NAVDF 2025

Clinical Investigation and Biomarker Discovery in Early Drug Development for Allergic Dermatitis

Nikki M. Thellman DVM, PhD

Part 2: Biomarkers and Translational Biology in Allergic Dermatitis

Disclosures

- I am a Zoetis Inc. colleague
- Content of this lecture reflects my own personal opinions
- Images created in BioRender.com are noted

Outline

- Clinical sampling techniques
- Biomarkers Allergic Dermatitis
- MicroRNA and Microbiome in Allergic Dermatitis
- Where to next?
- Q & A

Comparative Medicine & Translational Biology Approach to Biomarker Discovery

Human atopic dermatitis (hAD) is a complex disease with multiple clinical phenotypes making treatment challenging:

- moved to integrating biomarker research into the management of allergic dermatitis, with the goal of more effective and personalized treatments: PRECISION MEDICINE

Comparative Medicine

The American Veterinary Medical Association (AVMA) defines comparative medicine as the study of phenomena basic to the diseases of all species. This field is foundational to veterinary medicine, emphasizing the importance of understanding diseases across different species to improve both animal and human health.

Translational Biology

Innovative laboratory research the bridges the gap between scientific discoveries and medical applications. Interdisciplinary approach that combines the expertise of scientists, clinicians, and industry professionals.
Translating fundamental research to practical therapies / interventions.

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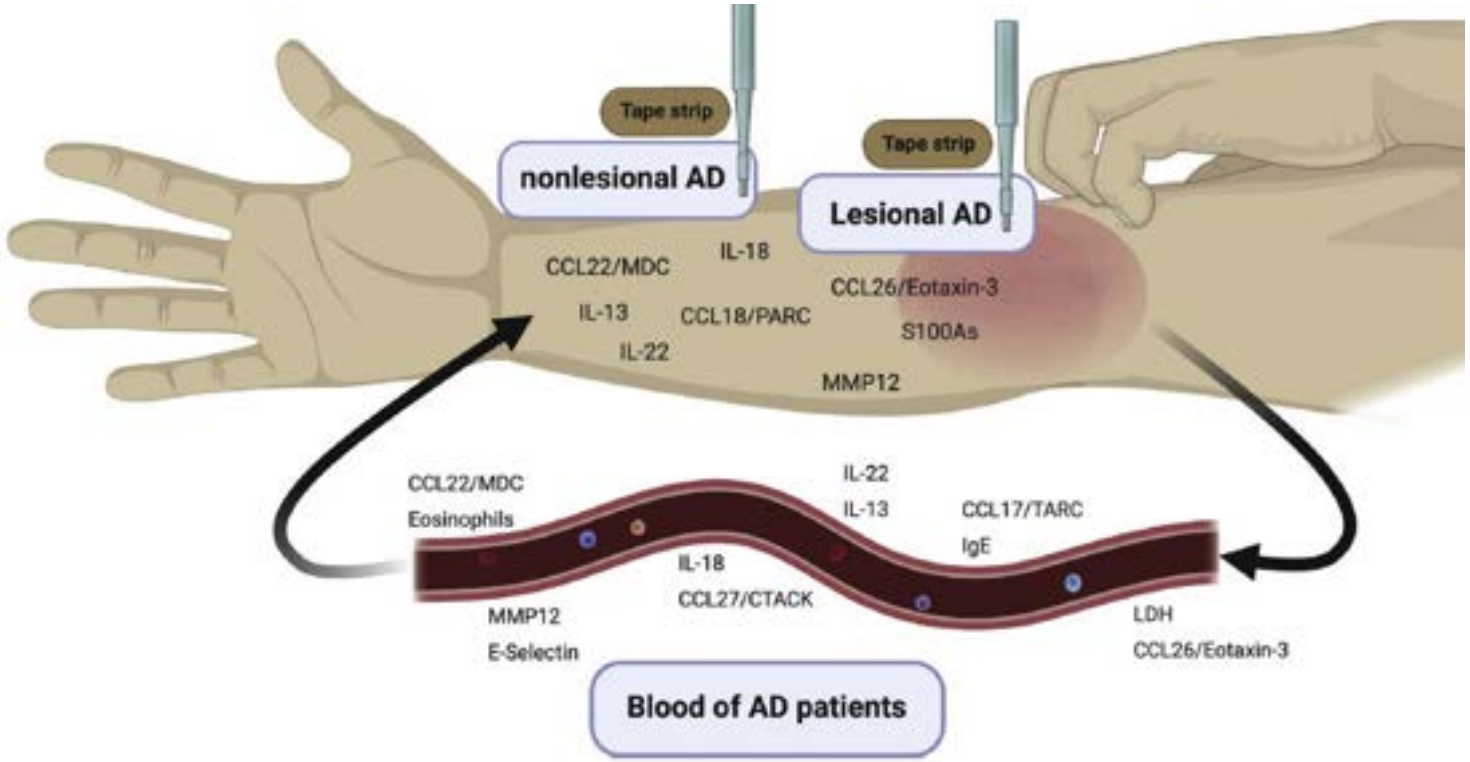
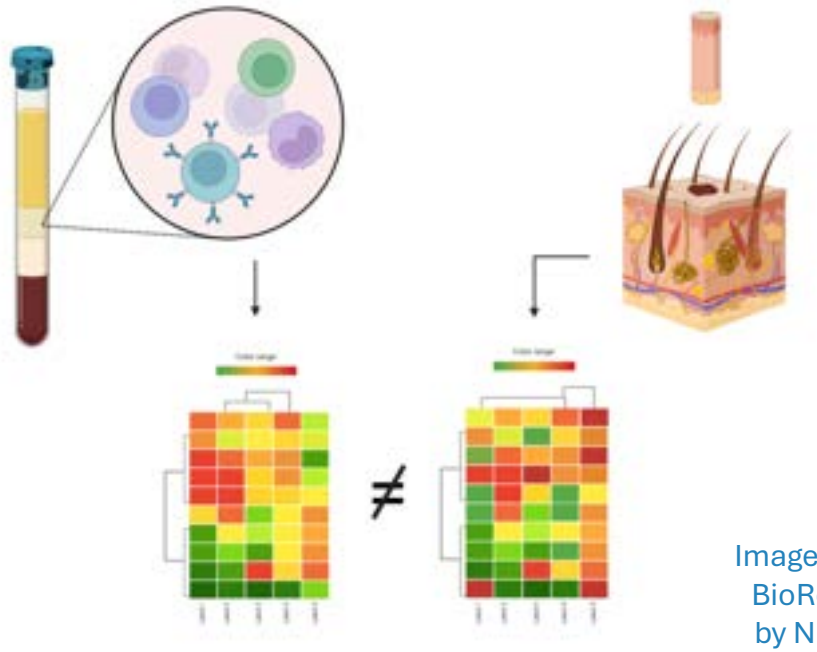
Clinical Sampling Techniques

