OCULAR SURFACE / EUROPEAN DRY EYE SOCIETY SYMPOSIUM

Dry eye and allergy

Andrea Leonardi
Department of Neuroscience
Ophthalmology Unit
University of Padua, Italy

800 1222-2022 ANNI
UNIVERSITÀ DEGLI STUDI DI PADOVA
DNS
Scuola di Medicina e Chirurgia
School of Medicine
SIUMI

Speaker's name :

☑ I have the following potential conflicts of interest to report:

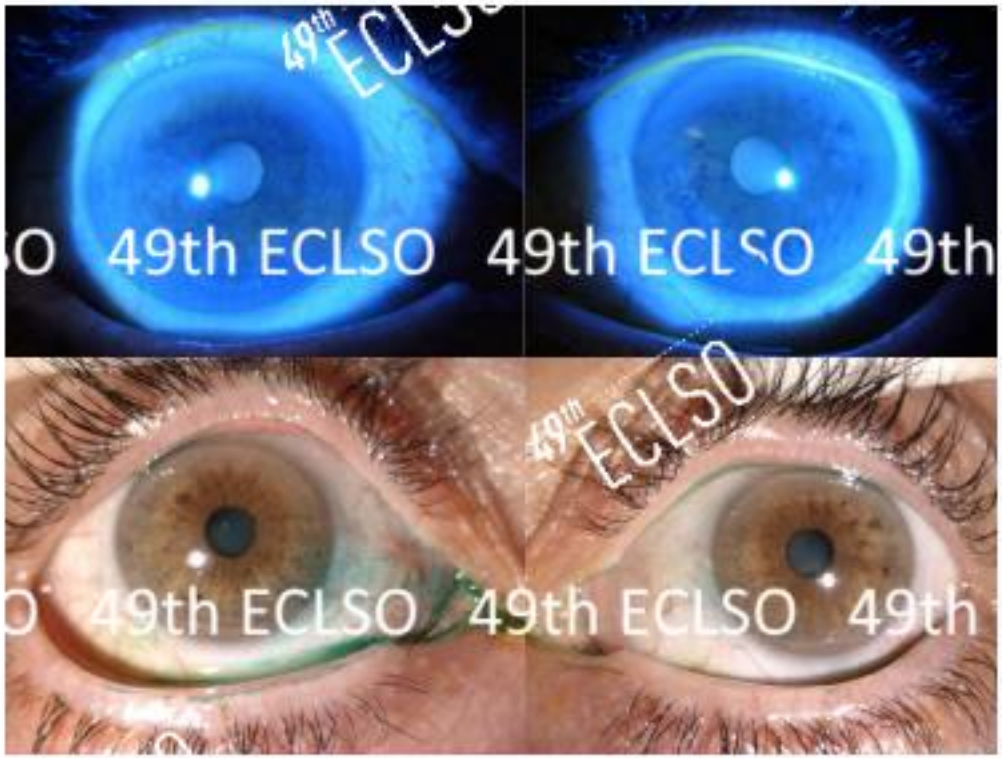
Prof Andrea Leonardi is a consultant for:

- Alcon
- Astra Zeneca
- Bausch & Lomb
- Santen Pharmaceutical Co. Ltd.
- Kedrion
- Laboratoires Théa
- URSA-pharma
- Seqirus
- SIFI
- SOOFT-Fidia

Dry eye and allergy

- 53, year old [illegible]use woman
- Burnin[illegible] almost no i[illegible]ion
- Al[illegible] a[illegible]ick t[illegible]
- Sl[illegible]
- Topi[illegible]es witho[illegible]

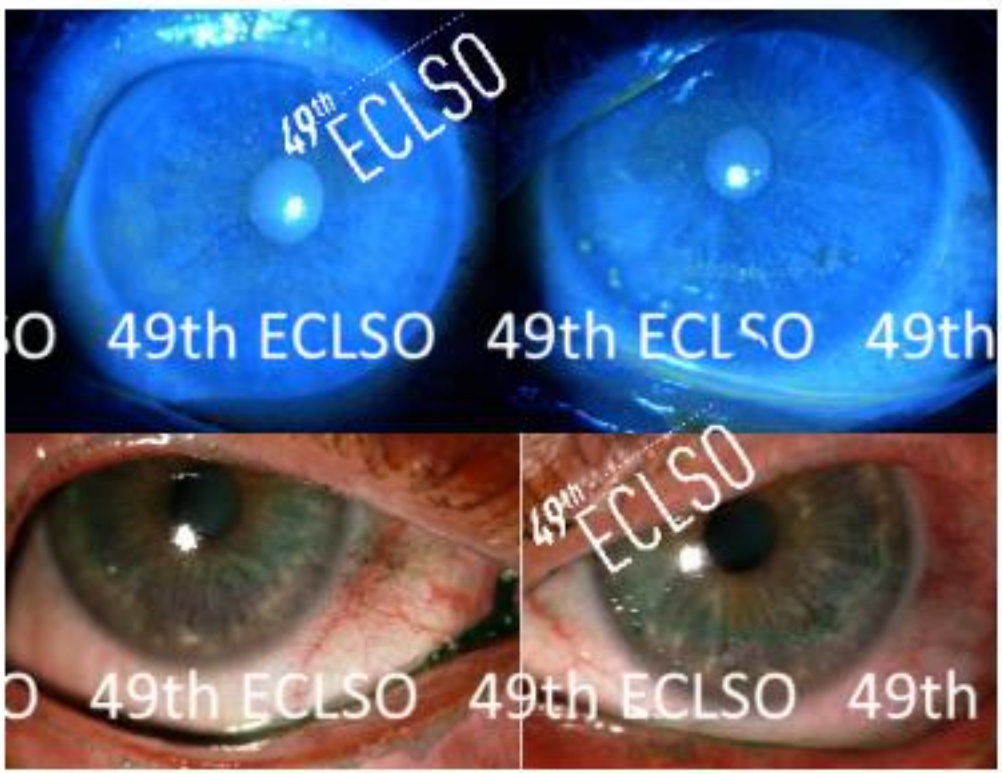
Signs and symptoms suggestive for dry eye in allergic patient



Dry eye and allergy

- 45, year ol… …by atopic derm…
- Bu… …
- …
- A…
- Sys… substi…

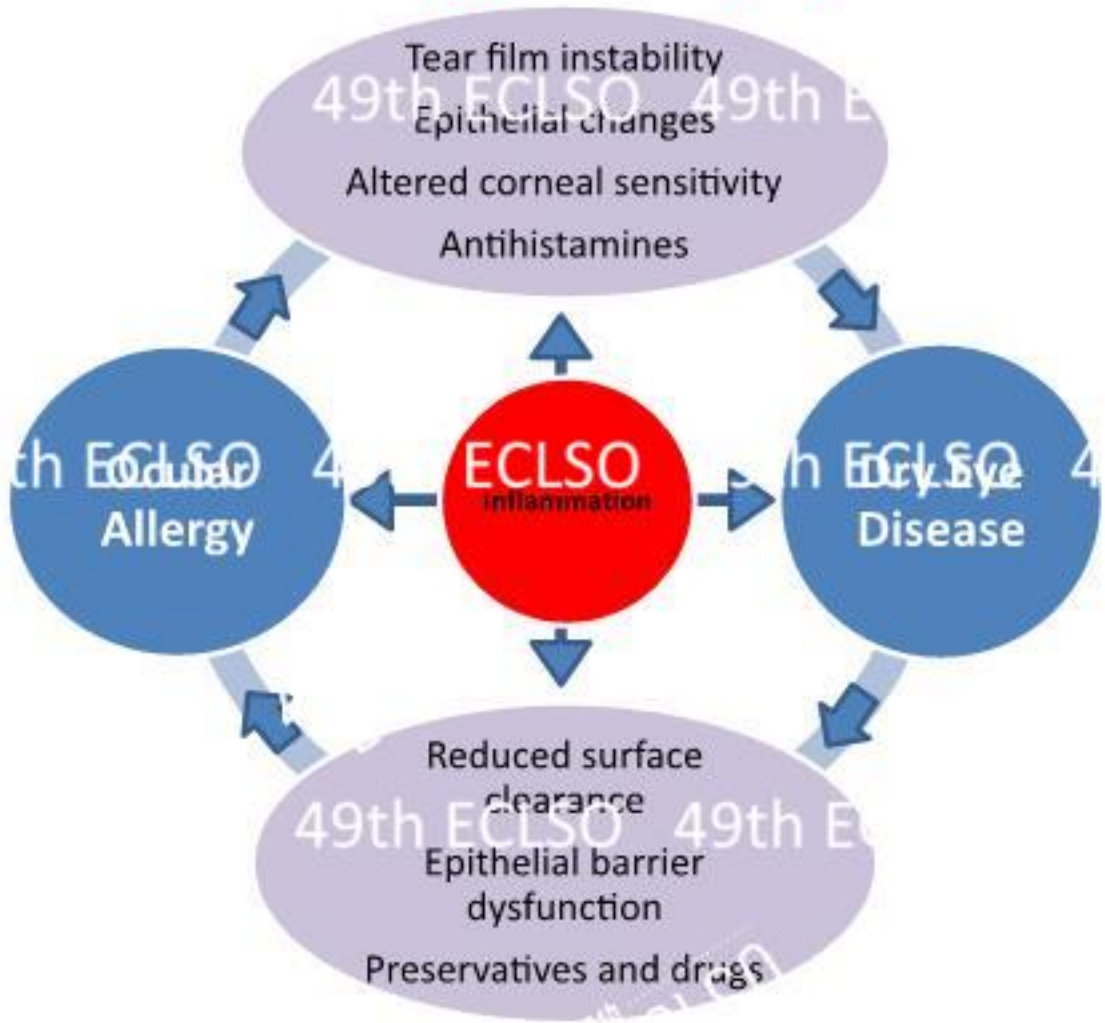
Signs and symptoms and clinical tests suggestive for an overlap syndrome atopic dermatitis and Sjoegren syndrome



