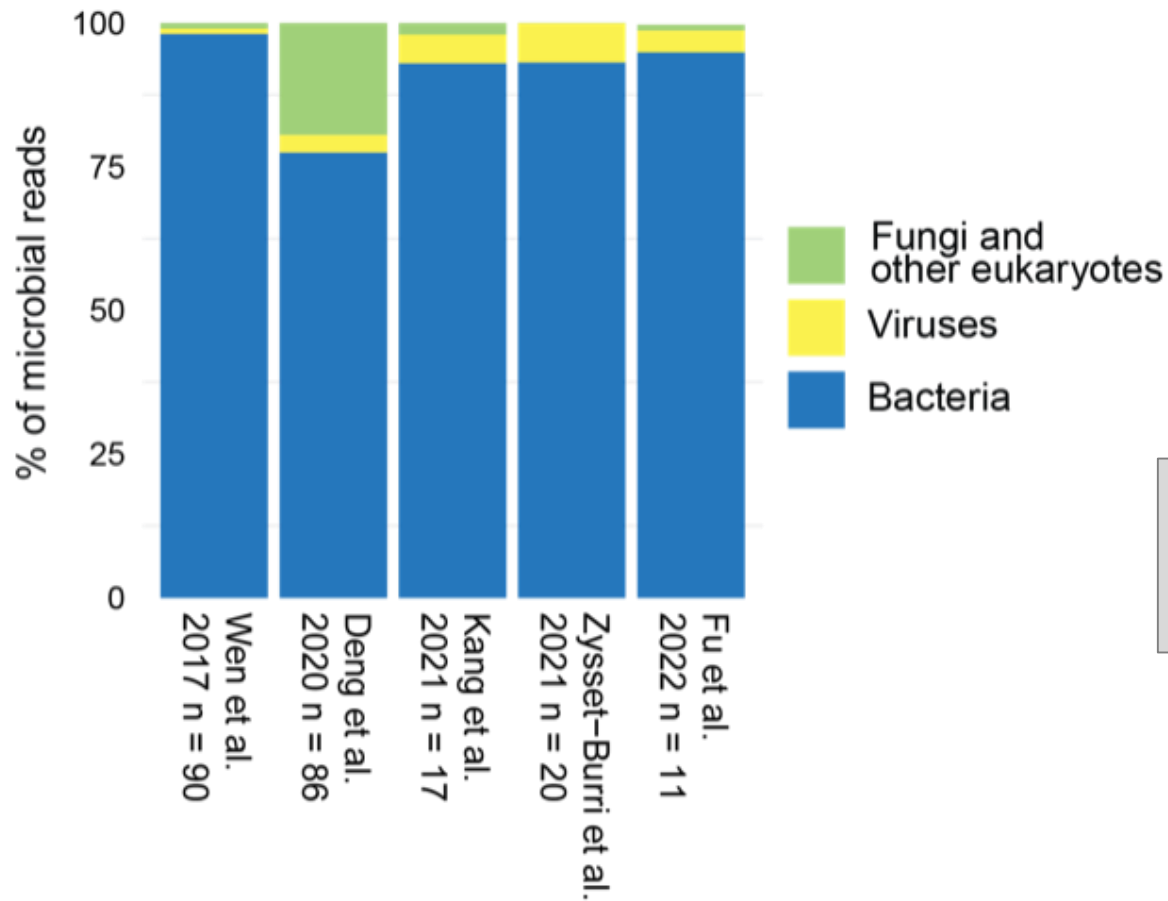


Next generation sequencing platform (University of Bern)

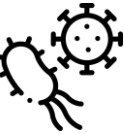
Stewart F. et al.: Addressing challenges in microbiome DNA analysis. *New England BioLabs Inc.*
<https://www.neb.com/tools-and-resources/feature-articles/addressing-challenges-in-microbiome-dna-analysis>



Taxonomic profiling: kingdom - The ocular surface microbiome in health

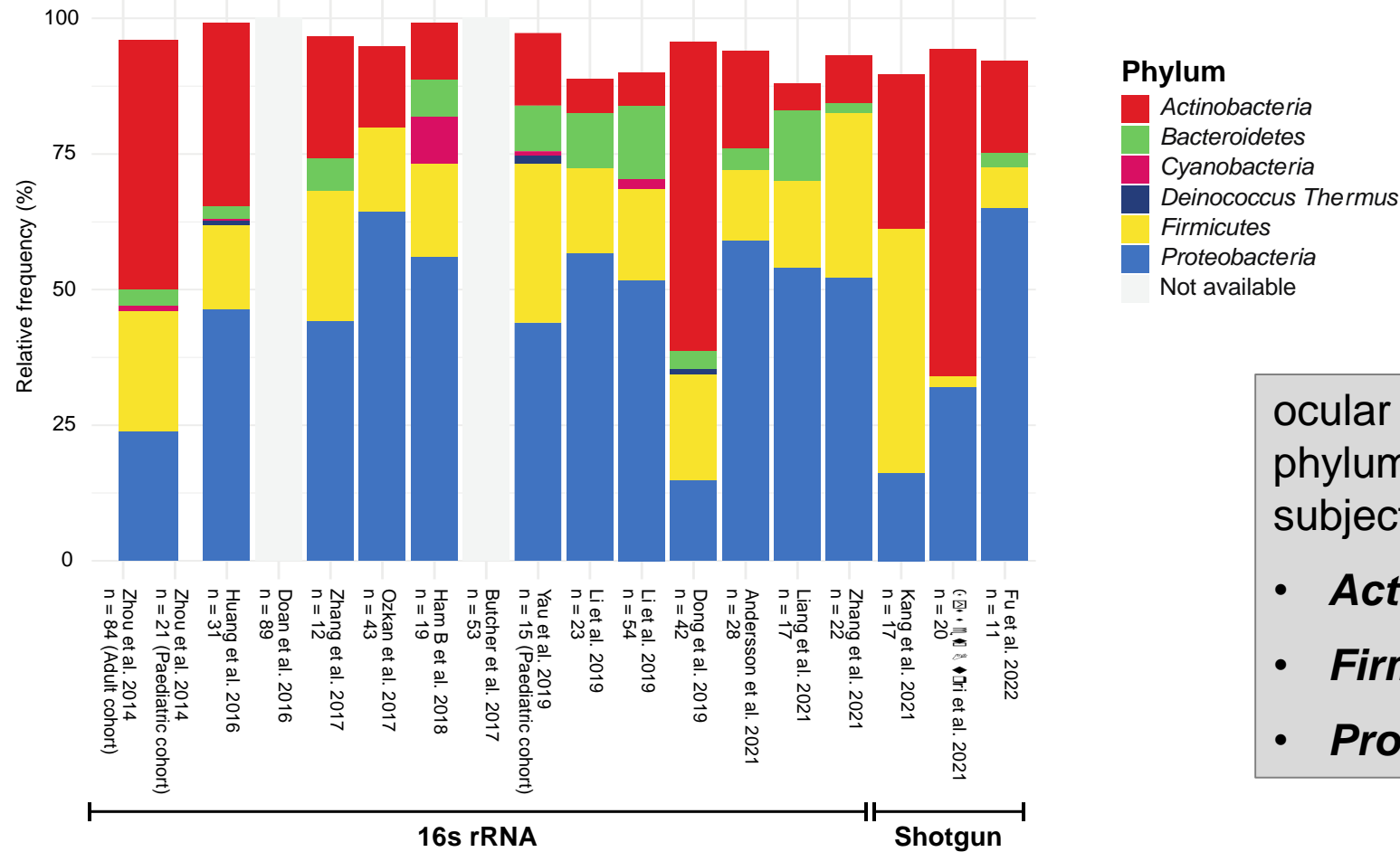


ocular surface microbes in *healthy* subjects: mainly **Bacteria**



Peter et al.: Investigating the ocular surface microbiome: What can it tell us? Clin Ophthalmol (2023)

Taxonomic profiling: phylum - The ocular surface microbiome in health



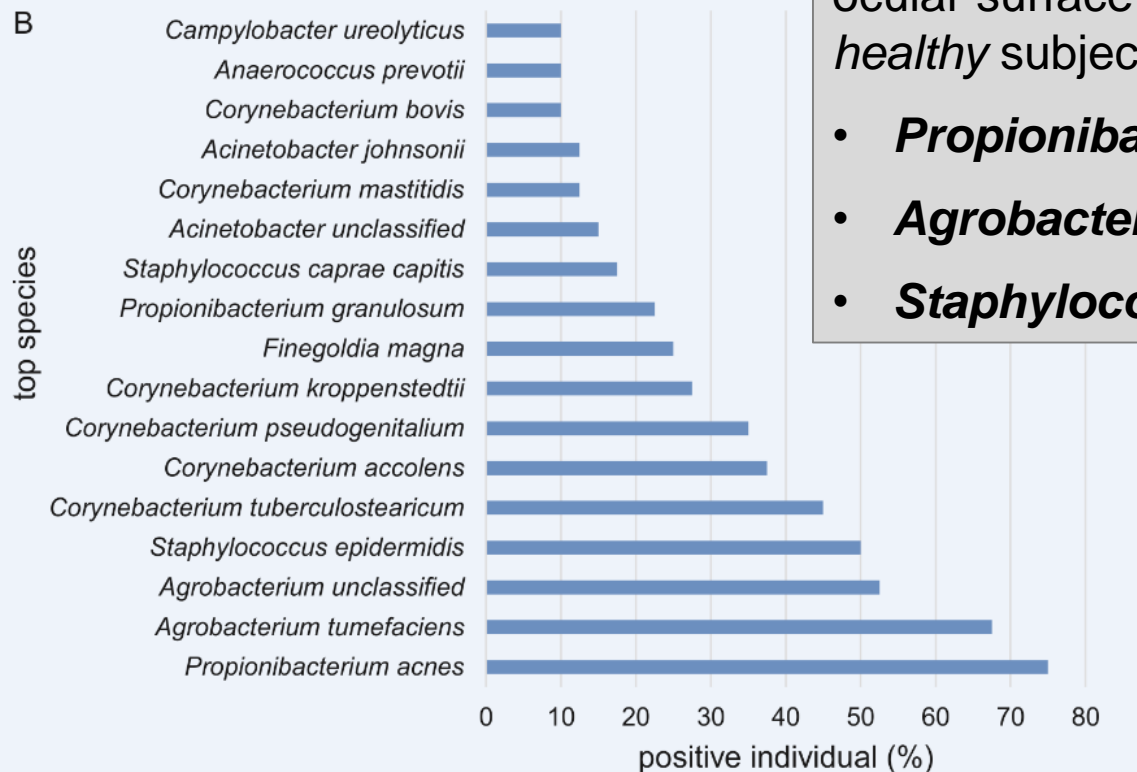
ocular surface bacteria at phylum level in *healthy* subjects: mainly