Clinical Sampling Considerations

The prominent sites for assessing biomarkers in patients with allergic dermatitis are either the **blood** or the **skin:**





Images created in BioRender.com by N. Thellman

- Detection of clinically relevant levels of soluble biomarkers may **not be a robust** enough
- Blood vs. Skin Biopsy have unique transcriptomic signatures

Renert-Yuval Y, et. al. *J Allergy Clin Immunol*. 2021;147(4):1174-1190.

Clinical Sampling Considerations

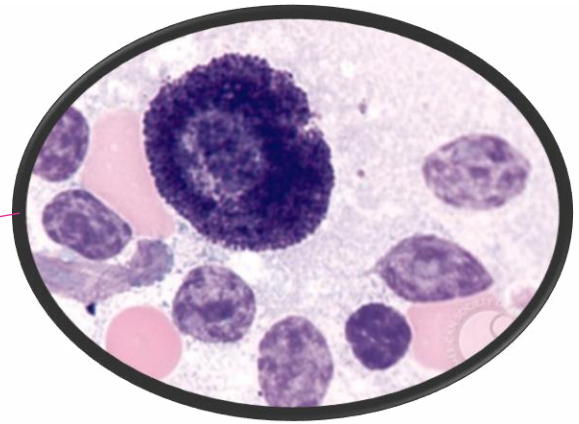
Immunity, Inflammation and Disease

Open Access

ORIGINAL RESEARCH

No difference in human mast cells derived from peanut allergic versus non-allergic subjects

Lau F. Larsen [1], Nanna Juel-Berg[1], Anker Hansen[2], Kirsten S. Hansen[1], E. N. Clare Mills[3], Ronald van Ree[4], Madeleine Rådinger[5], Lars K. Poulsen[1], & Bettina M. Jensen [1]