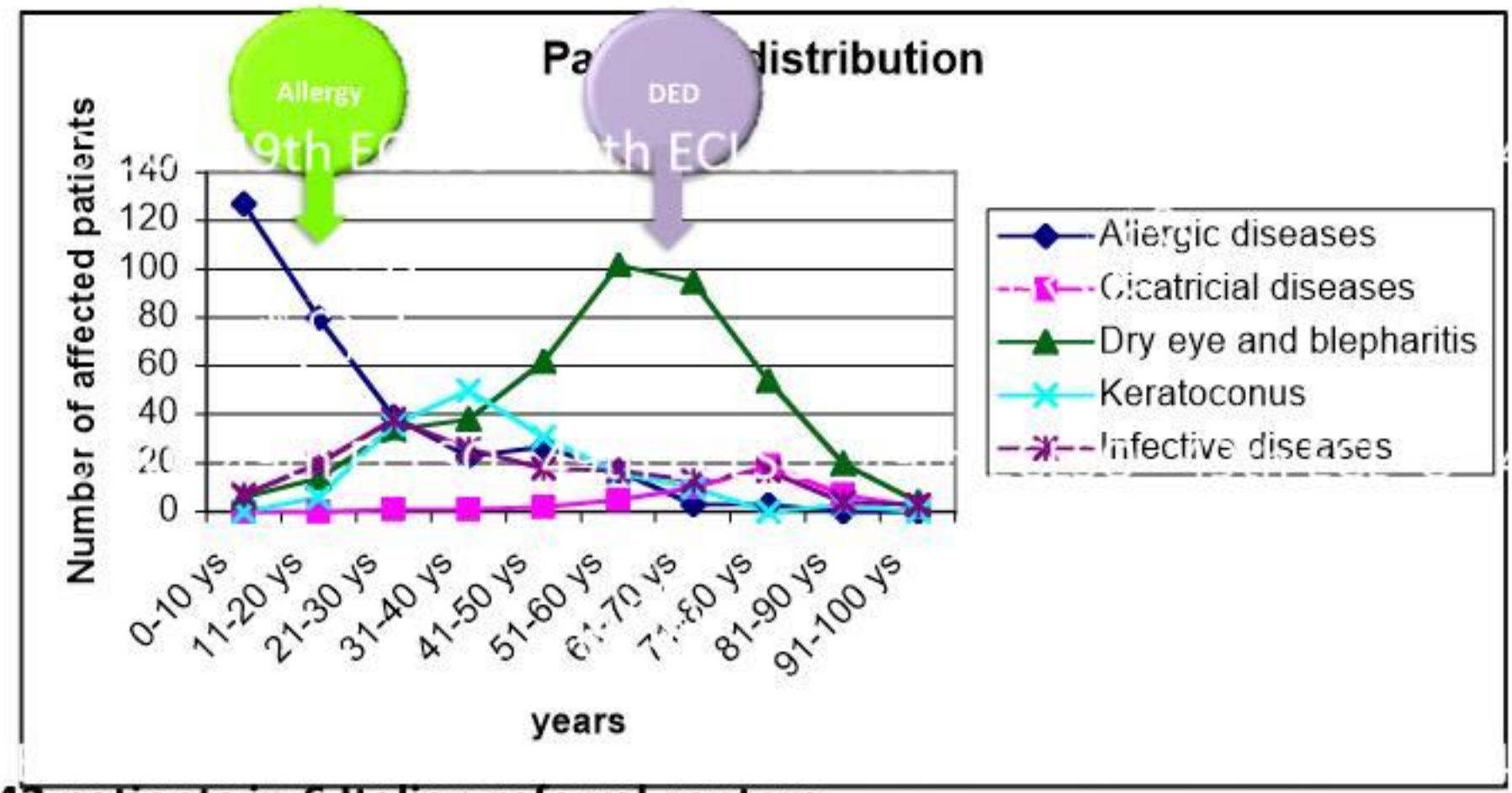
Dry Eye (DED) and Ocular Allergy (OA)

- Major ocular surface disorders affecting millions of people
- Sometimes share similar symptoms
- Common and different mechanisms

Allergy and Dry Eye



Modugno RL, Salami F, Leonardi A. Allergy and Dry Eye Disease. Ocul Immunol Inflamm. 2021;5:1-9



1442 patients in 6 Italian referral centers

Lambiase A, Minchiotti S, Leonardi A, et al. Prospective, multicenter demographic and epidemiological study on vernal keratoconjunctivitis: a glimpse of ocular surface in Italian population. Ophthalmic Epidemiol. 2009 Jan-Feb;16(1):38-41. doi: 10.1080/09286580802573177.

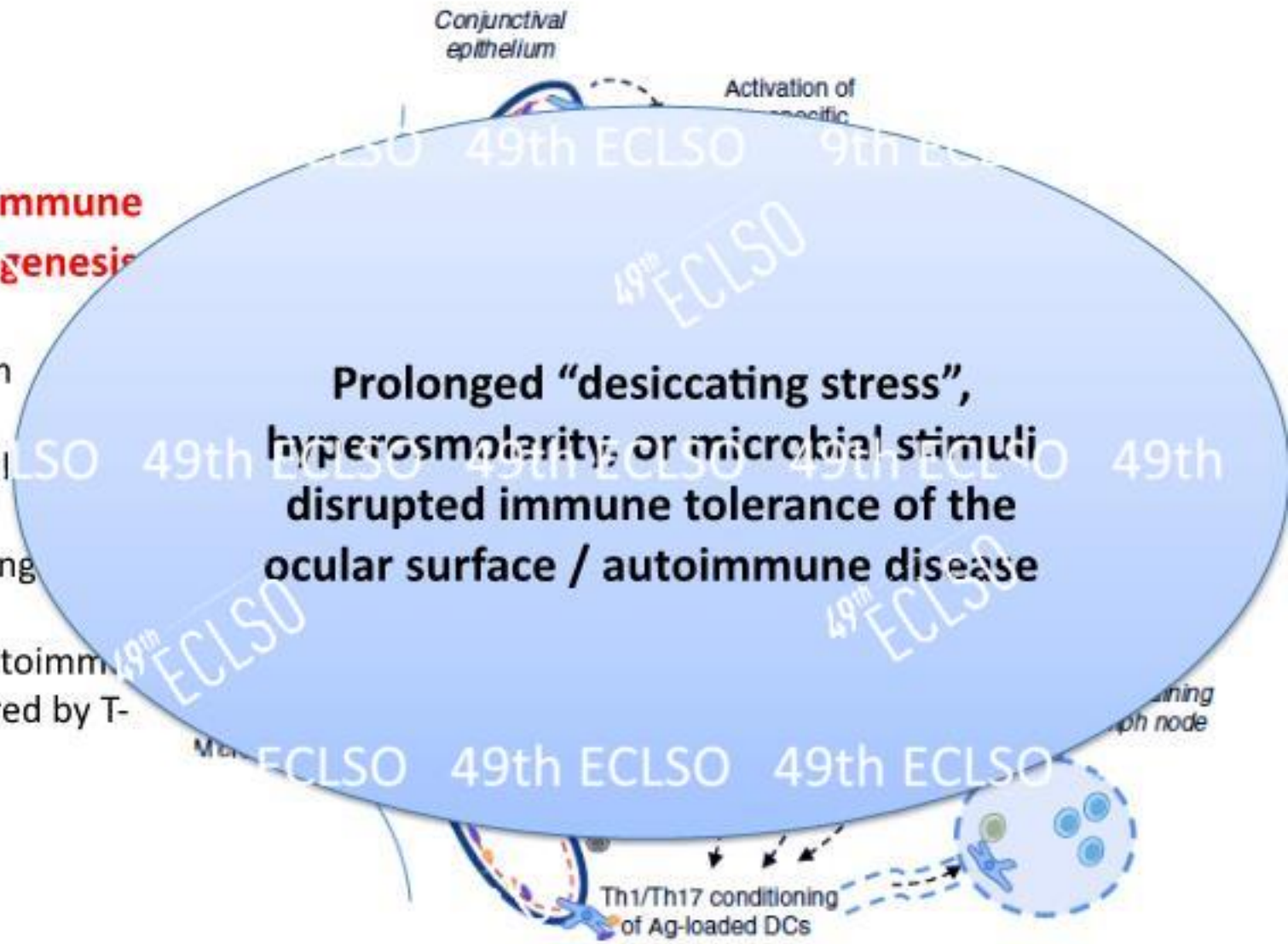
DED

Non-autoimmune/ Autoimmune hypothesis to DED pathogenesis

Desiccating stress and tear film abnormalities lead to tear hyperosmolarity acting on ocular epithelial cells:

a) Activation of specific signaling pathways

b) Induction of a (localized) autoimmune disease which can be transferred by T-cells



Galletti JG. Immunology, 150, 397–407

Can we all become dry?

DED

- **Both DED patients and co**
  - **increased corneal sta**
  - **decreased TBUT**
  - **increased IL-6, MMP-9, IL-**

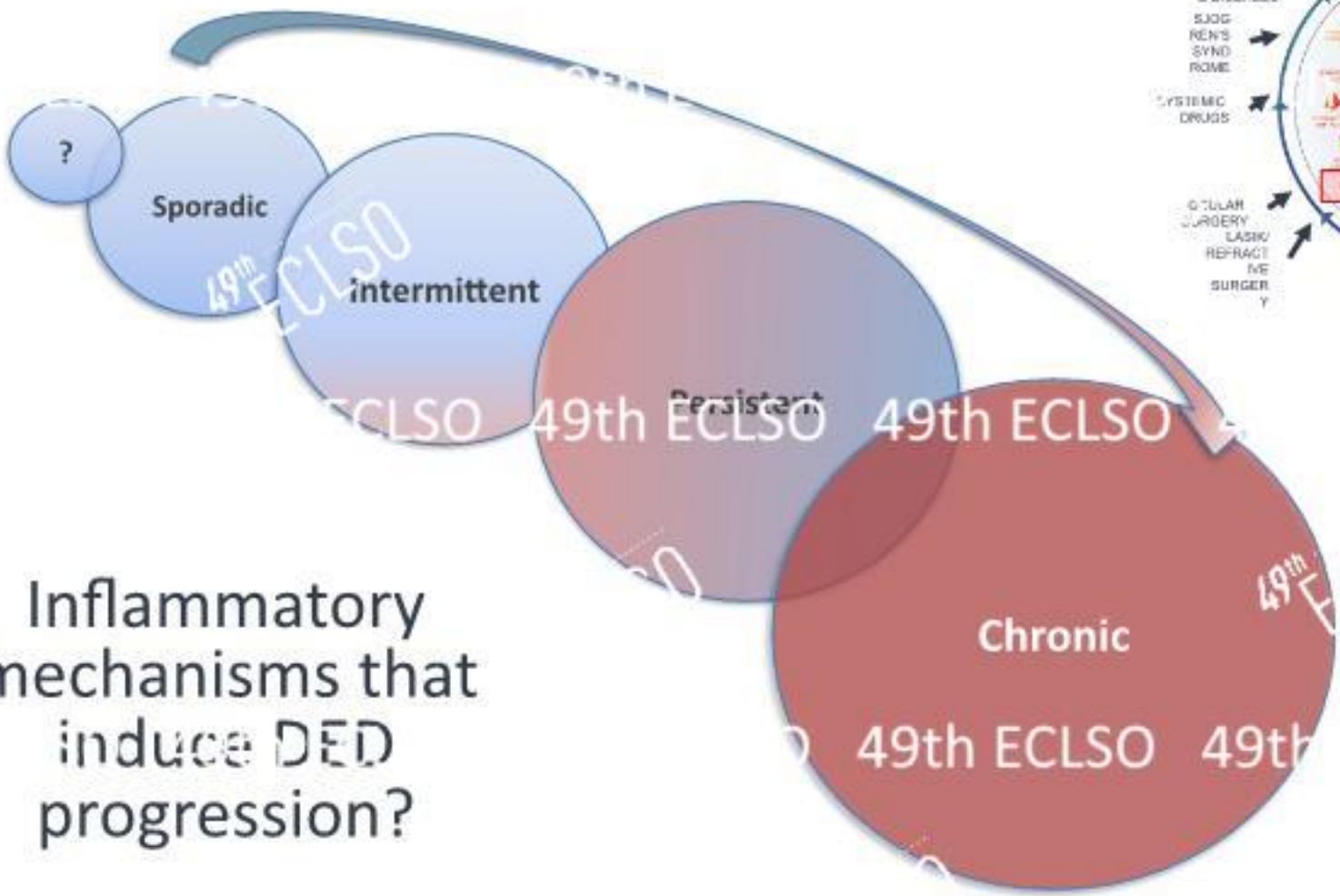
CAE exacerbates signs and symptoms of dry in a manner by dity,

desiccating stress readily induces ocular surface inflammation in healthy subjects

2016

Alex A et al IOVS 2013

Inflammation and disease progression



Inflammatory mechanisms that induce DED progression?