- Actinobacteria**
- Firmicutes**
- Proteobacteria**



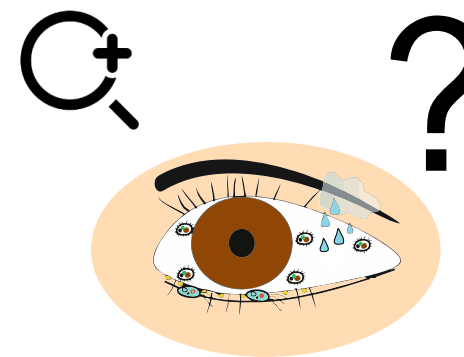
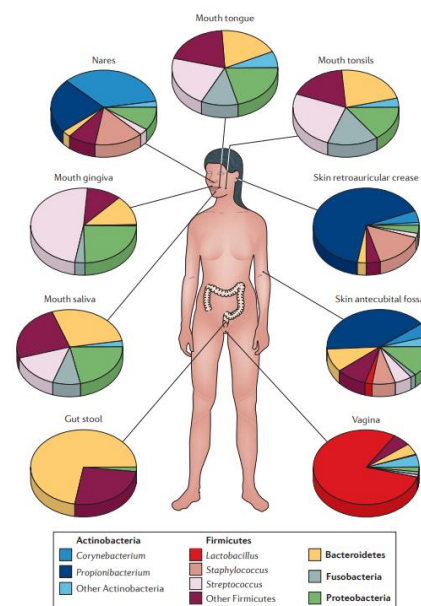
Peter et al.: Investigating the ocular surface microbiome: What can it tell us? Clin Ophthalmol (2023)

Taxonomic profiling: species - The ocular surface microbiome in health



ocular surface bacteria at species level in healthy subjects: mainly

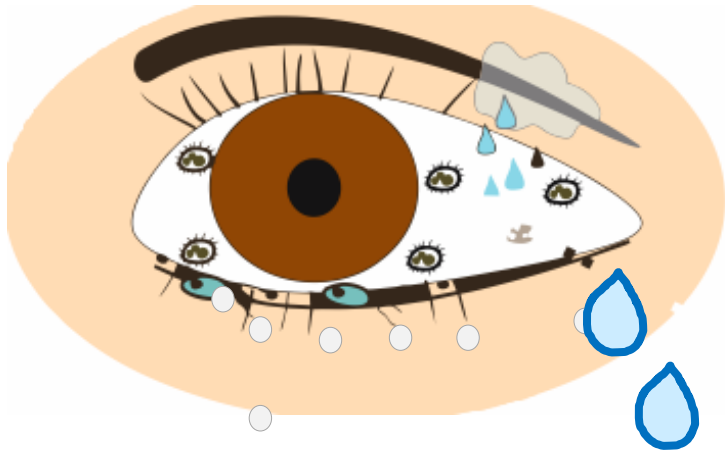
- **Propionibacterium acnes**
- **Agrobacterium spp.**
- **Staphylococcus epidermidis**



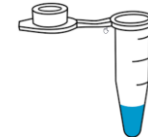
Yes, there is a *characteristic* ocular surface microbiome

Zysset-Burri et al.: Understanding the interactions between the ocular surface microbiome and the tear proteome. Invest Ophthalmol Vis Sci, 2021

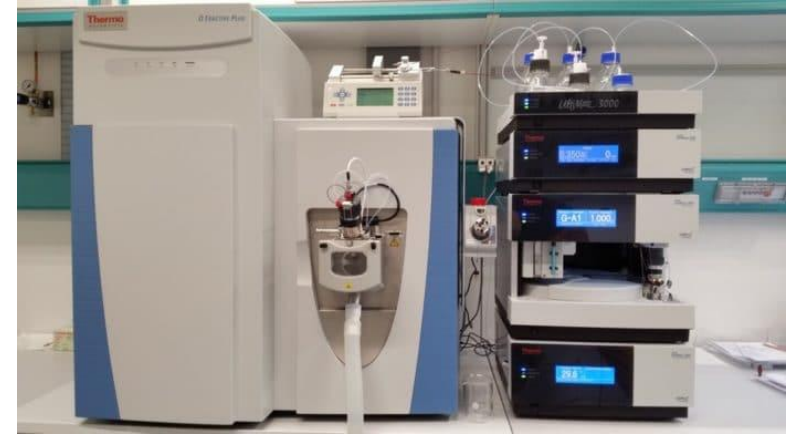
The human tear proteome: sampling



Centrifuge @ max speed



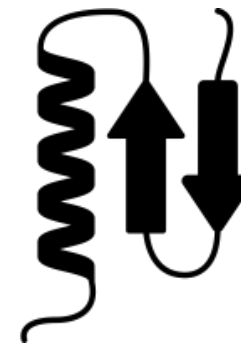
Chromatography tandem MS



Core Facility Proteomics & Mass Spectrometry (University of Bern)

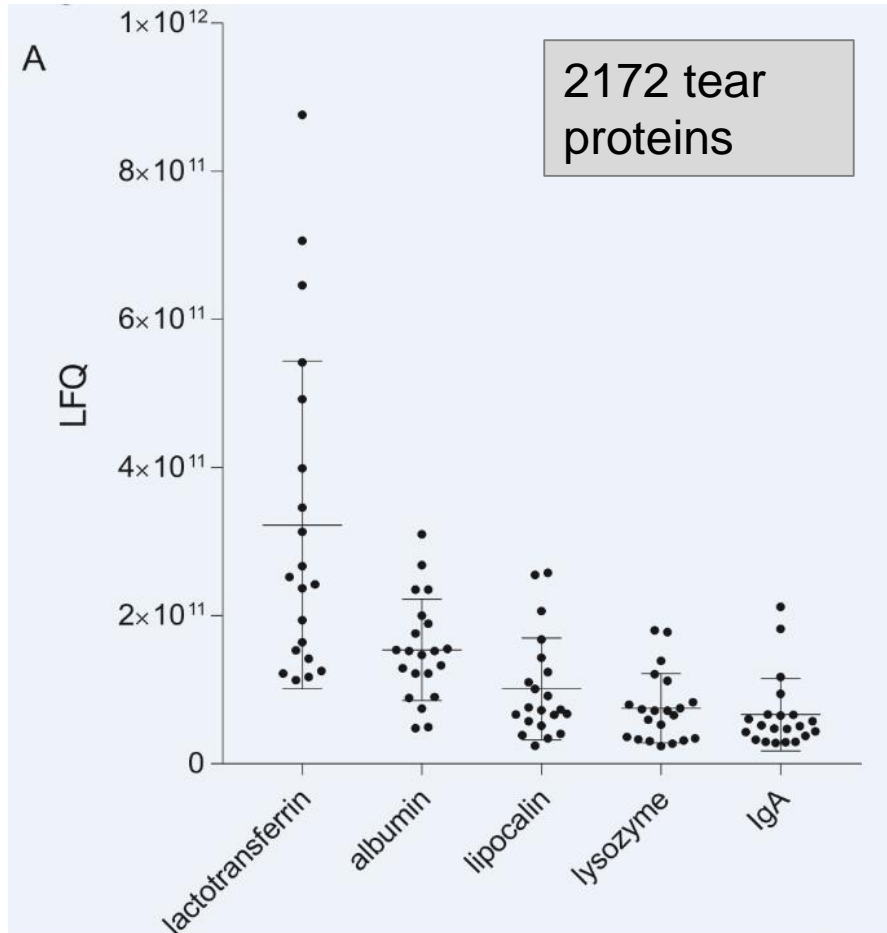


Quantification of tear proteins

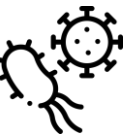
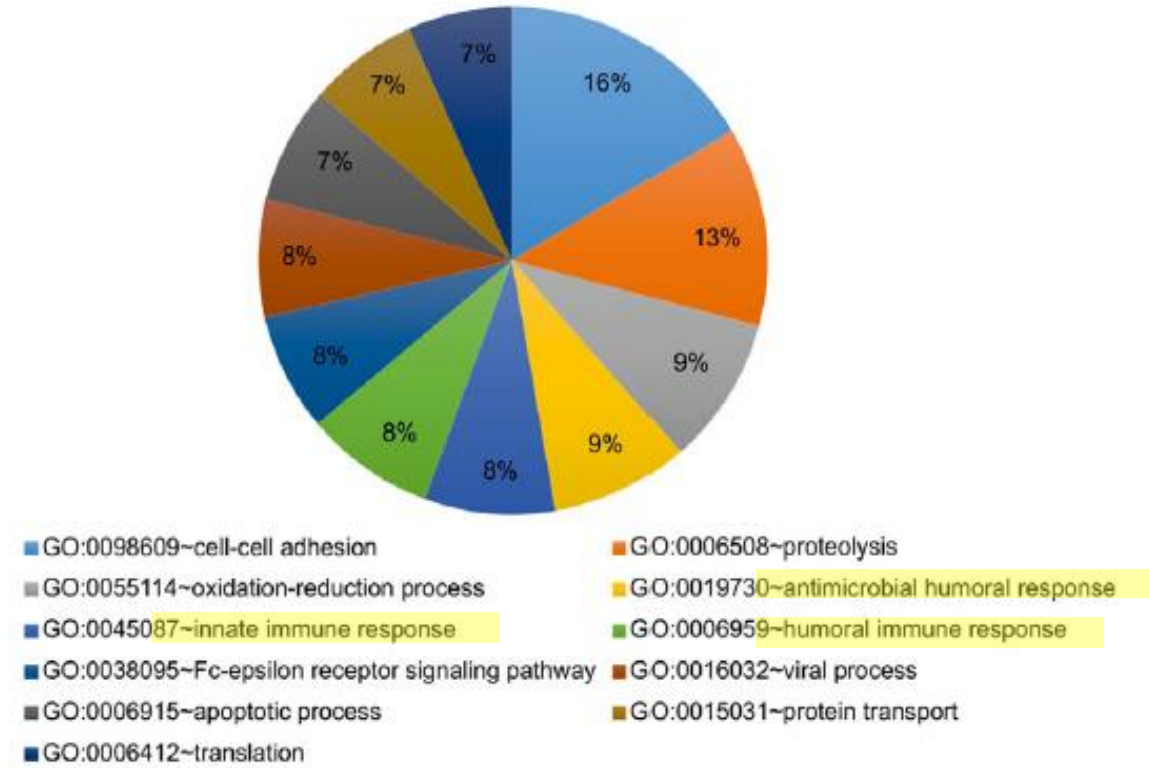