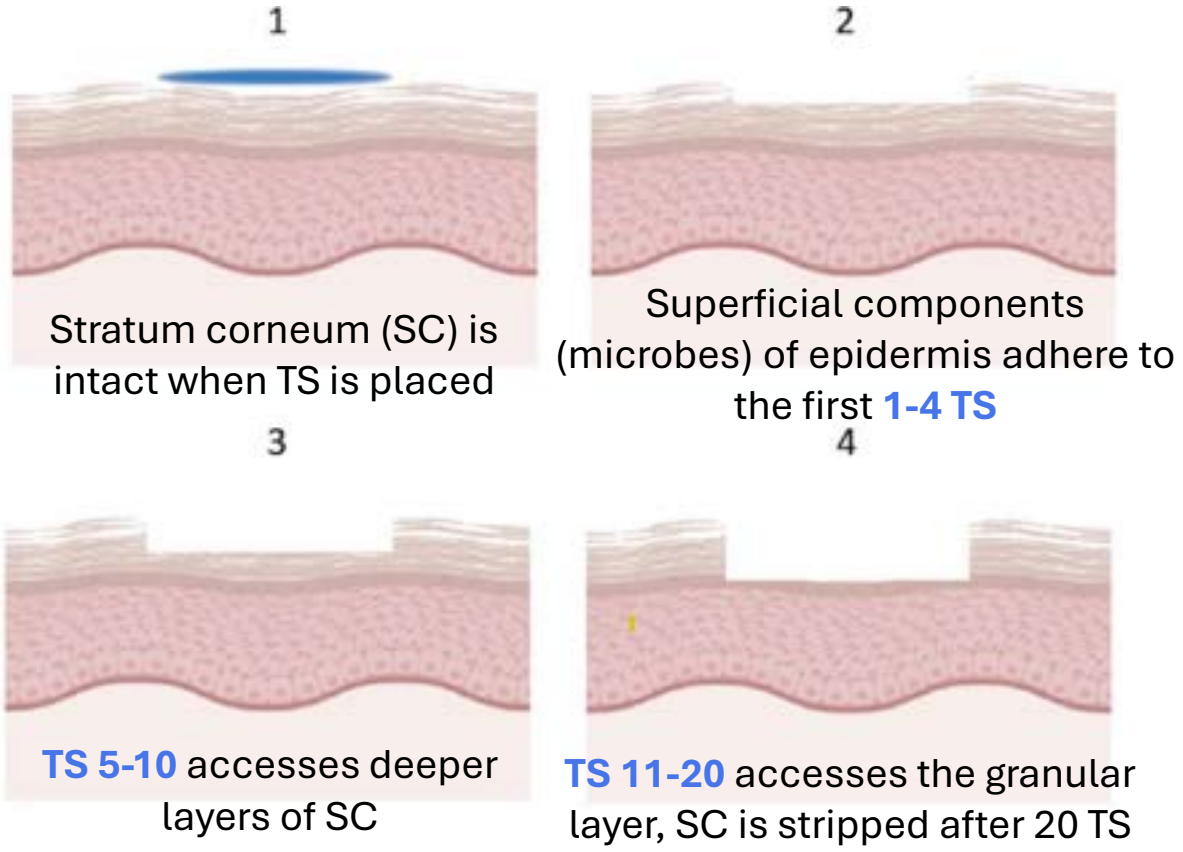
893 Parameters

- Receptor expression
  - CD48, CD88, CD117, C3aR
- Histamine Release
- Secretion of Cytokines / Chemokines
  - IL8, IL10, IL13, IL23, IL37, MCP1, VEGF, GMCSF
- MicroRNA
  - Resting or activated PBdMC

Only IL-31 was differentially expressed!

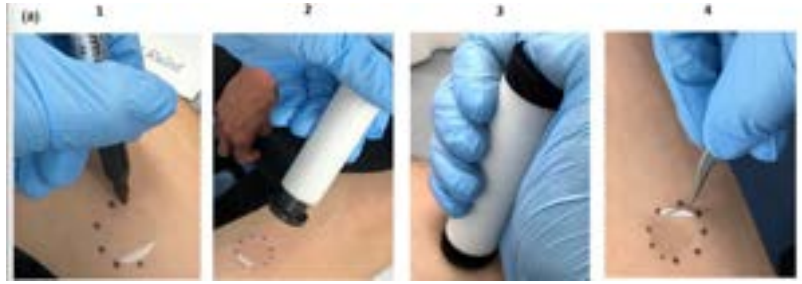
Minimally Invasive Methods for Skin

Minimally invasive methods are being implored to capture molecular profiles of lesional and nonlesional skin