Can we all become allergic?

All of us are exposed to allergens but in cases we

A specific immune response is necessary in predisposed subjects

- **Only allergic patients** develop
  - Red and itchy eye
  - Respiratory symptoms
  - Increased histamine tear levels

Inflammation in DED and OA

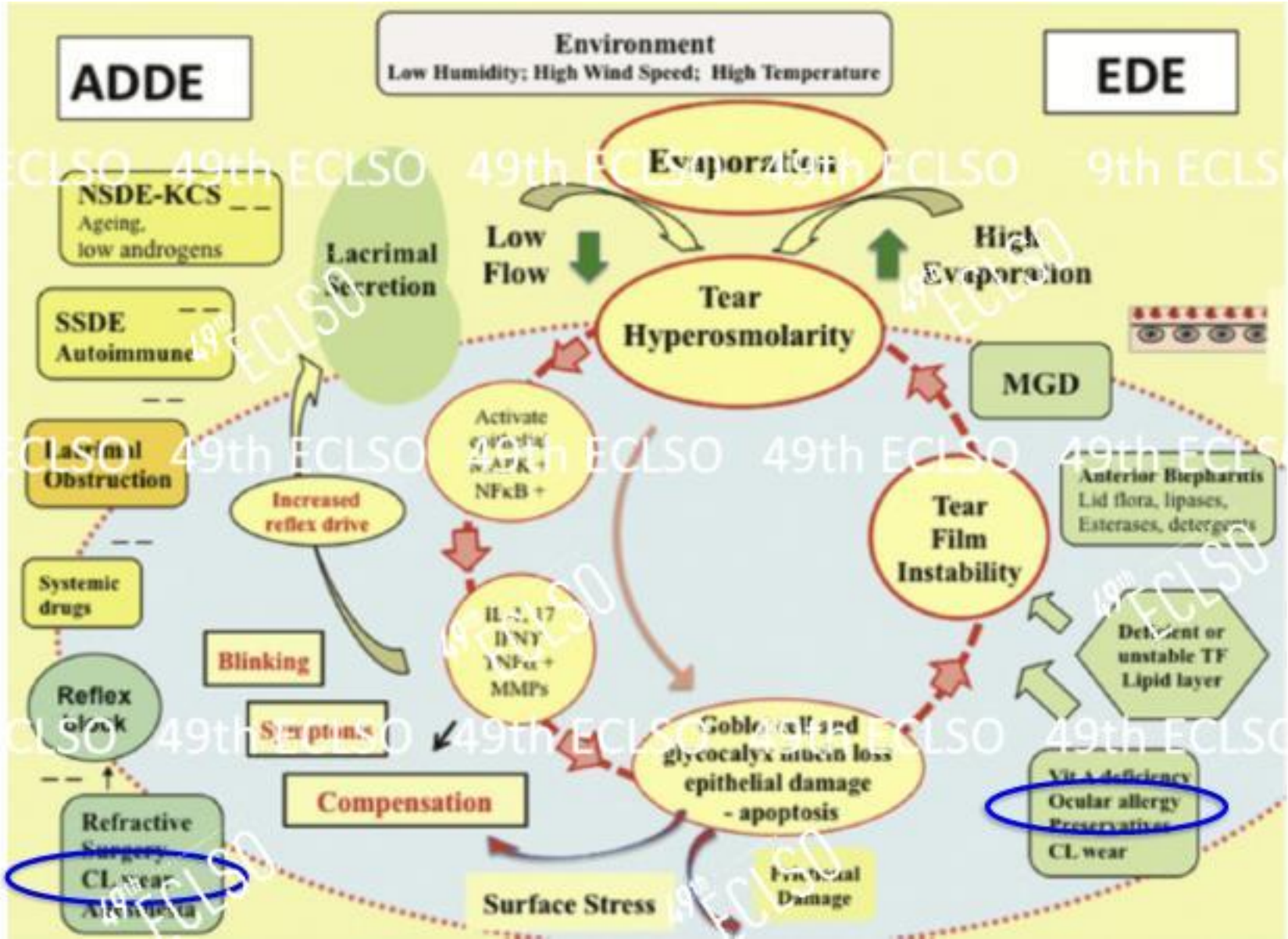
Dry Eye

- Type 1/3 immune response
- Dissecating stress / autoimmune reaction
- DC and CD4+ T cells conjunctival infiltration
- Increased levels of IFN-γ and IL-17
- Epithelial apoptosis and goblet cell loss
- Desquamation of apical epithelial cells leading to corneal barrier disruption MMPs-dependent
- Poorly wettable skin-like surface

Ocular Allergy

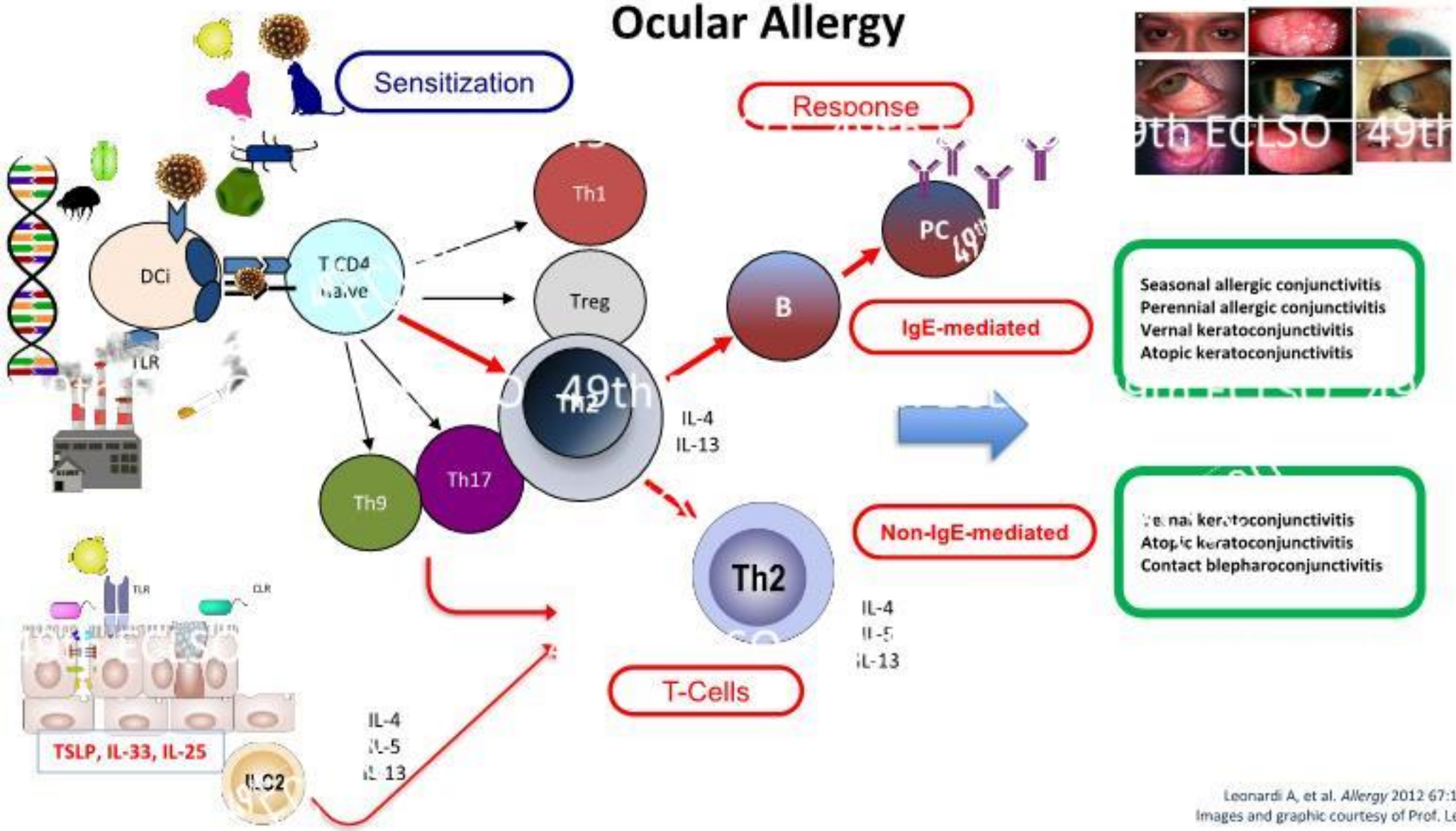
- Type 2 immune response
- Specific IgE and non-IgE (Th2) sensitization
- Specific mast cell activation
- Eosinophils and Neutrophils infiltration
- Th2 cells and Th2-type cytokines (IL-4, IL-5, IL-13)
- Th17 (IL-17) involvement
- Epithelial toxicity of eosinophil-derived proteins and MMPs
- Tissue remodeling

Leonardi A, Modugno RL, Salami E. Allergy and Dry Eye Disease. Ocul Immunol Inflamm. 2021;5:1-9