Zysset-Burri et al.: Understanding the interactions between the ocular surface microbiome and the tear proteome. Invest Ophthalmol Vis Sci, 2021

The human tear proteome



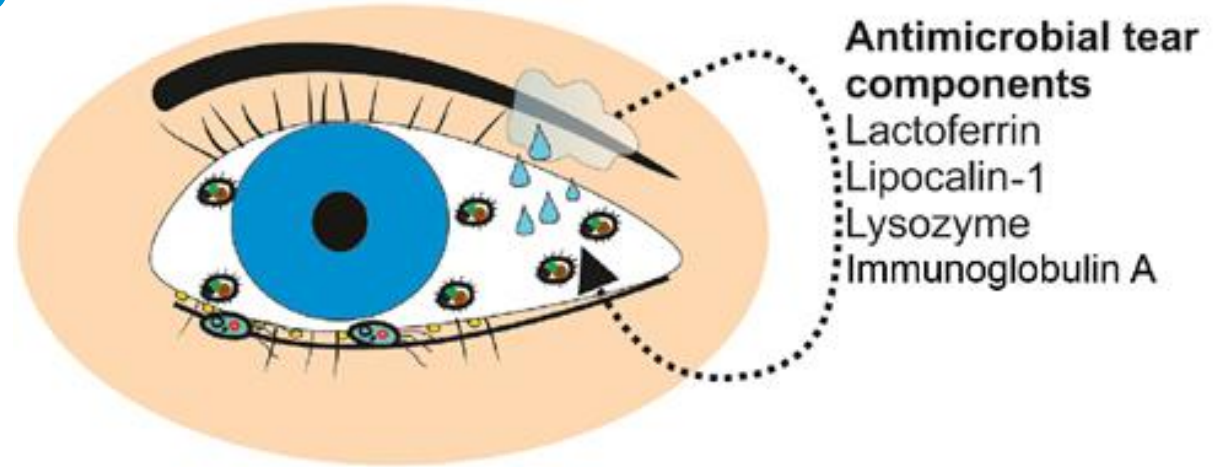
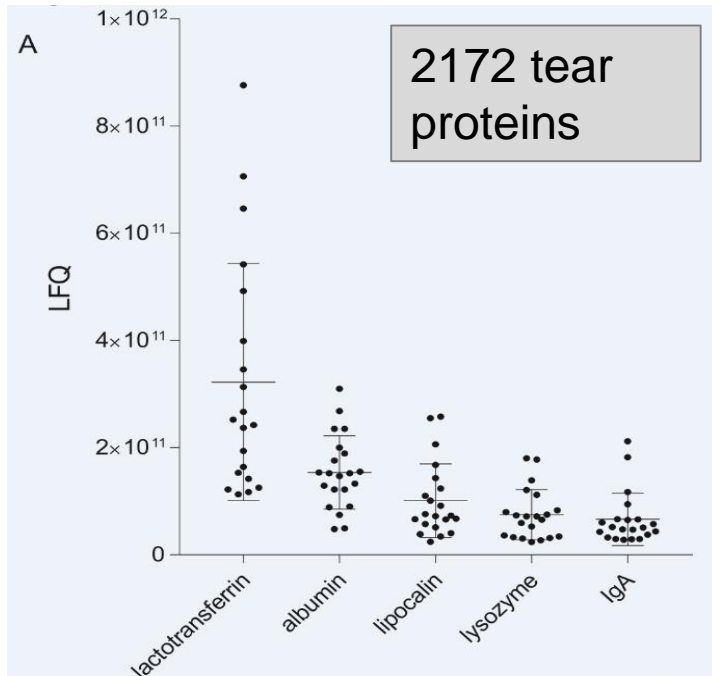
biological processes



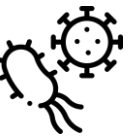
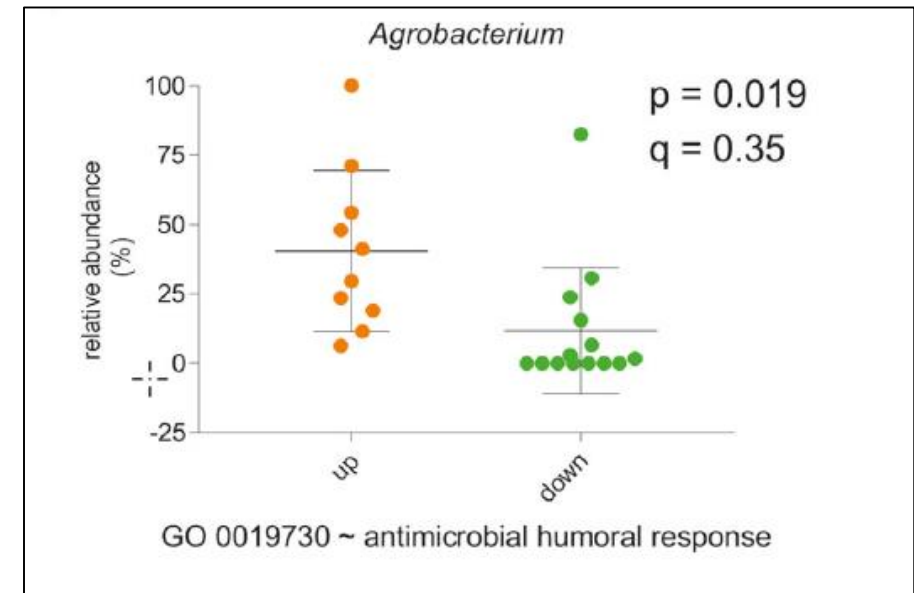
Many tear proteins are involved in defence mechanisms

Zysset-Burri et al.: Understanding the interactions between the ocular surface microbiome and the tear proteome. Invest Ophthalmol Vis Sci, 2021

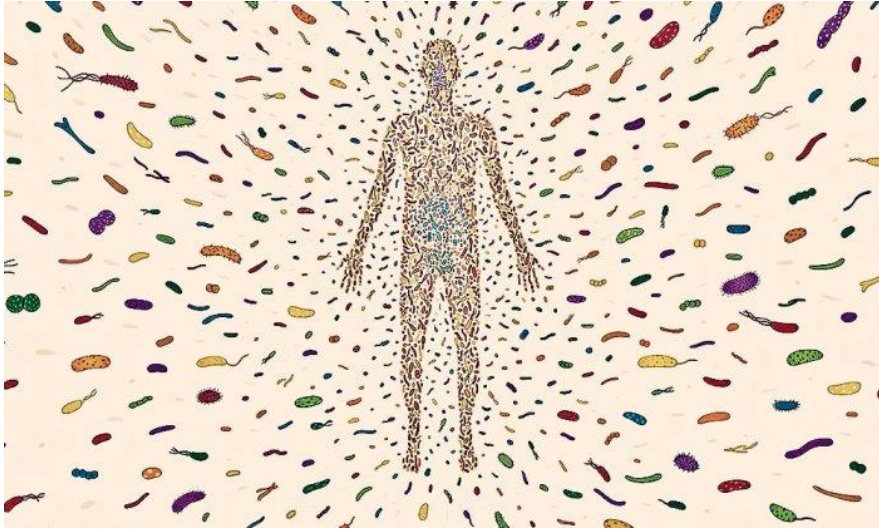
The human tear proteome



- The most abundant tear proteins have antimicrobial properties -> **chemical barrier**
- The tear proteome is associated with the ocular surface microbiome

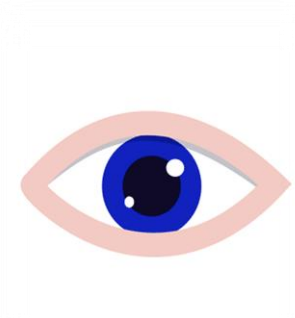
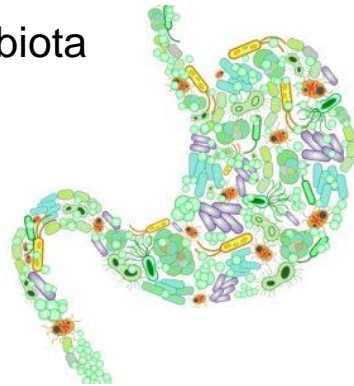