Skin tape strips (TS) and other sampling tools are commonly utilized in human dermatology research as minimally invasive techniques for collecting **epidermal** samples:

- TS can be used as an alternative to skin biopsies in certain circumstances
- TS can be used to determine **protein**, **RNA**, **lipid** and **microbial expression**
- There is currently no standardized protocol used for collecting and processing tape strips



Hughes AJ, et.al. *Br J Dermatol*. 2021 Jul;185(1):26-35.

Minimally Invasive Methods for Skin

Received: 3 July 2021 | Revised: 13 November 2021 | Accepted: 15 November 2021
DOI: 10.1111/cod.14015

ORIGINAL ARTICLE

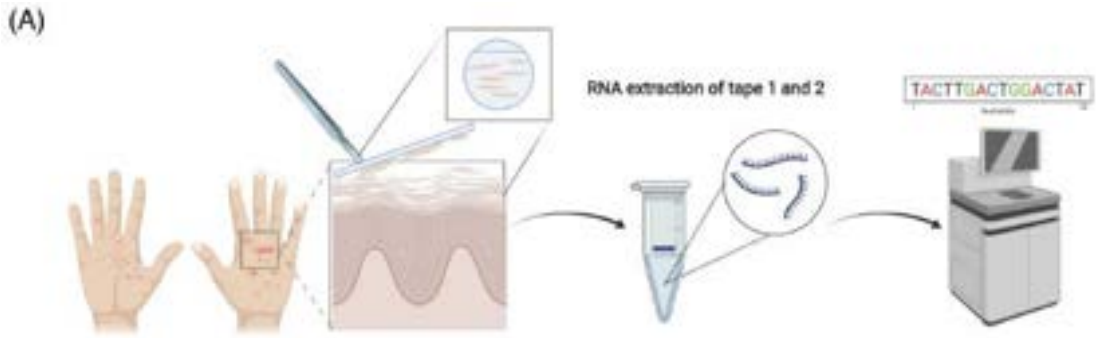
CONTACT DERMATITIS WILEY

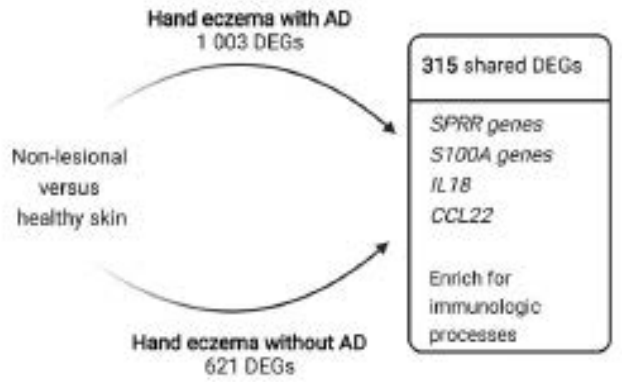
The transcriptome of hand eczema assessed by tape stripping

Julie B. K. Sølberg[1] | Anna S. Quaade[1] | Stine B. Jacobsen[2] | Jeppe D. Andersen[2] | Marie-Louise Kampmann[2] | Niels Morling[2] | Thomas Litman[3] | Jacob P. Thyssen[4] | Jeanne D. Johansen[1]

1. TS samples show the IMMUNOLOGY of lesions on the hands
2. Most prominent difference between HE +AD and HE –AD was at the NON-LESIONAL SITE
3. Transcriptional differences between the dorsum and palm were higher for eczema patients for lesion samples
4. TS samples detect GENE EXPRESSION differences between clinical subtypes of hand eczema:
   - Suggests that AD patients +HE have a larger activation of Mast Cells as well as INF-α
   - 6 genes were differentially expressed between Allergic Contact Dermatitis & Irritant Contact Dermatitis (EPHA1)

30 adults with hand eczema (HE)
- 12 with concurrent atopic dermatitis & 18 without AD
- (RNAseq) transcriptomics performed using TS from lesional, nonlesional, & healthy skin