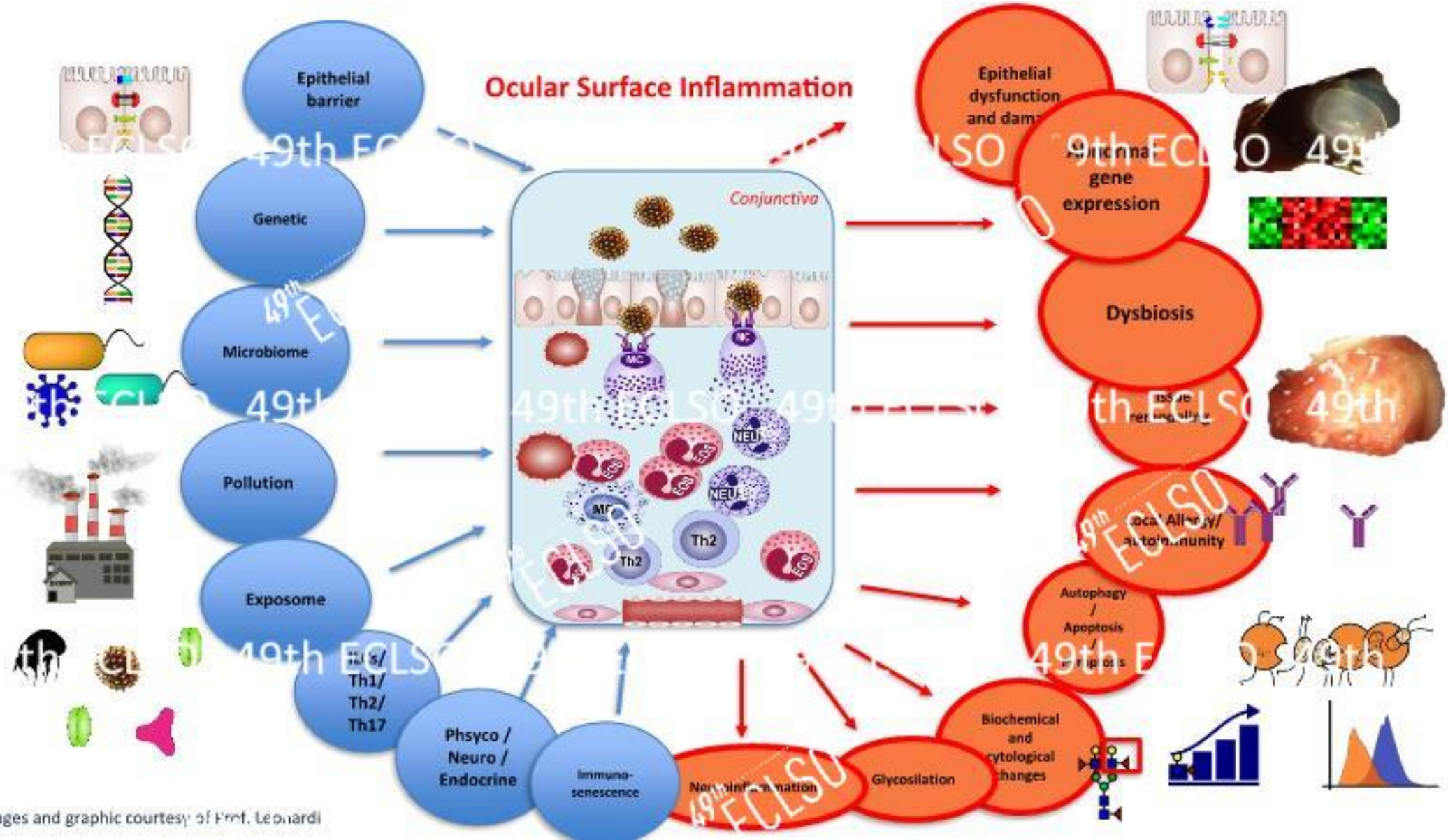

ADDE
Environment
Low Humidity; High Wind Speed; High Temperature
EDE
Evaporation
NSDE-KCS
Ageing,
low androgens
Lacrimal Secretion
Low Flow
High Evaporation
SSDE
Autoimmune
Tear Hyperosmolarity
MGD
Lacrimal Obstruction
Activate
NFκB +
Anterior Blepharitis
Lid flora, lipases,
Esterases, detergents
Increased reflex drive
Tear Film Instability
Systemic drugs
Blinking
Reflex block
Symptoms
Compensation
Goblet cell and glycocalyx mucin loss
epithelial damage
- apoptosis
Deficient or unstable TF Lipid layer
Vit A deficiency
Ocular allergy
Preservatives
CL wear
Refractive Surgery
CL wear
Surface Stress
Functional Damage

DEWS 2017

Ocular Allergy

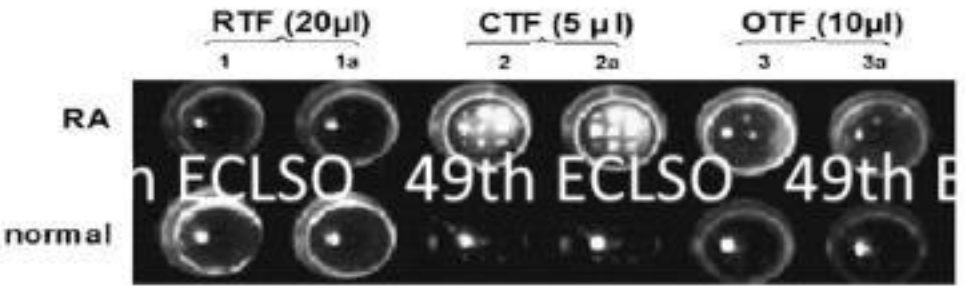


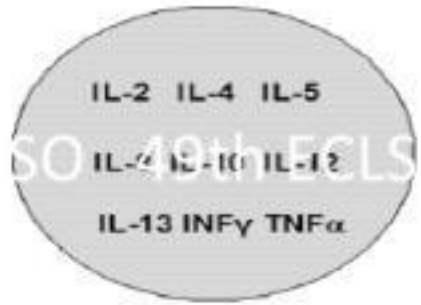
Leonardi A, et al. *Allergy* 2012 67:1327–7.
Images and graphic courtesy of Prof. Leonardi



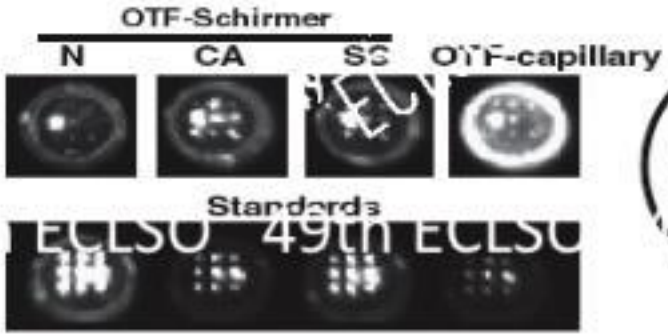
Images and graphic courtesy of Prof. Leonardi

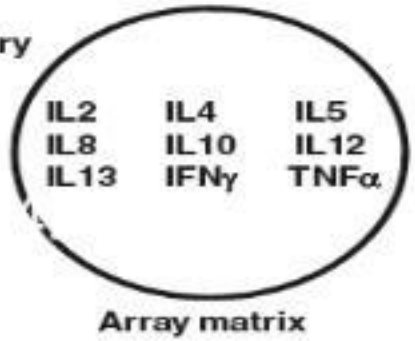
Allergy and Dry Eye: similarities and differences





Micro well plate array





Th1/Th2 in Allergic Conjunctivitis and Sjoegren Syndrome

Elevated cytokines in **DED**: IL-1α, IL-1β, IL-6, IL-23, TNF-α, IFN-γ, and IL-17

Increased cytokines in **ALLERGY**: IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-13, IL-23, TNF-α, IFN-γ, IL-17

Sack et al. ExpEyeRes 2007
Shimin et al. OptVis Sci 2008
Leonardi et al ClinExpAllergy 2006
Leonardi et al. Allergy 2009

Conjunctival gene expression in SS and VKC by Nanostring

DED 9th EC

Gene	FD	p
Upregulated		
IL6	16.7	0.01
CCL24	16.6	0.03
NOD2	8.8	0.001
CXCL9	7.1	0.01
IL23A	5.4	0.02
CCR1	4.9	0.03
CCL4	4.2	0.02
CXCR4	3.9	0.04
TNFA	3.8	0.004
CCL22	3.8	0.0002
NOX1	3.1	0.0009
ITK	2.5	0.006
HLA-DRA	2.4	0.0008
ITGB2	2.1	0.009
CXCL10	2.1	0.01
NOS2	2.1	0.004
HLA-DRB1	1.9	0.005
C1QB	1.9	0.03
CFB	1.9	0.0005
CXCL2	1.9	0.04
STAT1	1.8	0.003
MYD	1.8	0.02
C2	1.8	0.00006
TNFAIP3	1.78	0.02
CCL5	1.7	0.002
IL1RN	1.6	0.004
IL15	1.5	0.01

Liang et al. The Ocul Surf. 2019;17:516-525

Allergy LSO 4

Gene	Log2 FC	P
Upregulated		
CD1A	7,31	0,000802
CSF3R	7,24	0,0206
IL6	6,94	0,000802
CCL24	6,94	0,00357
FCGR2A	6,71	0,0206
LILRB2	6,55	0,0147
CCL18	6,43	0,00763
SELL	6,23	0,0341
IRAK3	6,04	0,000362
CXCR4	6,02	0,0451
CXCL1	5,92	0,000362
CLEC7A	5,84	0,0183
SOCS3	5,75	0,0341
CD274	5,42	0,00237
ITGAX	5,42	0,0463
NCF4	5,29	0,0347
TGFB1	5,16	0,00902
ICAM2	4,99	0,00163
MRC1	4,97	0,0347
ICAM1	4,92	0,0028
IL2RG	4,91	0,00463
CIITA	4,88	0,00237
CCL22	4,86	0,0312
IRF7	4,85	0,00362
JAK3	4,82	0,023
FYN	4,81	0,00463
CARD9	4,76	0,00439
CISH	4,75	0,0286

Leonardi et al. The Ocul Surf. 2021;19:241-248.

DED

Sjogren's Syndrome

Microbiome and DED

- Host immunity to pathogens is largely regulated by the commensal microbiota
- The microbiota implication in autoimmune response's regulation is still a matter of discussion
- **In the context of autoimmunity** could be **"protective, neutral, or provocative"**
  - Molecular mimicry
  - Pathogen-associated molecular patterns (PRRs)
  - Microbial metabolites

Can gut microbiota/immunity trigger autoimmune procedures at distal sites ?

Front Immunol. 2018; 9: 1106.