Low-abundant microbiome



95% of the human microbiota is found in the **gut**

Image: www.amnh.org

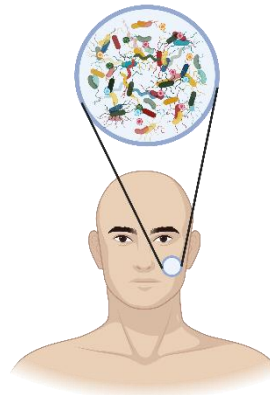


mechanical barriers

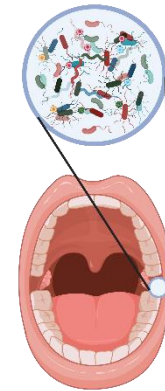


chemical barriers

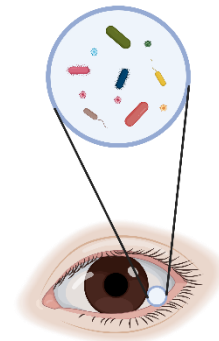
Antimicrobial tear components
Lactoferrin
Lipocalin-1
Lysozyme
Immunoglobulin A



Facial skin
16 bacteria /
human cell



Buccal mucosa
12 bacteria /
human cell



Conjunctiva
0.06 bacteria /
human cell

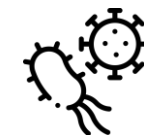


Doan et al.: Paucibacterial Microbiome and Resident DNA Virome of the Healthy Conjunctiva. IOVS (2016)

Low-abundant microbiome



- Low amount of input DNA
- Prone to environmental and human contaminations
- Generation of robust data to compare groups in case/control studies is challenging



Challenges and insights in the exploration of the low abundance human ocular surface microbiome

Elio L. Herzog^{1,2,3*}, Marco Kreuzer⁴, Martin S. Zinkernagel^{1,2} and Denise C. Zysset-Burri^{1,2}

¹Department of Ophthalmology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, ²Department for BioMedical Research, University of Bern, Bern, Switzerland, ³Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland, ⁴Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland

- n =12 *healthy* eyes / 6 participants
- lid and conjunctiva swabs at 12 time points
- whole-metagenome shotgun sequencing

Check for updates

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*CORRESPONDENCE
Elio L. Herzog
✉ elio.herzog@extern.insel.ch

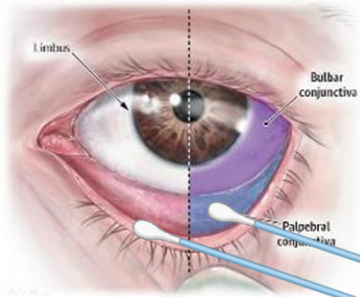
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CITATION

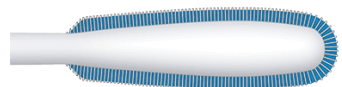
Low-abundant microbiome



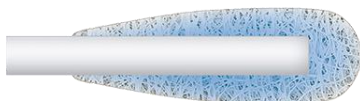
- Low amount of input DNA
- Prone to environmental and human contaminations
- Generation of robust data to compare groups in case/control studies is challenging



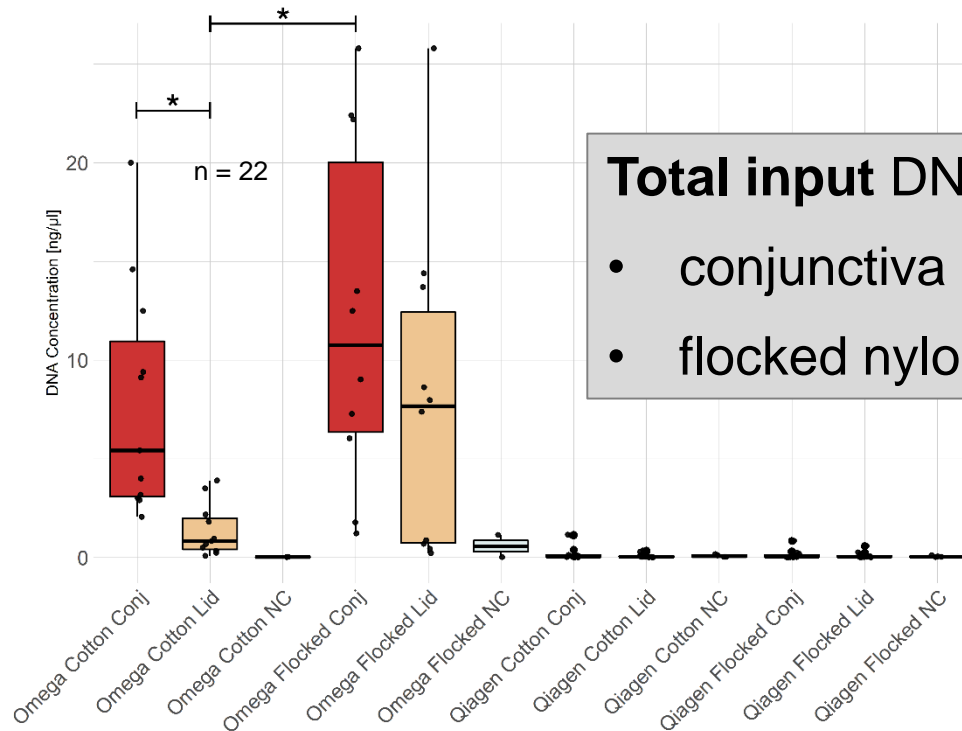
lower conjunctiva
lower lid margin



flocculated nylon swabs
(FLOQSwabs, Copan, Brescia, Italy)

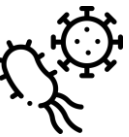


cotton swabs



Total input DNA concentration:

- conjunctiva > lid
- flocculated nylon > cotton swabs

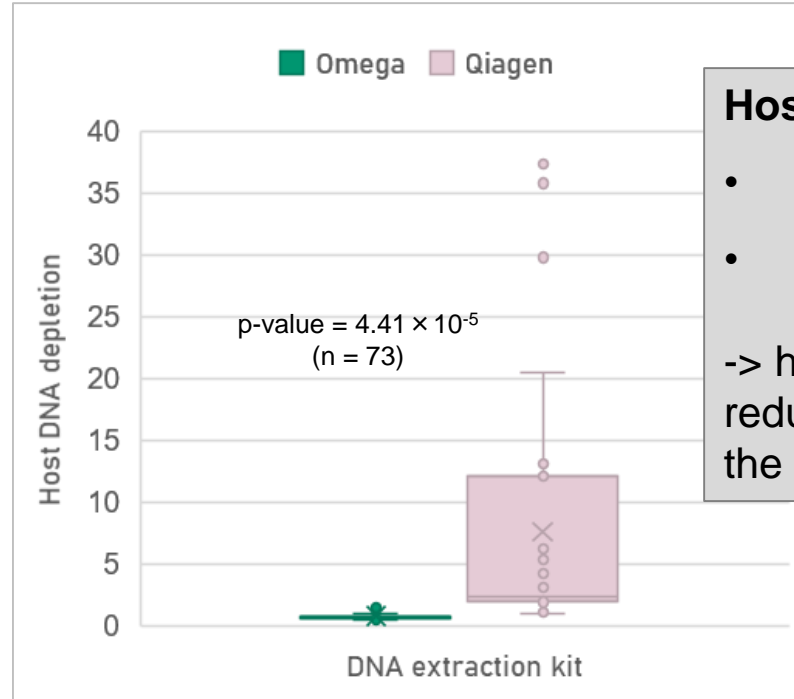
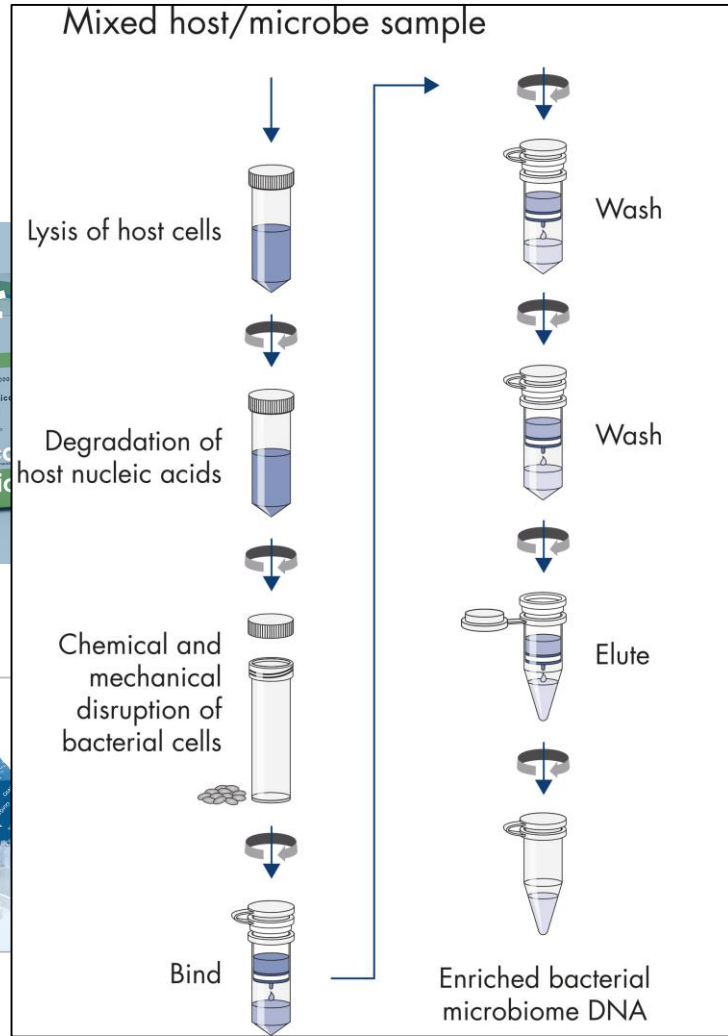


Herzog EL, Kreuzer M, Zinkernagel MS, Zysset D: Challenges and insights in the exploration of the low abundance human ocular surface microbiome. *Frontiers in Cellular and Infection Microbiology* (2023)

Low-abundant microbiome



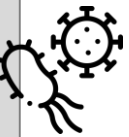
- Low amount of input DNA
- Prone to environmental and human contaminations
- Generation of robust data to compare groups in case/control studies is challenging



Host DNA contamination:

- Omega: $99.19 \pm 0.31\%$
- Qiagen: $92.38 \pm 9.89\%$

-> human contamination reduced by a factor of ~ 10 by the Qiagen kit



Herzog EL, Kreuzer M, Zinkernagel MS, Zysset D: Challenges and insights in the exploration of the low abundance human ocular surface microbiome. *Frontiers in Cellular and Infection Microbiology* (2023)

Low-abundant microbiome



- Low amount of input DNA
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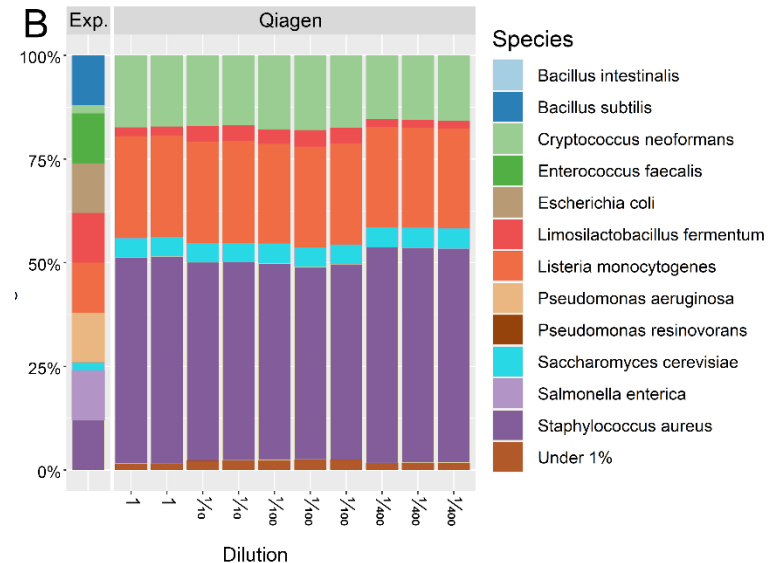
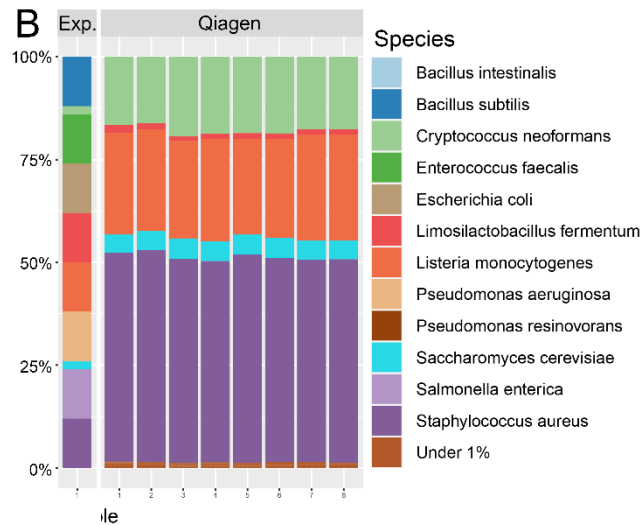
Microbial Community Standard

(ZymoBIOMICS, ZymoReserach, Irvine, CA, USA)

- 3 Gram-positive bacteria
- 5 Gram-negative bacteria
- 2 fungal species

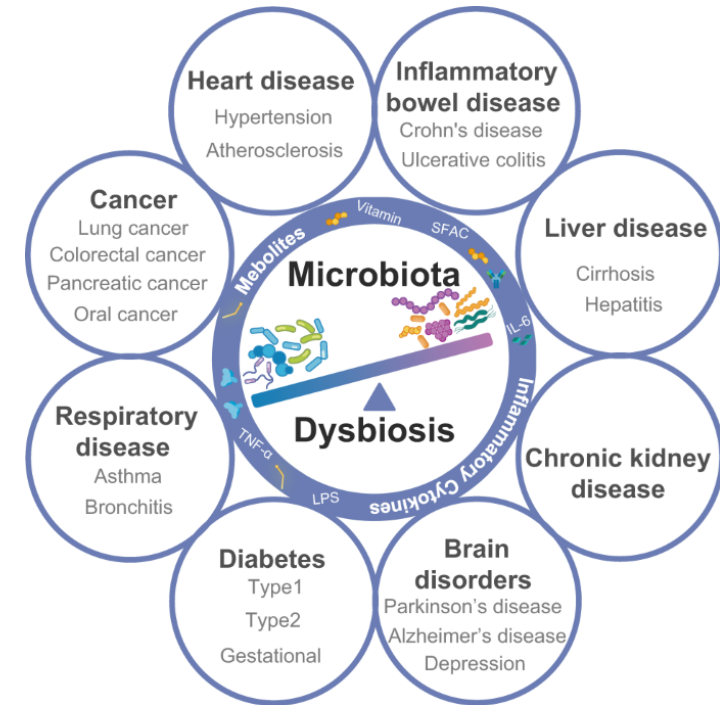
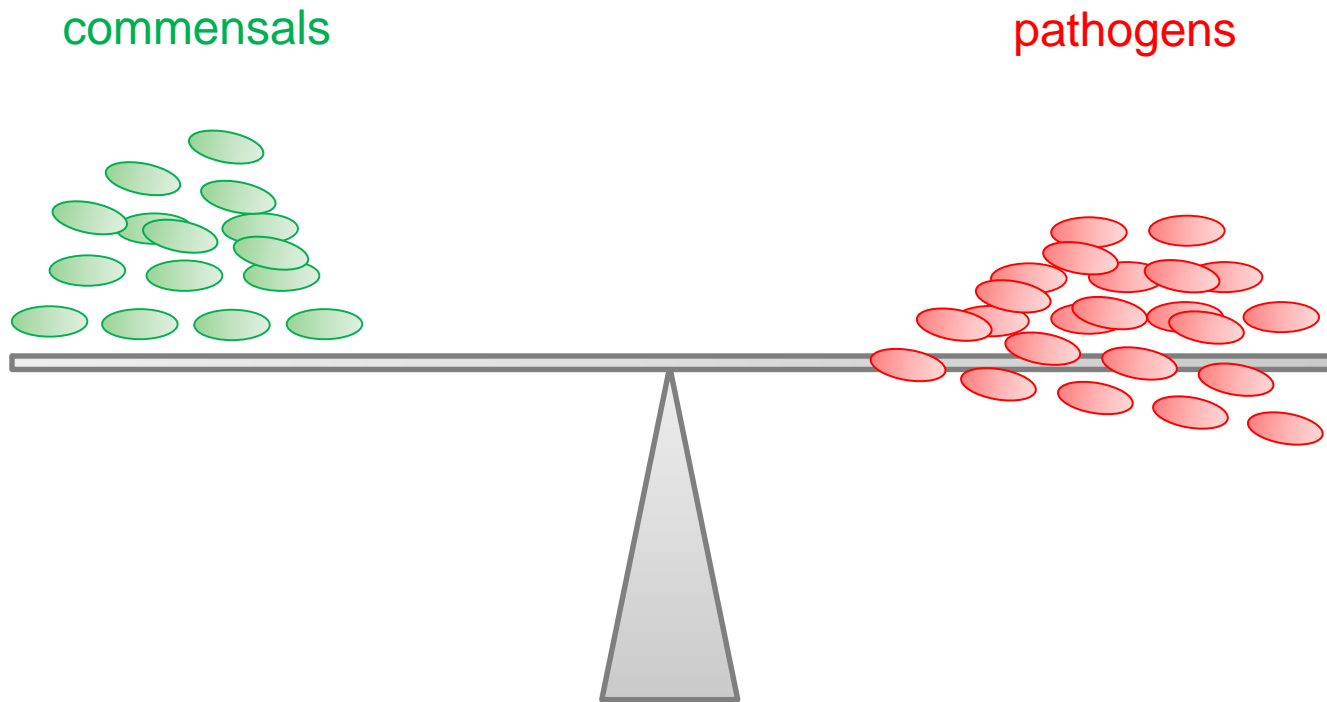
Taxonomic composition of the positive control ...

- ... stable over time, even at low-concentrations



Herzog EL, Kreuzer M, Zinkernagel MS, Zysset D: Challenges and insights in the exploration of the low abundance human ocular surface microbiome. Frontiers in Cellular and Infection Microbiology (2023)

Homeostasis vs. dysbiosis - The ocular surface microbiome in disease



Dry eye disease

- One of the most common ocular surface disease worldwide (prevalence up to 34%*)
- Pathogenesis incompletely understood
- Treatment options limited to: moisturizing eye drops, antibiotics, immune modulators

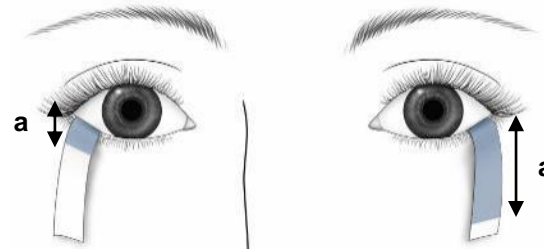


* Dana, R.; Bradley, J.L.; Guerin, A.; Pivneva, I.; Stillman, I.O.; Evans, A.M.; Schaumberg, D.A: Estimated Prevalence and Incidence of Dry Eye Disease Based on Coding Analysis of a Large, All-age United States Health Care System. *Am. J. Ophthalmol.* (2019)

Dry eye disease: diagnosis (I)

- *Objective* assessment:

Schirmer's test I and II



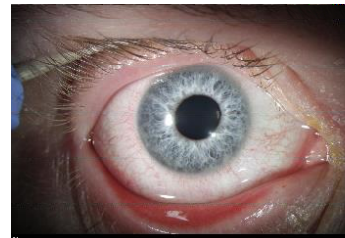
$a \leq 10\text{mm}$: DED
 $a > 10\text{mm}$: no sign of DED

Tearlab Osmolarity



$> 316 \text{ mOsmol/L}$: DED
 $\leq 316 \text{ mOsmol/L}$: no sign of DED

Slit lamp examination:



eyelid margins, conjunctiva, cornea



Dry eye disease: diagnosis (II)