Minimally Invasive Methods for Skin

Tape strips detect distinct immune and barrier profiles in atopic dermatitis and psoriasis

https://doi.org/10.1016/j.jaci.2020.05.048

Helen He, MD,[a,b]* Robert Bissonnette, MD,[c]* Jianni Wu, BS,[a,b,d]* Aisleen Diaz, MD,[a,e] Etienne Saint-Cyr Proulx, MD,[c] Catherine Maari, MD,[c] Carolyn Jack, MD, PhD,[c] Maudeline Louis, BS,[c] Yeriel Estrada, BS,[a] James G. Krueger, MD, PhD,[b] Ning Zhang, MD, PhD,[a] Ana B. Pavel, PhD,[a] and Emma Guttman-Yassky, MD, PhD[a,b] *New York, NY; Montreal, Quebec, Canada; and Ponce, Puerto Rico*

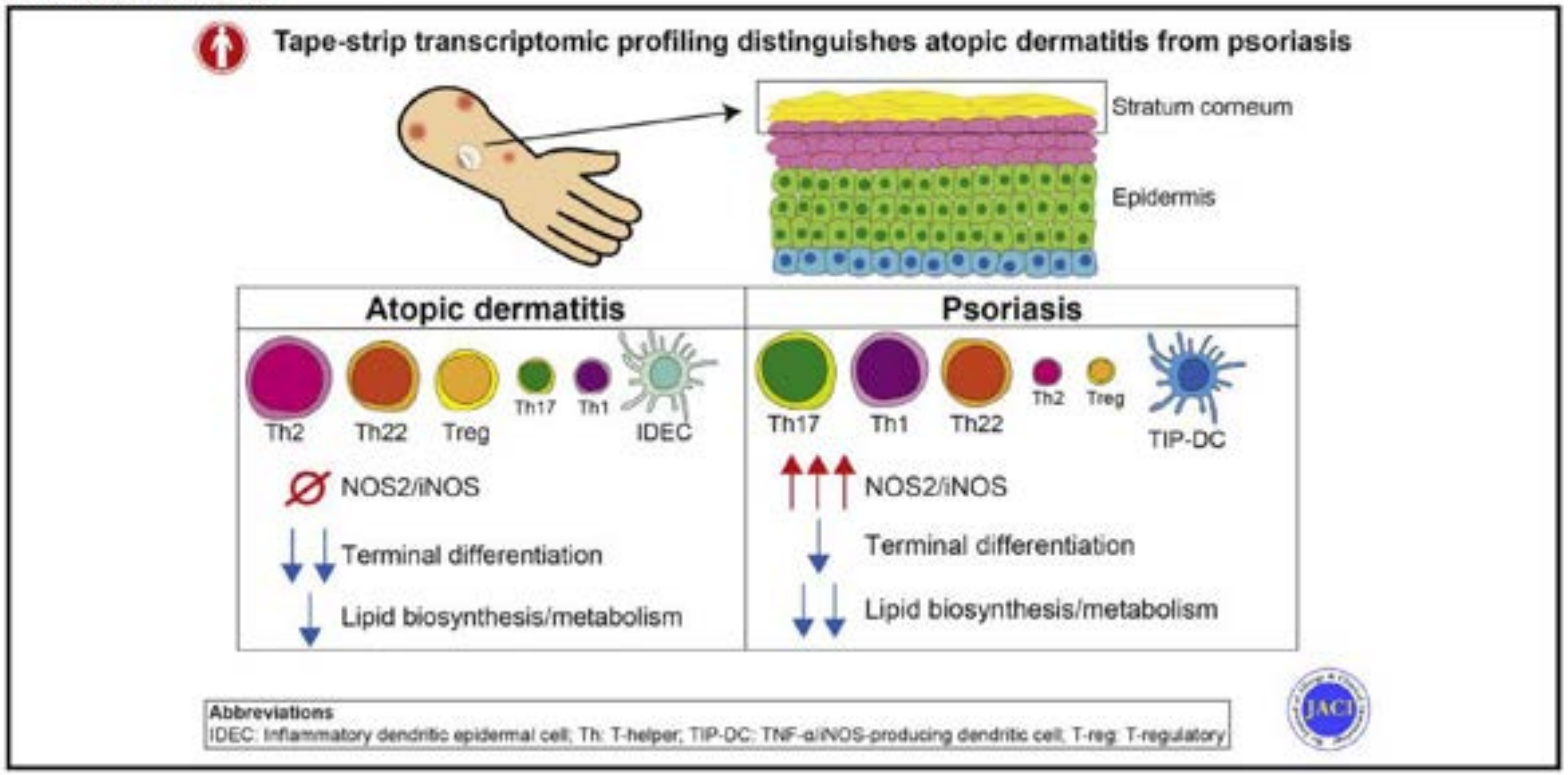
RNAseq transcriptomics and qRT-PCR performed using TS:

- lesional & nonlesional in **AD**, **Psoriasis**, and **healthy** controls (n=20 each)

1. Tape strips successfully defined a cutaneous molecular phenotype of AD and psoriasis aligned with published biopsy data.
2. Demonstrated some biomarkers showed even larger differentiation in diseased vs healthy TS skin than biopsies
   - likely because of increased synthesis in upper epidermis

- CCL17/TARC and FCER1A in **AD**
- CXCL9/10 in **Psoriasis**
- IL-34 in **both**

GRAPHICAL ABSTRACT



Minimally Invasive Methods for Skin

Received: 11 August 2021 | Accepted: 18 December 2021
DOI: 10.1111/srt.13135

ORIGINAL ARTICLE

WILEY

Minimally invasive skin sampling and transcriptome analysis using microneedles for skin type biomarker research

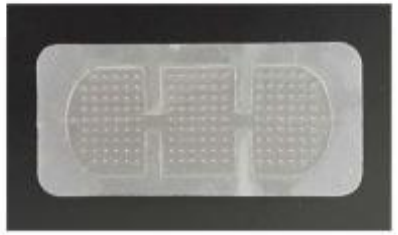
Seo Hyeong Kim[1,†] | Ji Hye Kim[1,†] | Sung Jae Lee[1] | Min Sook Jung[1] | Do Hyeon Jeong[2] | Kwang Hoon Lee[1,3]

33 subjects with **different skin conditions** (skin aging, skin hydration, skin pigmentation, oily skin & sensitive skin)

- Skin samples from microneedles on face
- Total RNA extracted for microarray for gene expression

1. Confirmed that the sodium hyaluronate-based microneedle can be used as a skin sampling method for transcriptomic biomarker analysis.
2. Example: various RNA biomarkers related to age (Figure D & E).
3. Next steps: skin type-related biomarkers in subjects who have symptoms of skin diseases.

A



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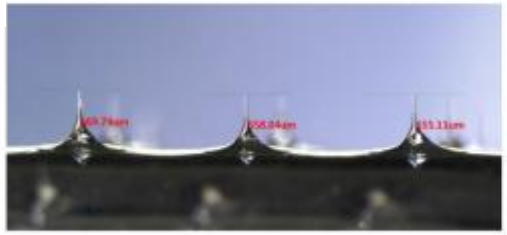

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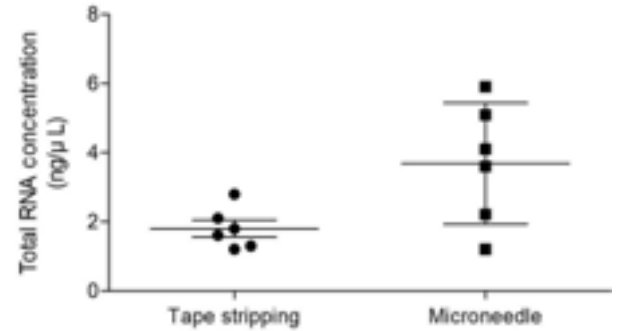
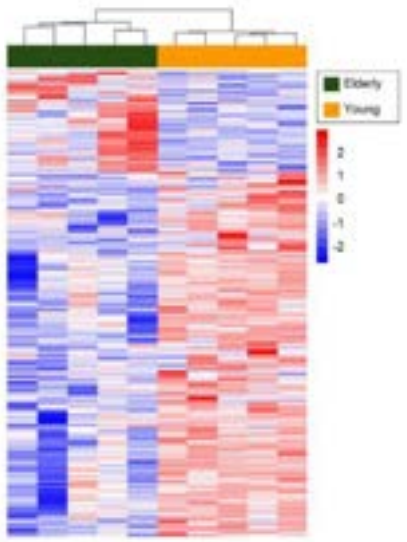