DED

Altered Mucosal Microbiome Diversity and Disease Severity in Sjögren Syndrome

Cintia S. de Paiva[1], Dan B. Jones[1], Michael E. Stern[2], Fang Bian[1], Ouianta L. Moore[1], Shani Corbiere[1], Charles F. Streckfus[4], Diane S. Hutchinson[5], Nadim J. Ajami[5], Joseph F. Petrosino[5] & Stephen C. Pflugfelder[1]

Scientific Reports | 6:23561 | DOI: 10.1038/srep23561

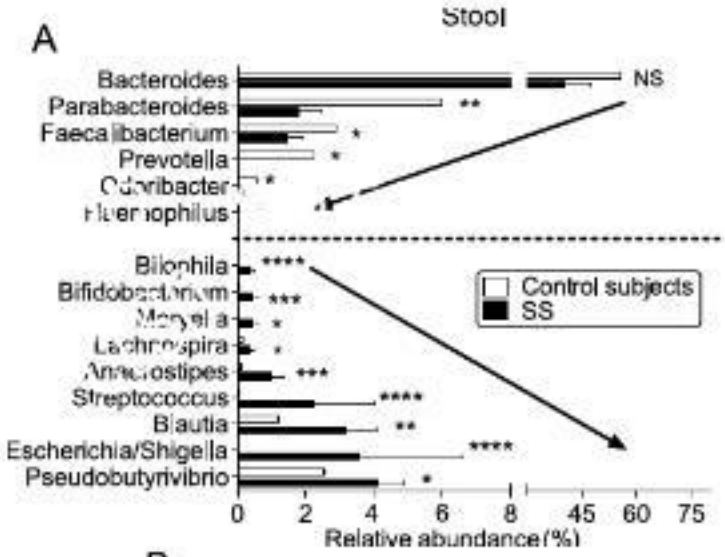
Aims

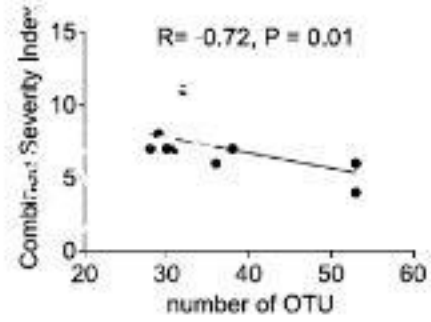
Characterized conjunctival, tongue and fecal microbiome profiles of patients with SS

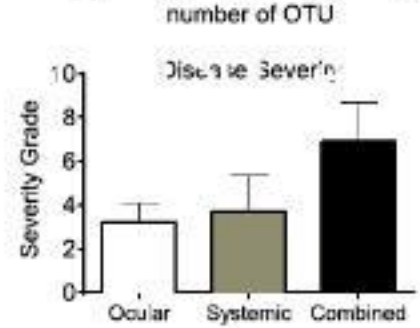
S.S. patients:

- Dysbiotic intestinal microbiome
  - low relative abundance of commensal bacteria
  - high relative abundance of potentially pathogenic genera

- The severity of SS disease was inversely correlated with microbial diversity





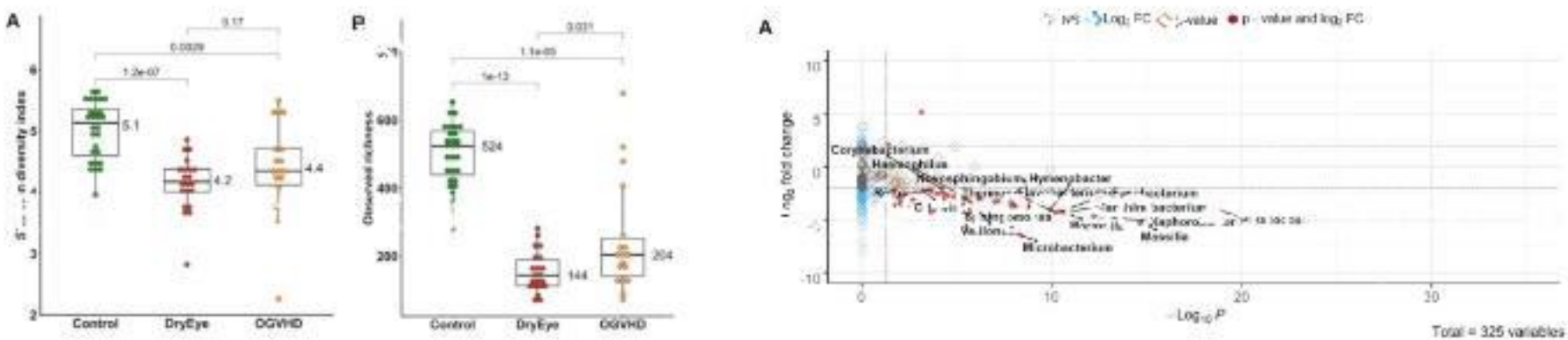


Ocular surface microbiota in patients with aqueous tear-deficient dry eye

Jasmine Andersson [a,*], Josef K. Vogt [b], Marlene D. Dalgaard [c], Oluf Pedersen [b], Kim Holmgaard [a], Steffen Heegaard [a,d]

The Ocular Surface 19 (2021) 210–217

DED



Ocular surface microbiota in patients with DED:

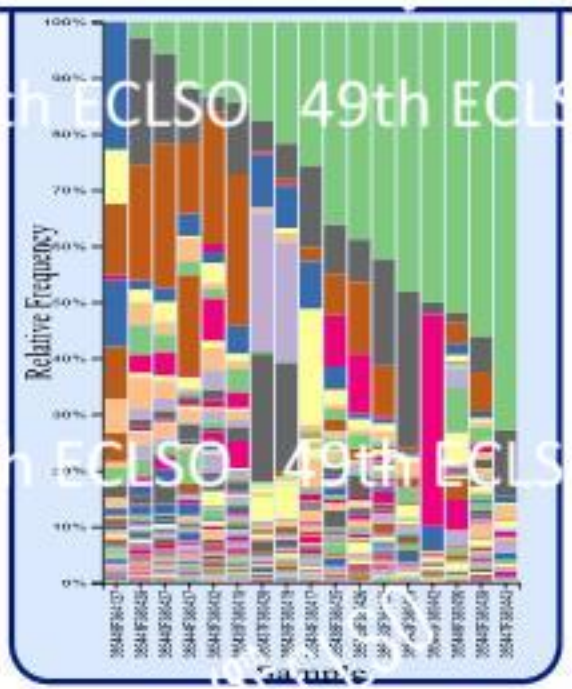
- **Decreased microbial diversity**
- **Several genera were less abundant compared to controls**

Higher microbial alfa and beta diversities in VKC than CT

Higher diversity inVKC

Lower diversity in CT

Increased bacterial dysbiosis in VKC vs CT



Leonardi et al. Allergy 2021 doi: 10.1111/all.14963

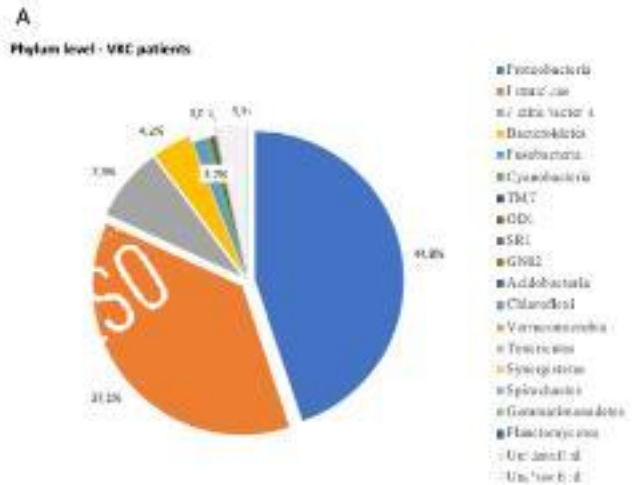
Allergy

Metagenomic analysis of the conjunctival bacterial and fungal microbiome in vernal keratoconjunctivitis

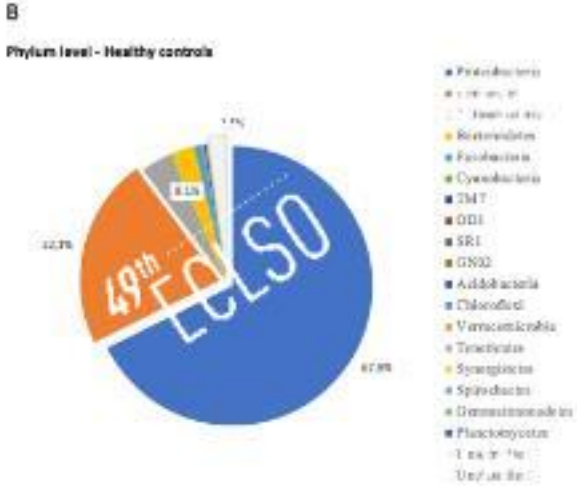
Andrea Leonardi, Rocco Luigi Modugno, Fabiano Cavarzeran, Umberto Rosani

First published: 28 May 2021 | https://doi.org/10.1111/all.14963

VKC



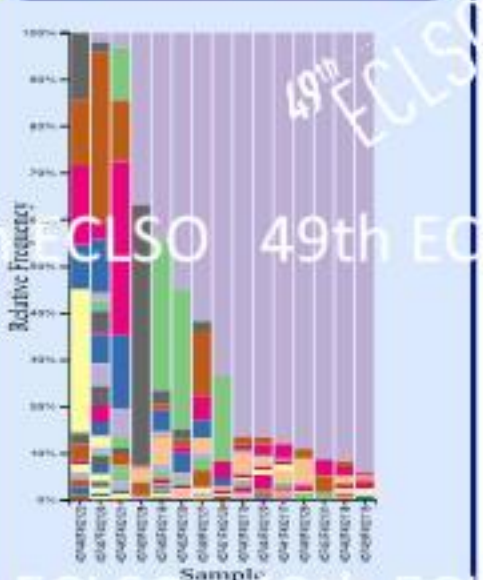
CT

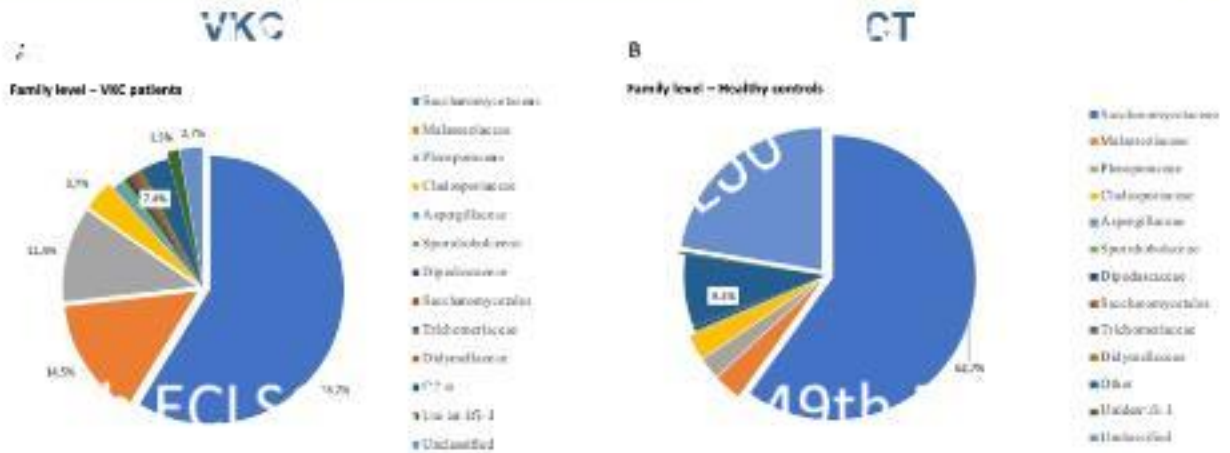


Proteobacteria (VKC 44.2%; HC 67.7%; p<0.0001)
Firmicutes (VKC 36.6%; HC 22.1%; p<0.0001)
Actinobacteria (VKC 7.8%; HC 3.7%; p<0.0001)
Bacteriodetes (VKC 4.1%; HC 2.7%, p<0.0001)
accounted for > 90% of all the reads