- **Objective assessment:**

Tear break-up time

= nb of seconds between the last blink and the appearance of the first dry spot in the tear film (cornea)

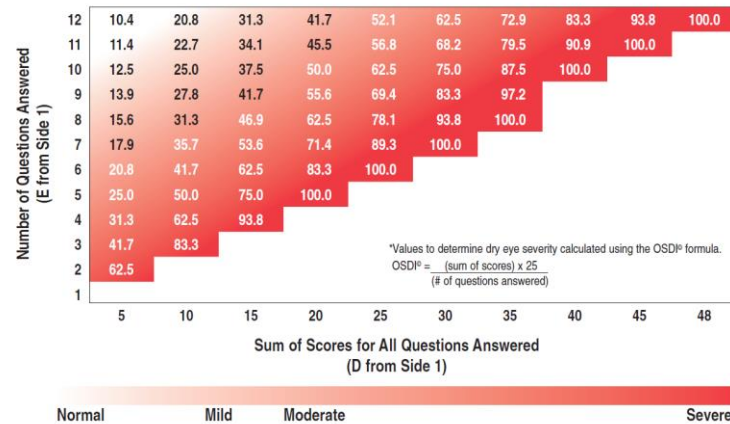


10-35s: normal
 < 10s: tear film instability



- **Subjective assessment:**

Ocular Surface Disease Index[®] (OSDI[®])



Ocular Surface Disease Index[®] (OSDI[®])²

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

Have you experienced any of the following during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light? ..	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5 (A)

Have problems with your eyes limited you in performing any of the following during the last week?	All of the time	Most of the time	Half of the time	Some of the time	None of the time	N/A
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A

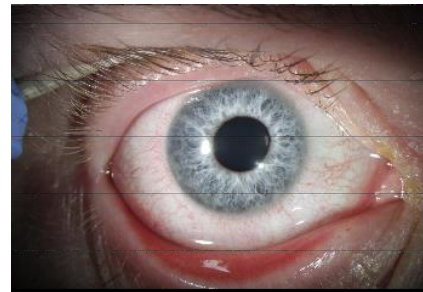
Dry eye disease: definition

- 2017: TFOS DEWS II Definition and Classification Subcommittee*: *multifactorial* disease of the ocular surface with symptoms originating from:

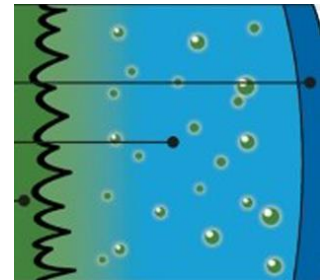
tear film instability and hyperosmolarity

ocular surface inflammation and damage

neurosensory abnormalities

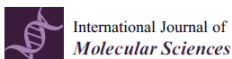


lipid layer (meibom glands)
aqueous layer (lacrimal gland)
Mucous layer (conjunctival goblet cells)



* Craig, J.P., et al., TFOS DEWS II Definition and Classification Report. *Ocul Surf*, 2017. 15(3): p. 276-283.

Study design



Article

The Human Ocular Surface Microbiome and Its Associations with the Tear Proteome in Dry Eye Disease

Irina Schlegel ^{1,†}, Claire M. F. De Gouyon Matignon de Pontourade ^{1,†}, Joel-Benjamin Lincke ¹, Irene Keller ^{2,3}, Martin S. Zinkernagel ^{1,2} and Denise C. Zysset-Burri ^{1,2,*}

¹ Department of Ophthalmology, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland; irina_schlegel@hotmail.com (I.S.); claire.degouyon@insel.ch (C.M.F.D.G.M.d.P.); joel-benjamin.lincke@insel.ch (J.-B.L.); martin.zinkernagel@insel.ch (M.S.Z.)

² Department for BioMedical Research, University of Bern, 3010 Bern, Switzerland; irene.keller@unibe.ch

³ Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, 3012 Bern, Switzerland

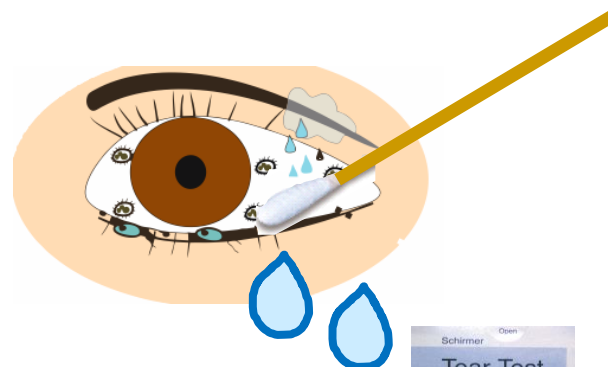
* Correspondence: denise.zysset@insel.ch

† These authors contributed equally to this work.

Abstract: Although dry eye disease (DED) is one of the most common ocular surface diseases worldwide, its pathogenesis is incompletely understood, and treatment options are limited. There is growing evidence that complex interactions between the ocular surface microbiome (OSM) and tear fluid constituents, potentially leading to inflammatory processes, are associated with ocular surface diseases such as DED. In this study, we aimed to find unique compositional and functional features of the OSM associated with human and microbial tear proteins in patients with DED. Applying whole-metagenome shotgun sequencing of forty lid and conjunctival swabs, we identified 229 taxa, with Actinobacteria and Proteobacteria being the most abundant phyla and Propionibacterium acnes the dominating species in the cohort. When DED patients were compared to controls, the species Corynebacterium tuberculoostearicum was more abundant in conjunctival samples, whereas the family Propionibacteriaceae was more abundant in lid samples. Functional analysis showed that genes of L-lysine biosynthesis, tetrapyrrole biosynthesis, 5-aminoimidazole ribonucleotide biosynthesis, and the super pathway of L-threonine biosynthesis were enriched in conjunctival samples of controls



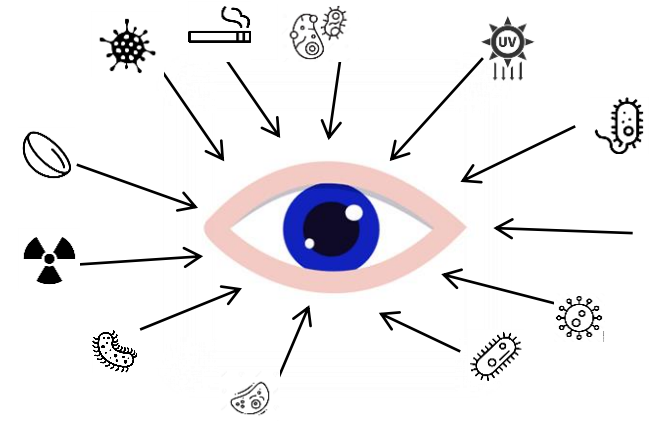
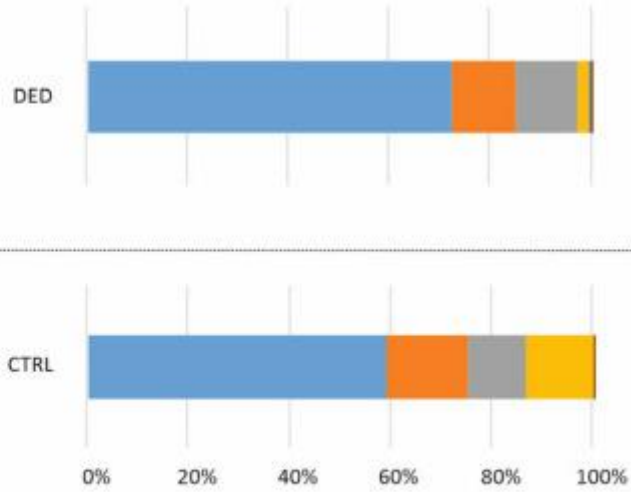
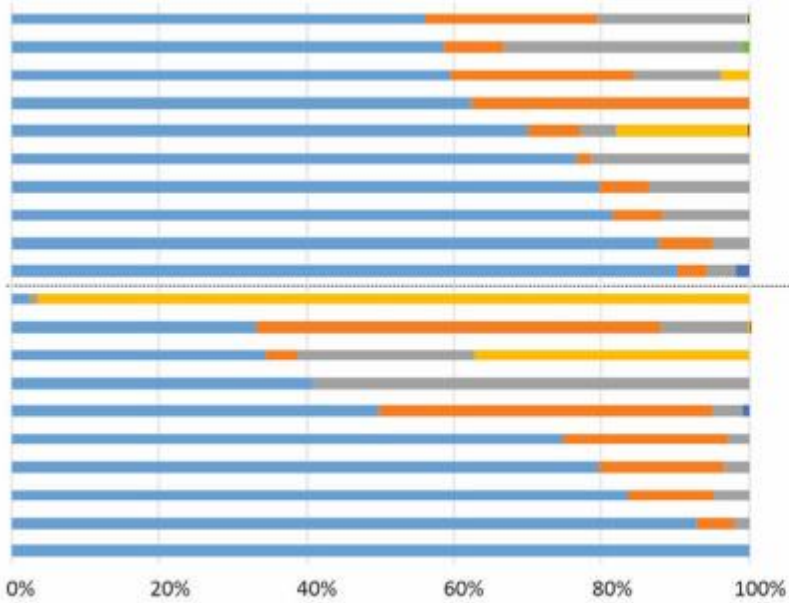
Citation: Schlegel, I.; De Gouyon Matignon de Pontourade, C.M.F.;



- n = 20 eyes / 10 DED patients compared to 10 age- and sex-matched controls
- lid and conjunctiva swabs
- whole-metagenome shotgun sequencing
- associations with the tear proteome