D

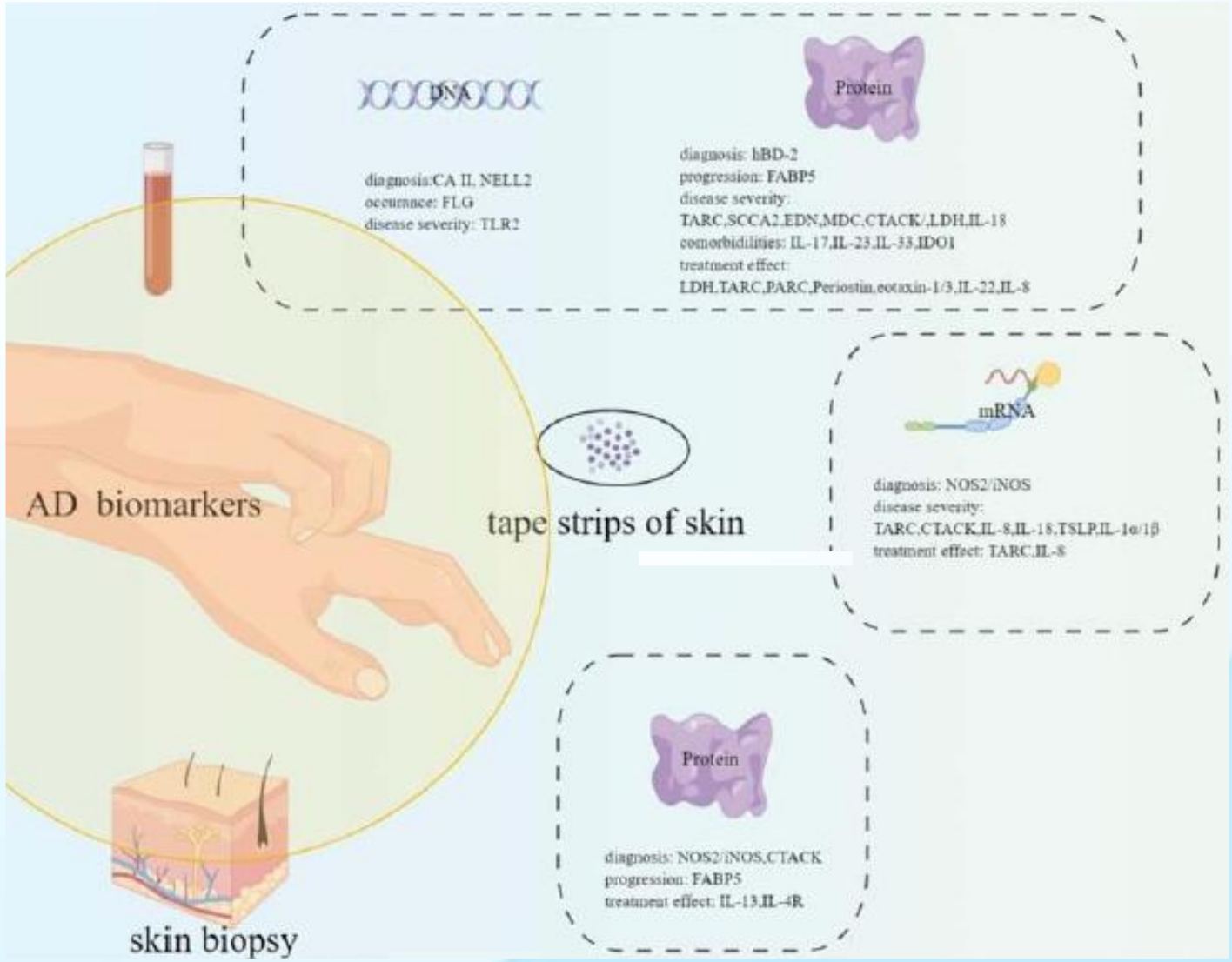
	Elderly	Young
Number (N)	5	5
Sex		
Male	0	2
Female	5	3
Age (yr)	60.80 ± 5.72	24.20 ± 1.15
PRIMOS (Ra)	32.10 ± 3.69	18.24 ± 3.23
Cutometer (R2)	0.61 ± 0.06	0.76 ± 0.02