Conjunctival fungal microbiome

Malasseziaceae OTUs were significantly higher in VKC vs CT





Saccharomycetaceae (VKC 61.5%; HC 62.7%; p<0.0001)
Malasseziaceae (VKC 16.5%; HC 3.3%; p<0.0001)
Pleosporaceae (VKC 5.0%; HC 2.6%; p<0.0001)
Cladosporiaceae (VKC 4.2%; HC 3.0%; p<0.0001)

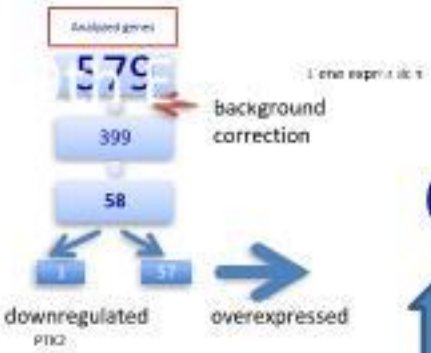
Conclusions

Higher diversity and dysbiosis highlights the role of the host-microbes interaction in VKC pathogenesis.

Leonardi et al. Allergy 2021 doi: 10.1111/all.14963

Transcriptomic

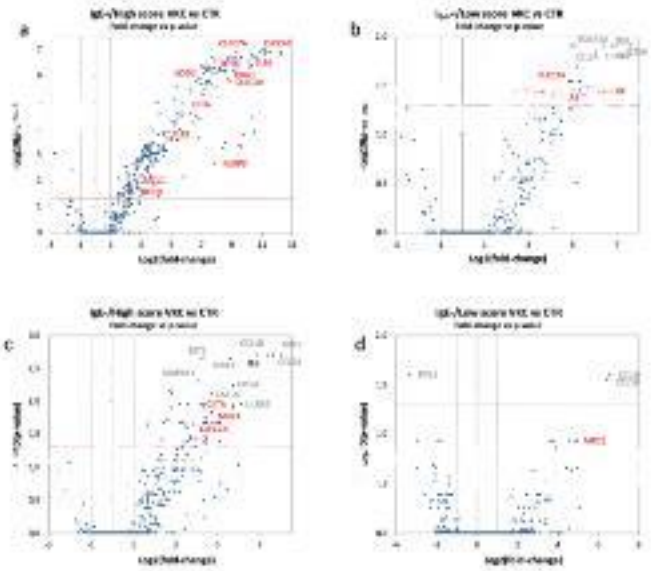
Allergy

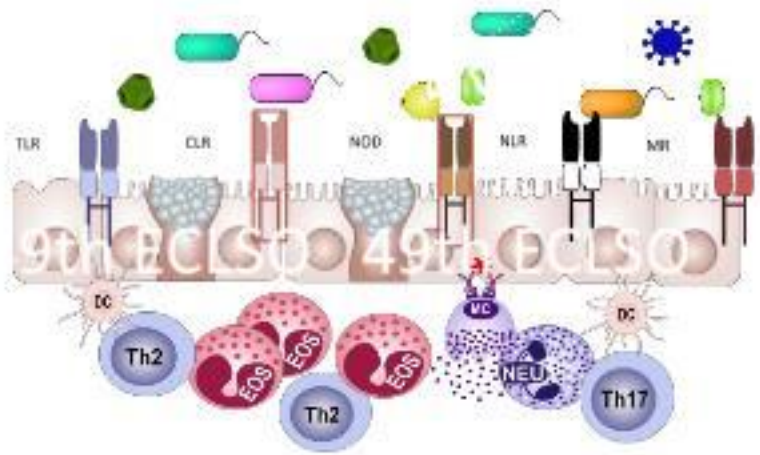


Increased expression of TLRs, NLRs and CLRs in both IgE+ and IgE- VKC

pattern recognition receptors (PRRs)

TLR4, TLR8, Dectin1/CLEC7A, CLEC4A, Mincle/CLEC4E, Dectin3/MCL1, MRC1/CLEC13D, NOD2, NLRP3





Leonardi et al. Conjunctival transcriptome analysis reveals the overexpression of multiple pattern recognition receptors in vernal keratoconjunctivitis. Ocul Surf. 2021;19:241-248.

The innate immune system interacts with local microbiome

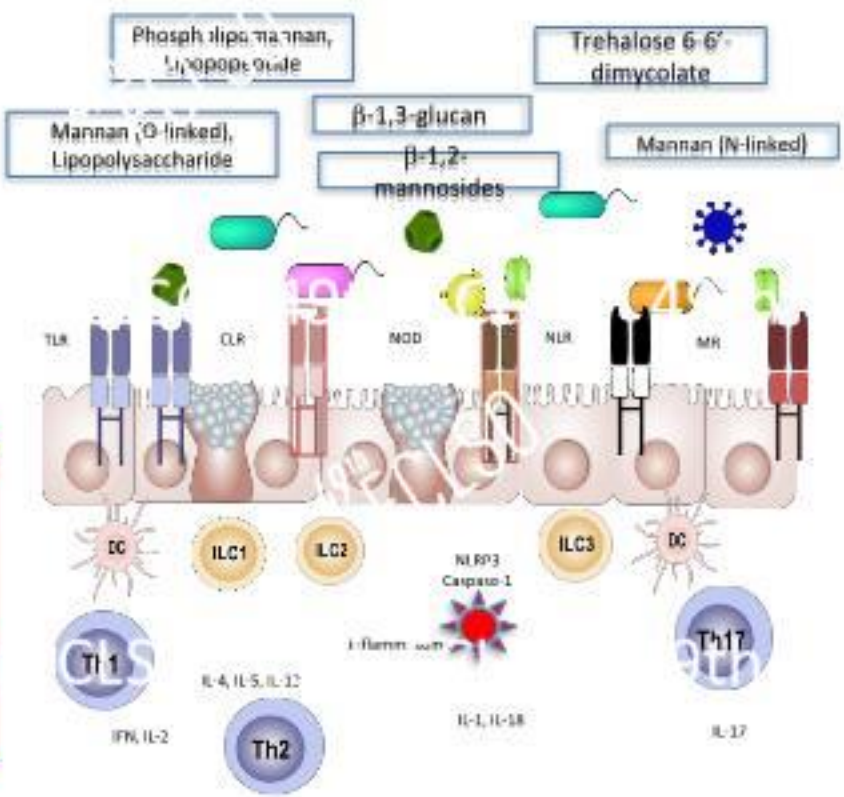
Pattern recognition receptors (PRRs) recognize:

- Pathogens-associated molecular patterns (PAMPs)
- Damage-associated molecular patterns (DAMPs)

The PRRs are divided into four families:

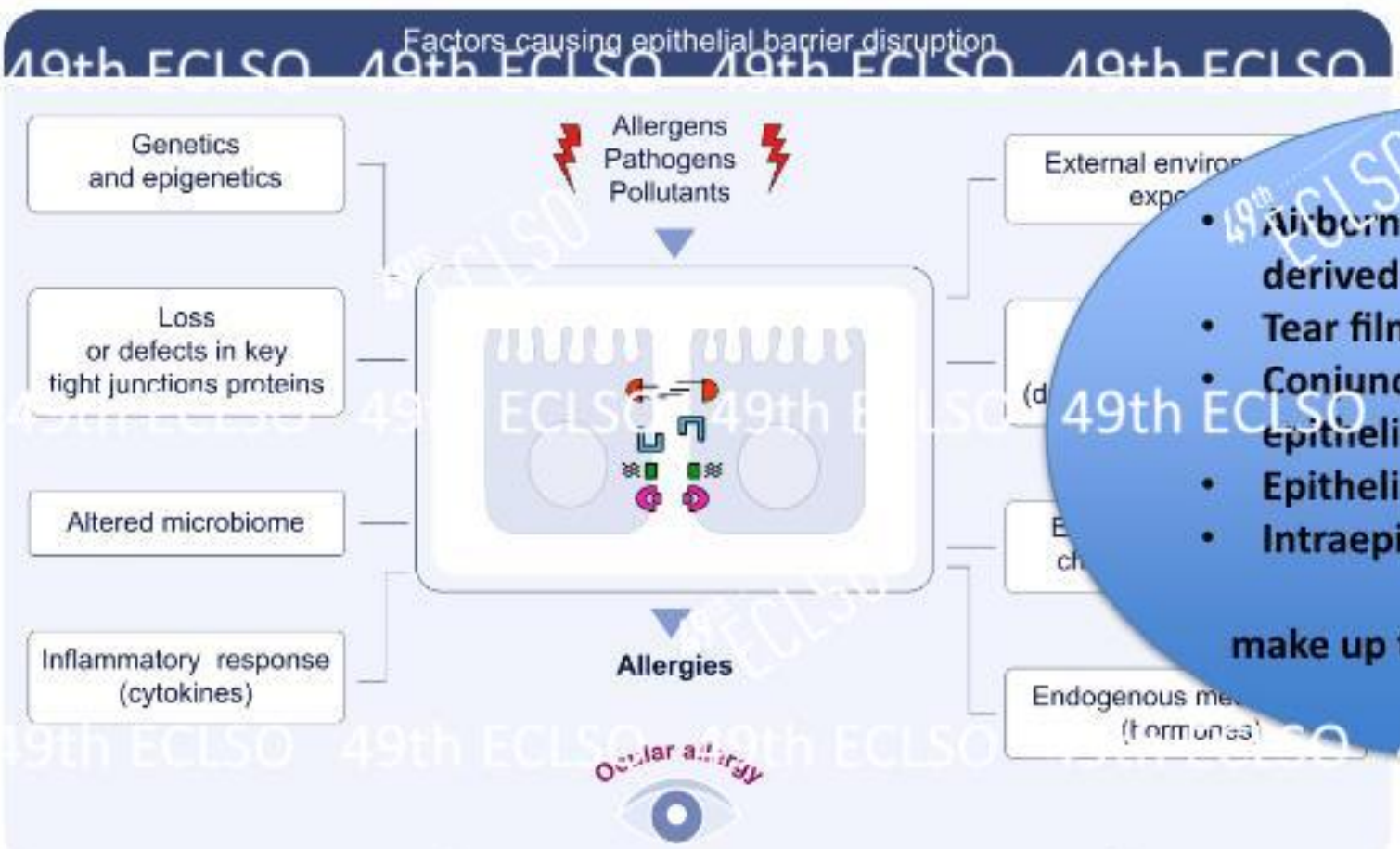
- Toll-like receptors (TLR)
- Nucleotide-binding oligomerization domain-like receptors (NLR)
- C-type lectin receptors (CLR)
- RIG-1 like receptors (RLR)

Hypothesis:
glycan, phospholipid, carbohydrate residues of allergens, microbes or proteins, can engage innate receptors on epithelial and dendritic cells priming a T1, T2, T3 response or trigger inflammasome response



Leonardi et al. Allergy 2021 doi: 10.1111/all.14963; Leonardi et al. The Ocul Surf 2021;19:241-243.