Results: differences in taxonomic composition

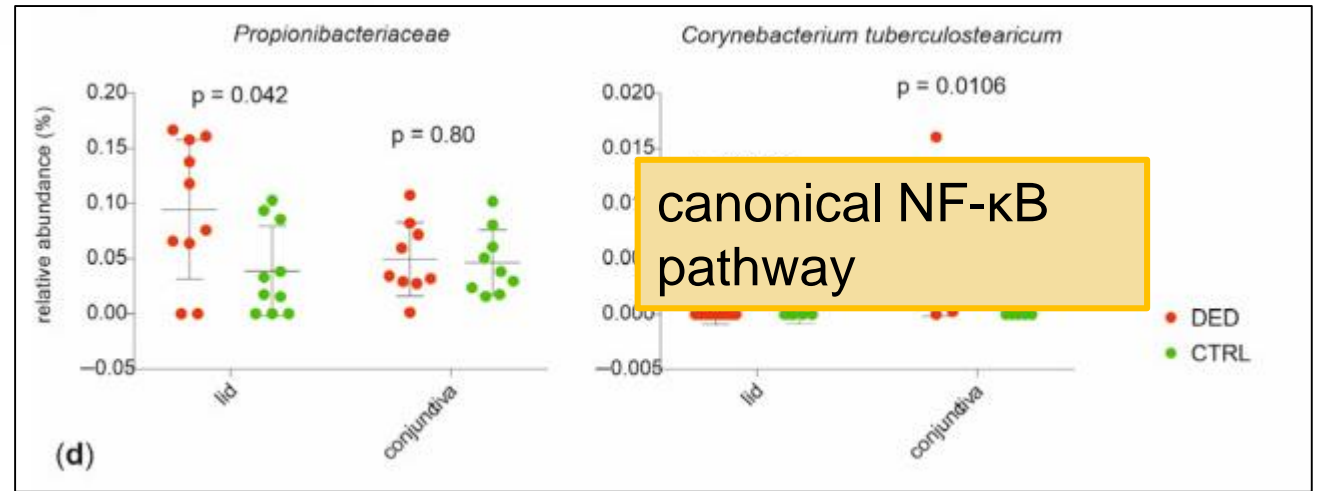


Taxonomic composition of the ocular surface microbiome as expected



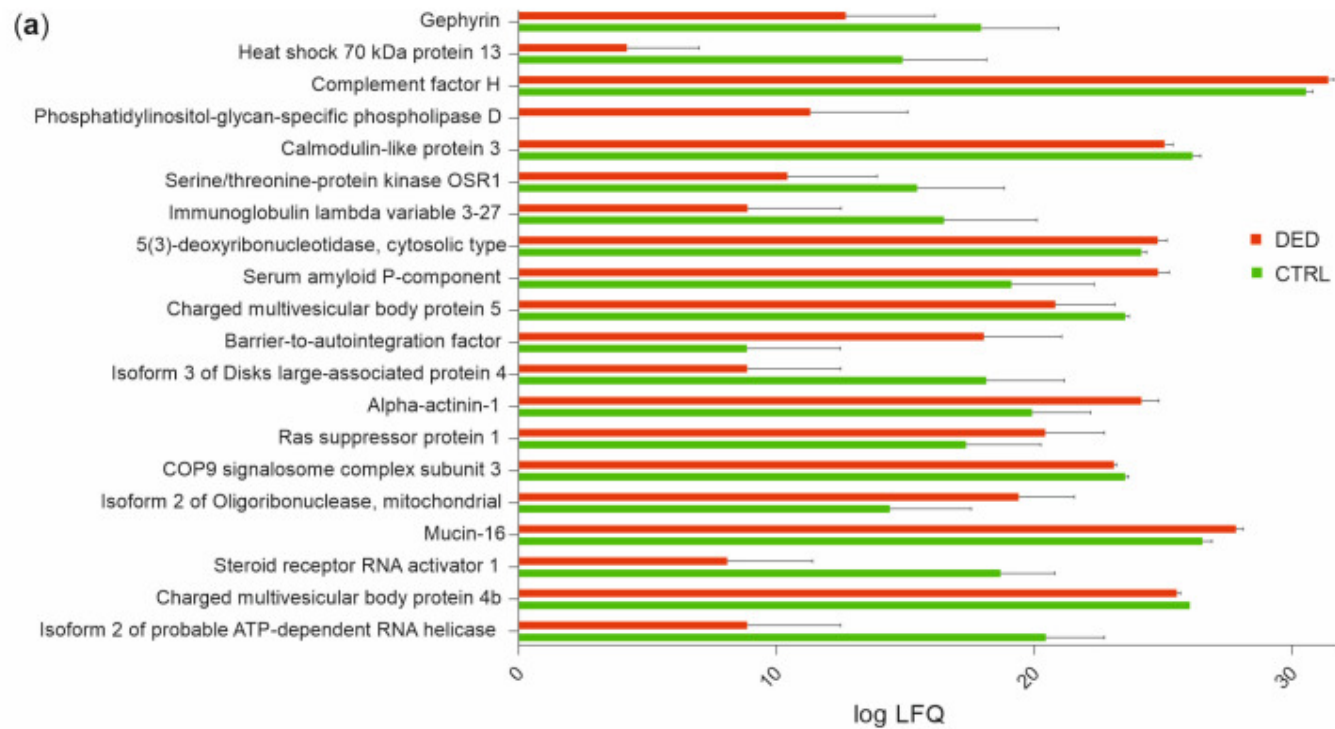
- Actinobacteria
- Proteobacteria
- Firmicutes
- Viruses noname
- Bacteroidetes
- Deinococcus Thermus
- Ascomycota
- Basidiomycota

Specific differences in the ocular surface microbiome of patients vs. controls



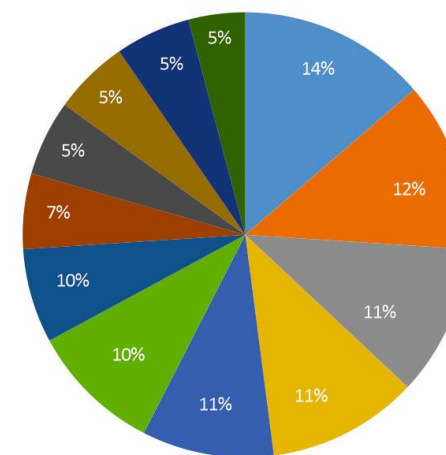
Schlegel I., et al. The Ocular Surface Microbiome and Its Associations with the Tear Proteome in Dry Eye Disease. International Journal of Molecular Sciences. 2023

Results: differences in the tear proteome



Specific differences in the tear proteome of patients vs. controls...