Epithelial barrier

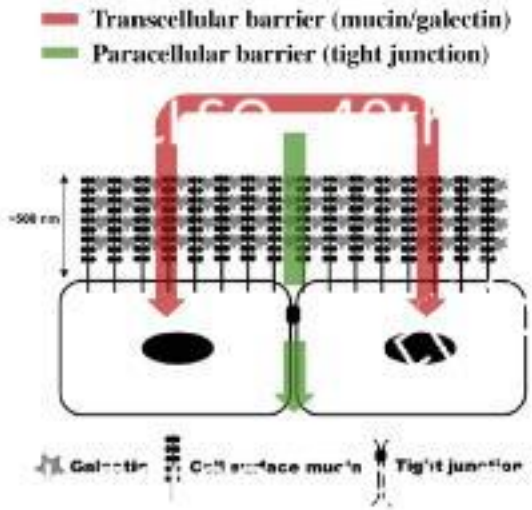


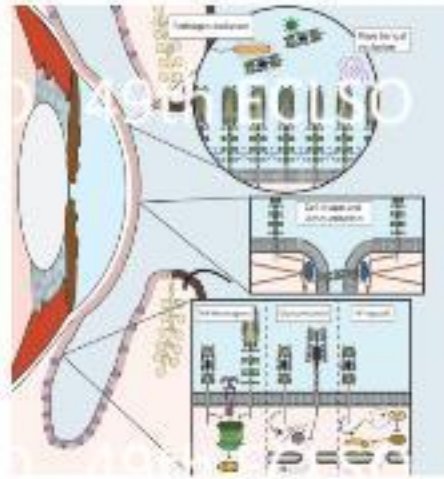
- Airborne and microbiota-derived antigens
- Tear film
- Conjunctival and corneal epithelia and goblet cells
- Epithelial dendritic cells
- Intraepithelial lymphocytes

make up the epithelial barrier

Singh N, Diebold Y, Sahu SK, Leonardi A. Epithelial barrier dysfunction in ocular allergy. Allergy. 2023 Nov 10. doi: 10.1111/all.15174.

Epithelial Barrier





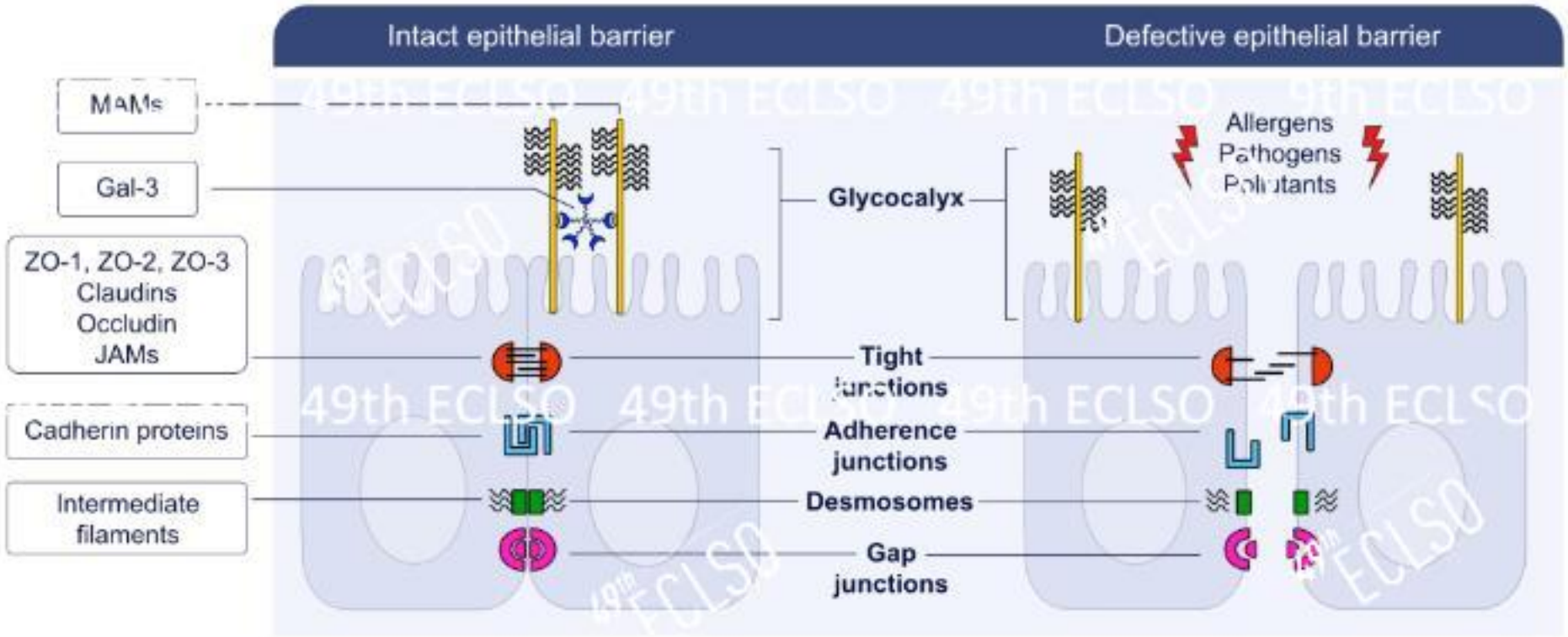
- Transcellular Barrier: membrane-associated mucins (MAMs: MUC1, MUC4, MUC16, MUC21, MUC22) and galectin-3 (LGALS3)
  - MAMs cross-linked by LGALS3
- Paracellular Barrier: stratified epithelia sealed by tight junctions (TJs), adherens junctions (AJs) and desmosomes

Functions

- Mechanical
- Pathogen exclusion
- Regulation of inflammation
- Determination of cell shape and desquamation
- Stabilization of ion channels

Argueso et al. J Biol Chem. 2009; 284:23037e23045; Martinez-Carrasco et al. The Ocular Surface 2021 21:313–330; Singh N, Diebold Y, Sahu SK, Leonardi A. Allergy. 2021. doi: 10.1111/all.15174

Epithelial Barrier function and dysfunction



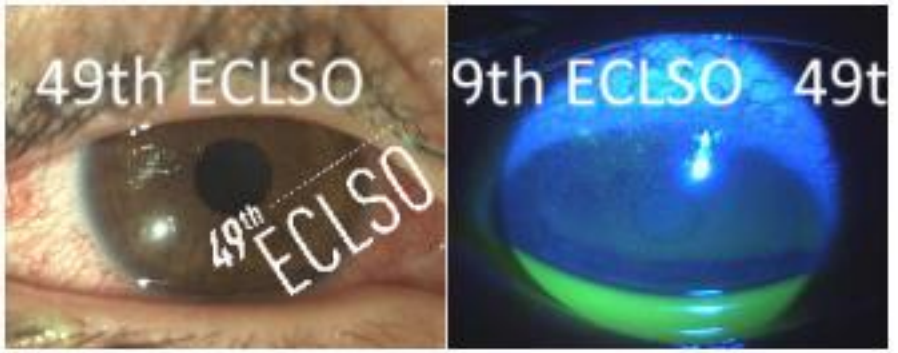
Consequences of barrier disruption

- Facilitation of allergen penetration and sensitization
- Increased susceptibility to pathogens
- Tissue remodeling
- Disease chronicity

Singh N, Diebold Y, Sahu SK, Leonardi A. Allergy. 2021. doi: 10.1111/all.15174

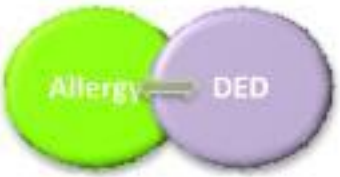
Keratitis in Dry Eye Disease

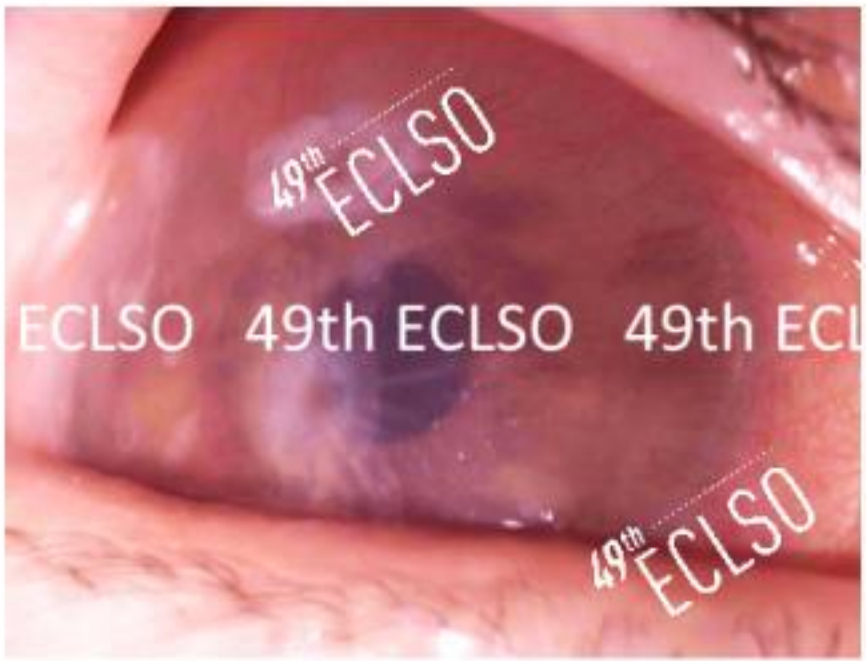
1. Deficiency of MUC16 and/or galectin-3, or of other constituents of the mature glycocalyx
2. Loss of tight junction integrity
3. Desquamating, damaged or dead cells
4. Surface irregularities or defects
5. Increased expression of proteases (MMP9)

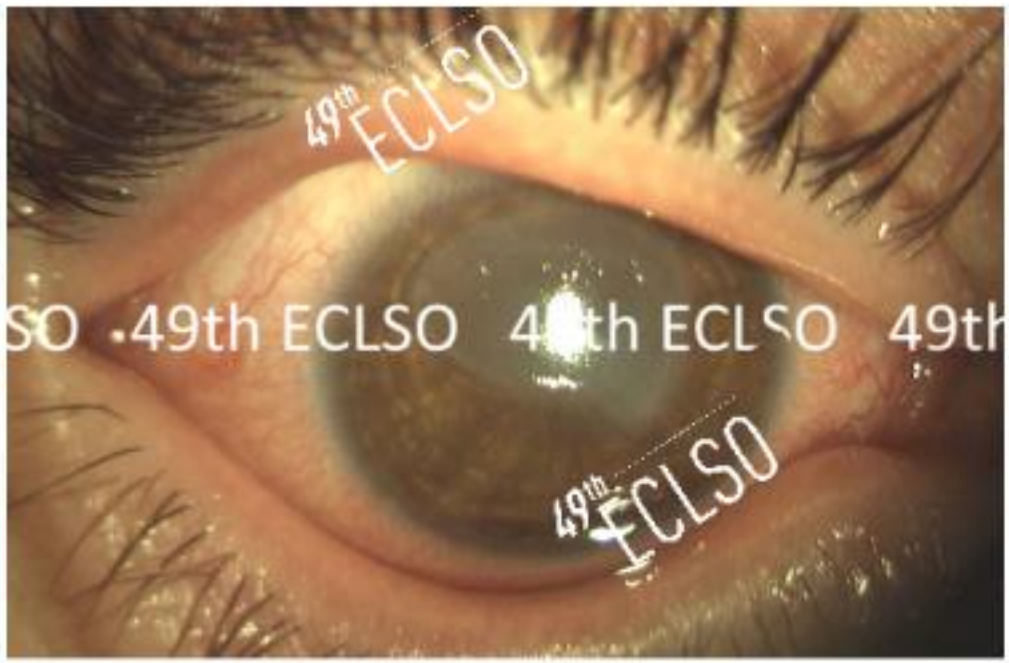


Keratitis in Ocular allergy

1. Loss of epithelial barrier
2. Increased expression of cytokines and chemokines by epithelial cells
3. Cytotoxic effect eosinophil-derived granule proteins (EDGPs)
4. Altered expression of mucins and glycans
5. Increased expression of proteases (MMPs)
6. Lower levels and activity of anti-proteases







Keratitis in Dry Eye Disease (SS)

Keratitis in Ocular allergy (AKC)

Allergy

EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY

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Evidence of epithelial remodeling but not epithelial mesenchymal transition by transcriptome profiling in vernal keratoconjunctivitis

Andrea Leonardi, Philippe Daull, Umberto Rosani, Fabiano Cavarzeran, Elena Salami, Jean-Sebastien Garrigue, Paola Brun ... See fewer authors

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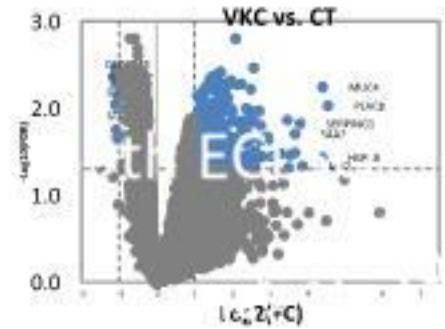
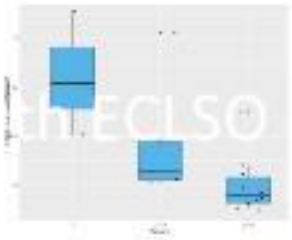


Higher VKC severity = Higher number of DEGs

2

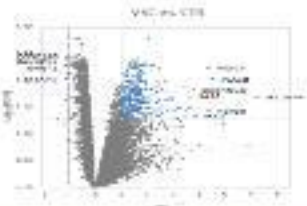
The 3 phenotypes have few DEGs in common

3

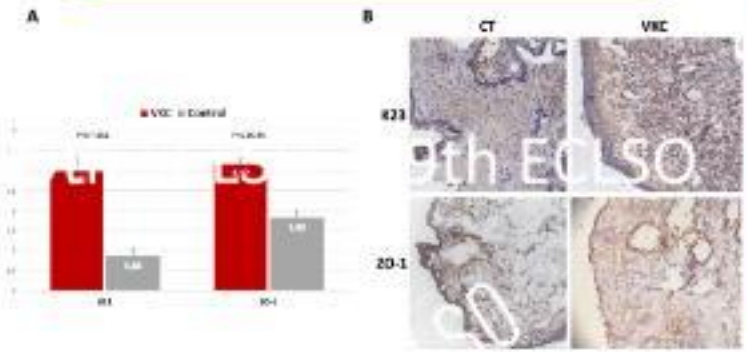


Higher VKC severity = Decreased epithelial function

Epithelial remodeling and EMT in VKC



- The affymetrix analysis tested 21,448 probes
- 325 DEGs: 93 over-expressed and 241 down-regulated



Epithelial barrier dysfunction

Increased expression of:

- Occludin
- Tight junction protein 1
- Cingulin
- Claudin 1
- Cadherins 1, 3, 26, CDHF3
- PAR 6 beta
- Galectin 3
- MUC1, MUC3A, MUC4, MUC16

Decreased expression of:

- Claudin -8, -10, -34
- Cadherin 9
- Catenin alfa, p-120
- PAR-6 alfa

EMT**

Increased expression of:

- MUC5AC
- E-Cadherin
- Amphiregulin
- HIF1A
- Fibronectin type III

Normal expression

- Vimentin
- N-Cadherin
- Fibronectin 1
- Zeb1

Decreased expression of:

- Snail2

Leonardi et al. Allergy 2022

EMT = epithelial mesenchymal transition

How does Allergy become DED?

- **Tear film instability**
- **Altered MUC expression**
- **Drug-induced (anti-H1; anti-M)**
- Secondary blepharitis
- Mechanical

Tear film abnormalities in OA

Suzuki et al. Tear film lipid layer alterations in allergic conjunctiviti… 25(3):277-80.

- Tear film lipid layer alterations in allergic c…
- In SAC vs CT:
  - Increased lipid layer thickness
  - Reduced mean BUT (3.4 +/- 1.5 vs…
  - Negative correlation between lipid…

Reduced BUT in allergic subjects

Chen et al. High incidence of dry eye in young child… …na. Acta Ophthalmol 2016; 94:e727–e730.

- The incidence of dry eye by BUT is higher in ch… …e than CT
- Subjective symptoms of dry eye were inconsistent with objective signs

Altered MUC expression in Ocular Allergy

- Reduction in goblet cell counts related to instability
- Up-regulation of MUCs 1, 2, 4 and 16 in atopic patients compared with CT
- Increased expression of MUCs 1, 3A, galectin 3
- Down-regulation of MUC5AC mRNA in eyes with corneai ulcers compared with CT

Altered expression of membrane-associated mucins (MAMs)

Primary or secondary?

Dogru et al. Allergy 2008; Leonardi et al. Allergy 2022

Drug-induced dryness in Ocular Allergy

Ocular drying associated with oral antihistamines (Lora
Welch et al. *Cornea* 2000;19: (Suppl): S135.

- **Tear flow and volume are decreased as a r**

An evaluation of the ocular drying effects o
cetirizine hydrochloride. Ousler et al. *Ann A*

- **In healthy individuals loratadine and c**
  associated with ocular dryness: increas
  conjunctival staining, and decreased TFt
- **Loratadine induces significantly more conji**

Topical and systemic anti-histamines have an anti-muscarinic activity

How does DED become Allergy?

- Antigen remains longer than usual on the conjunctiva (poor tear clearance)
- Altered epithelial barriers
- Drug-induced allergy

Allergy and Dry Eye:
similarities and differences

	ALLERGY	DRY EYE
Age	young adults	>50
M/F	= (VKC: M>F)	F>M
Prevalence	15-30%	5-35%
Family history	positive	negative
Personal history	positive	negative (autoimmune)
Pathogenesis	IgE-MC / Th2	Epithelial stress Inflammation Th1/ Th17/ autoimmune

Allergy and Dry Eye:
similarities and differences

Clinical	Allergy	Dry Eye
Itching	+++	++
Burning/Foreign body sensation	+	+++
Tearing	++	-
Redness	diffuse	Inter-palpebral
Papillae / follicles	papillae	follicles
Cornea	No in SAC / PAC Only in VKC and AKC	Punctate keratopathy

Allergy and Dry Eye:
similarities and differences

Clinical Test	ALLERGY	DRY EYE
Schirmer	-	++
TF-BUT	+	+++
Staining	SAC/PAC - VKC/AKC +++	+++
Cytology	Eosinophils	Abnormal epithelial cells
IgE	+++	-
Tear Histamine	++	+/-

Ocular Allergy and Dry Eye

- OA more frequent in young subjects, DED in adults
- Different pathophysiologies
- Similar symptoms, different histories
- Overlaps/ coexistence of the two diseases

- Department of Ophthalmology
  - Iva Fregona
  - Daniele Violato
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  - Paola Brun
  - Elena Tarricone
- Department of Biology
  - Paola Venier
  - Umberto Rosani
- Department of Laboratory Medicine
  - Mario Plebani
  - Diego Faggian
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  - Marc Abelson
  - Lisa M Smith
- Institute of Polymers, Composites and Biomatherials, CNR, Catania
  - Domenico Garozzo
  - Angela Messina
- SUNY University, New York, US
  - Robert Sack
- Department of Ocular Biology & Therapeutics (ORBIT), UCL Institute of Ophthalmology, London, UK
  - Virginia Calder
- GE3 Genomics, University of Genève
  - Mylène Docquier
- Department of Human Anatomy & Cell Science University of Manitoba, Winnipeg, Canada
  - Saehid Ghavami
- R&D Santen, Evry, France
  - Philippe Daull
  - Jean-Sébastien Garrigue

Thank you


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