biological processes

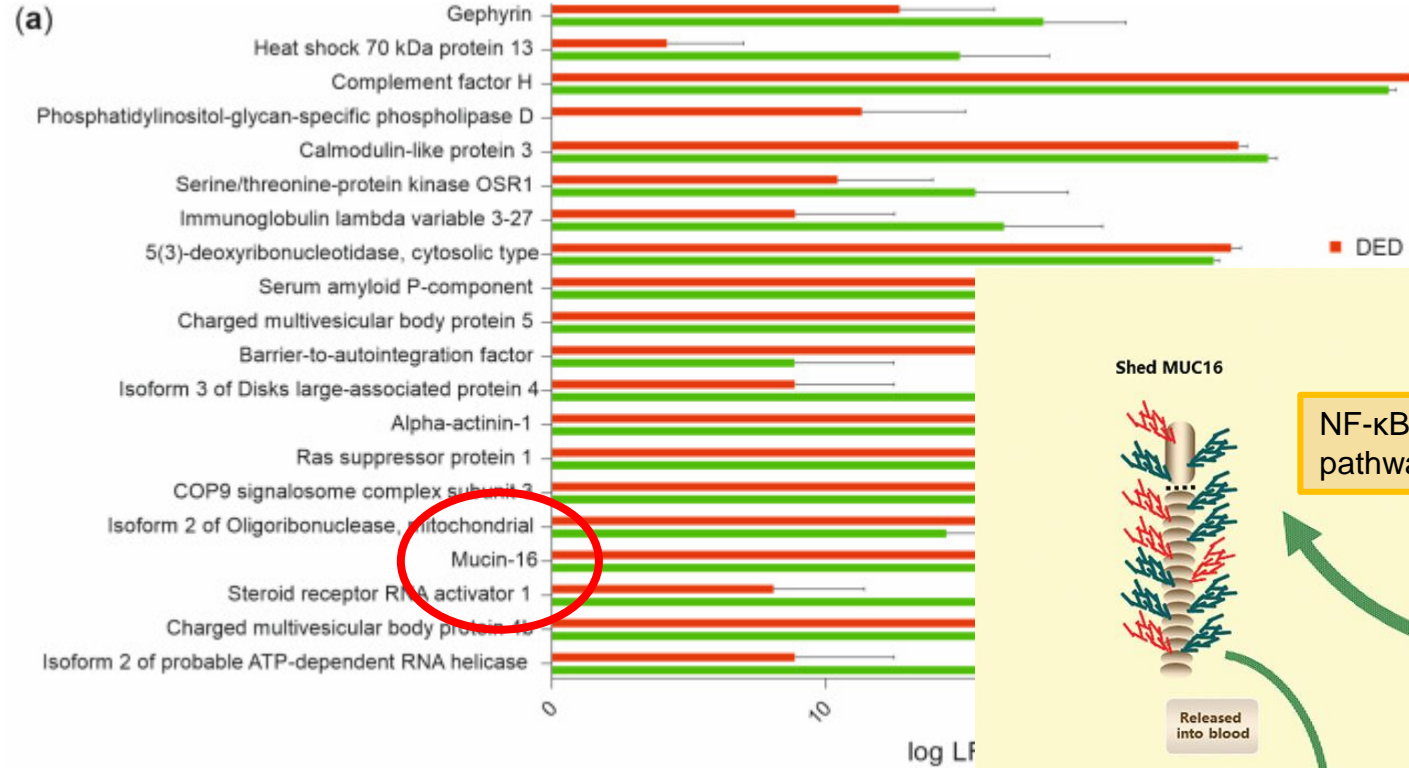


... with an involvement in immune responses

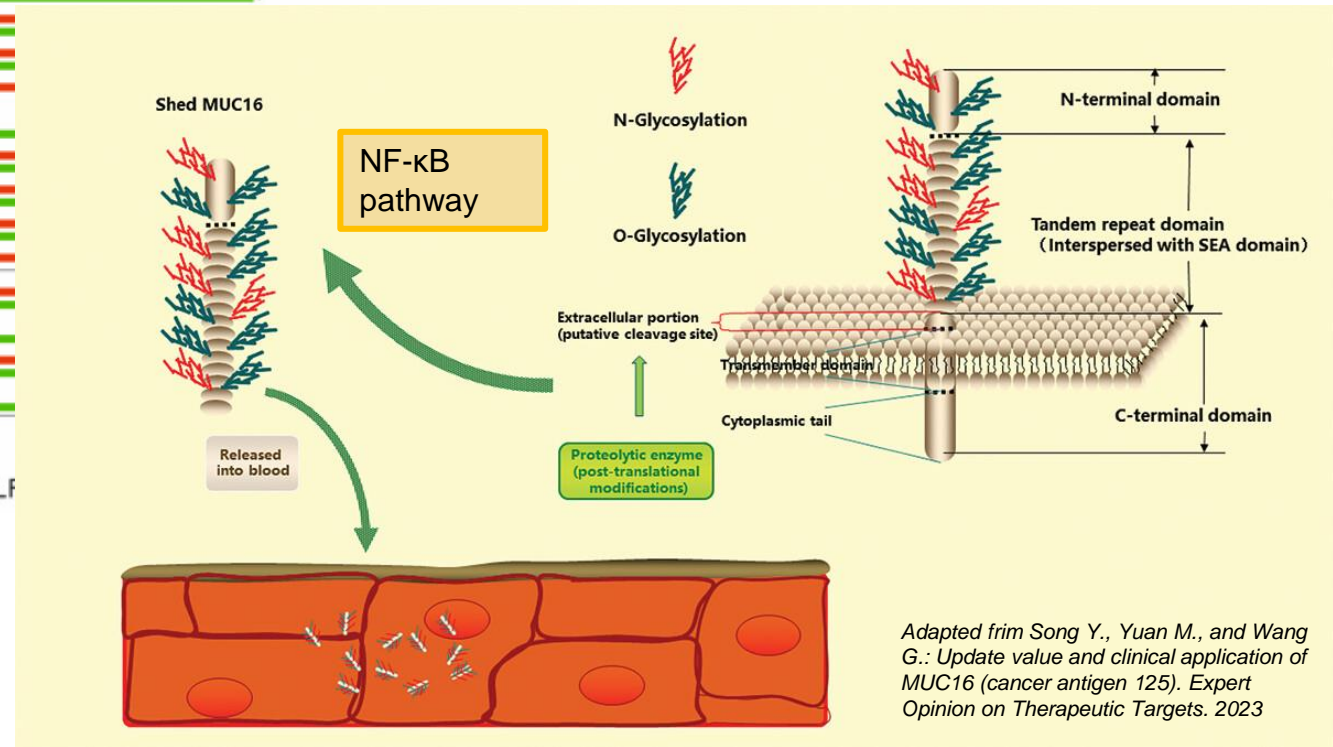


Schlegel I., et al. The Ocular Surface Microbiome and Its Associations with the Tear Proteome in Dry Eye Disease. International Journal of Molecular Sciences. 2023

Results: differences in the tear proteome



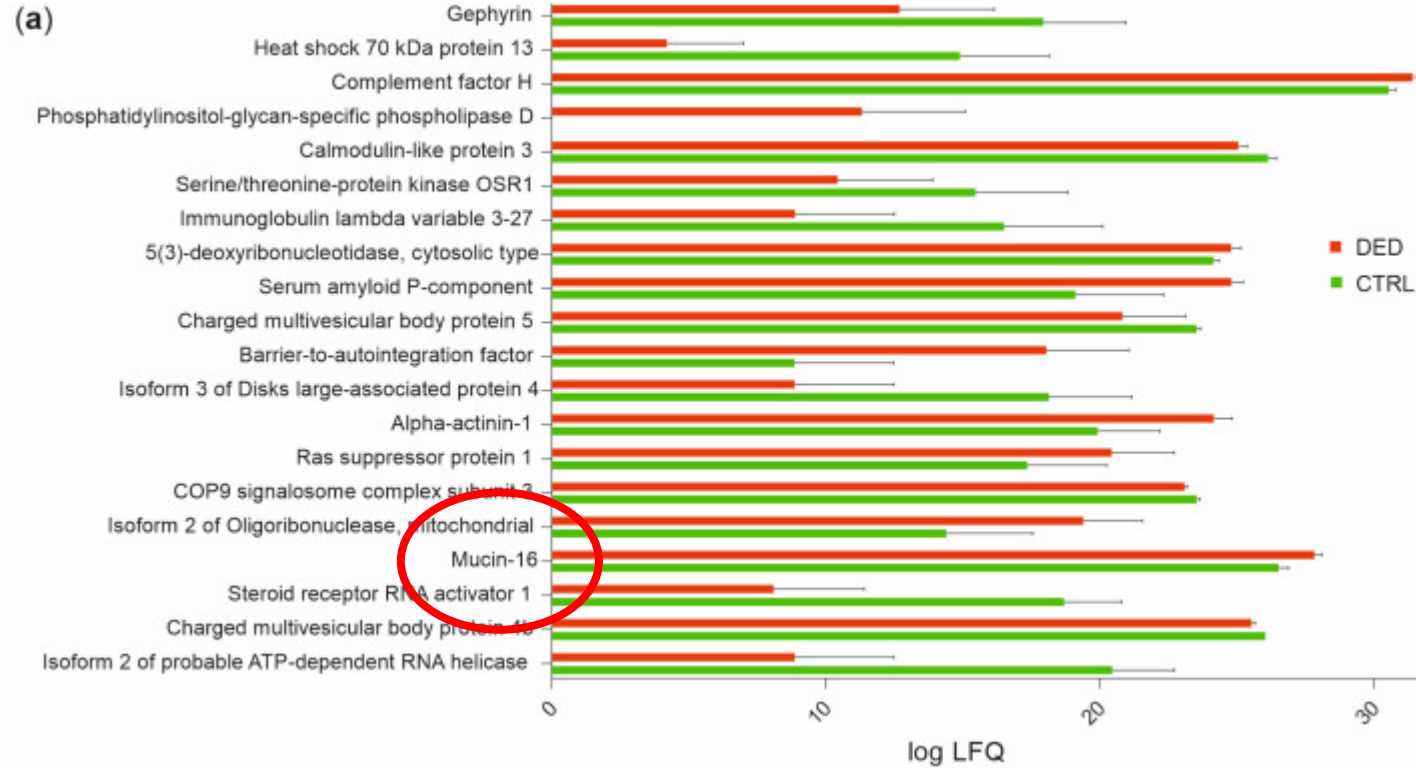
Specific differences in the tear proteome of patients vs. controls



Adapted from Song Y., Yuan M., and Wang G.: Update value and clinical application of MUC16 (cancer antigen 125). Expert Opinion on Therapeutic Targets. 2023

Schlegel I., et al. The Ocular Surface Microbiome and Its Associations with the Tear Proteome in Dry Eye Disease. International Journal of Molecular Sciences. 2023

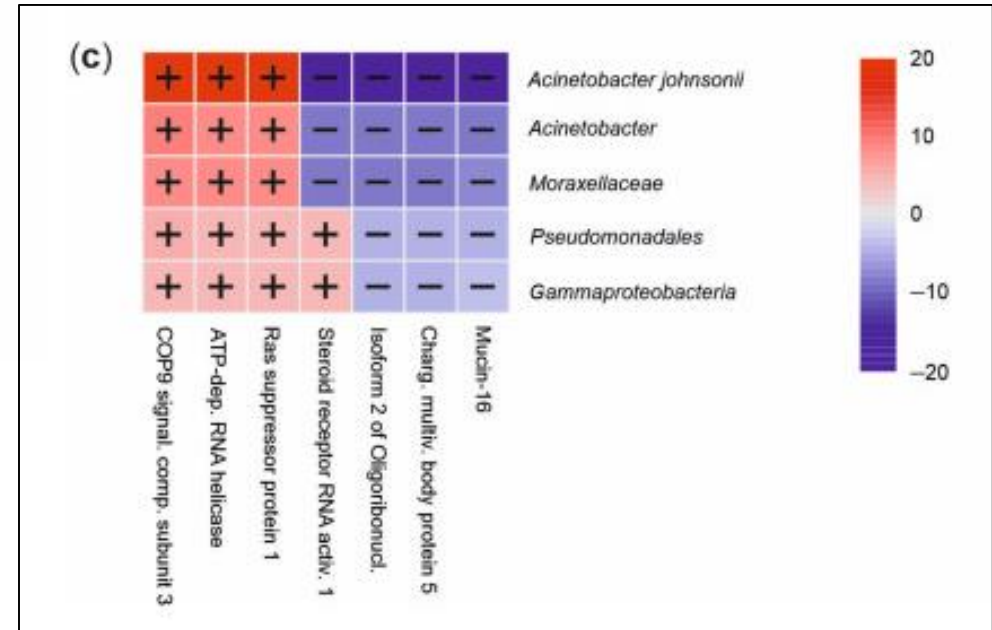
Results: differences in the tear proteome



Specific differences in the tear proteome of patients vs. controls



Specific differences in the tear proteome correlate with components of the ocular surface microbiome

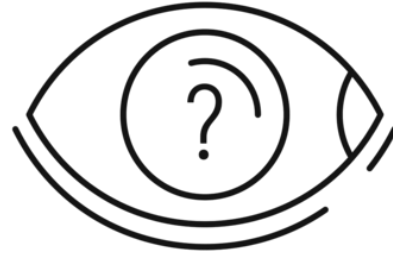


Schlegel I., et al. The Ocular Surface Microbiome and Its Associations with the Tear Proteome in Dry Eye Disease. International Journal of Molecular Sciences. 2023

Thank you

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- Dr. Marco Kreuzer

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