E



Biomarkers in Allergic Dermatitis

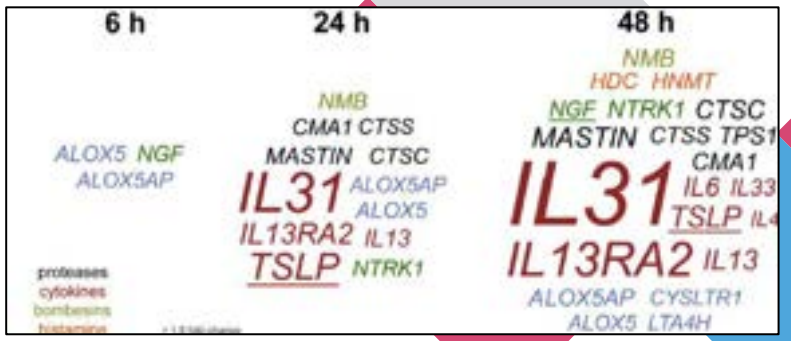
NAVDF 2025



Yu L and Li L (2022) Potential biomarkers of atopic dermatitis. *Front. Med.* 9:1028694.

Interleukin IL-31 “The Itch Cytokine”

IL-31 is a cytokine known to cause severe itch in humans, dogs, and other animal species

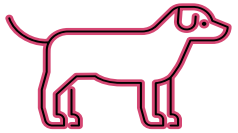


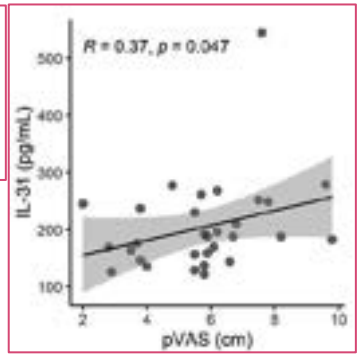
Olivry, T. et.al. *J Invest Derm* Vol. 136 (2016)
Expression of selected pruritogenic genes after house dust mite (HDM) challenge with significantly greater than 1.5-fold change in gene expression in HDM laboratory model.

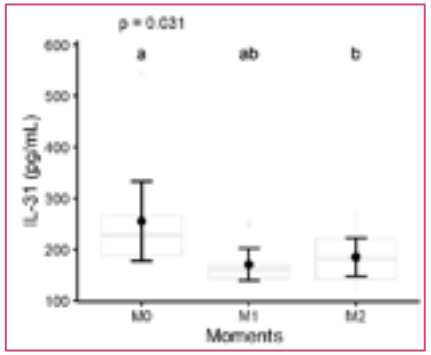
Canine:

- ⇧ gene expression in lesioned **skin** (models & spontaneous disease)
- Mod ⇧ **serum** levels correlation to disease severity (pruritus) & ⇩ levels w/ treatment from Lokivetmab
- but serum levels ✓ aren’t always detectable in AD dogs → **57% dogs (**Gonzales et.al 2013)

Calesso, J.R., et.al. Polish J Vet Sci Vol. 26, No. 2 (2023)
IL-31 detected in all dogs with mean value of 260 pg/mL







Interleukin IL-31 "The Itch Cytokine"

IL-31 is a cytokine known to cause severe itch in humans, dogs, and other animal species

Feline:

- **serum** IL-31 ✓ detected in a larger number of cats with allergic dermatitis than healthy cats
- concentrations *trend* to be ⇧ in allergic cats
- but ✓ not all cats have detectable levels

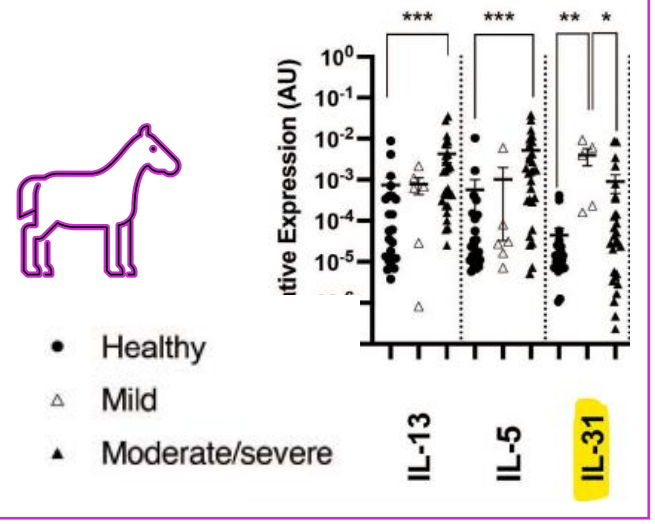
Equine:

- ⇧ gene expression in **skin** from horses with insect bite hypersensitivity (IBH) in mild to moderate/severe cases only
  - IL-31 receptor A (IL31RA) ⇧ was one of the top differentially expressed genes correlating with IBH severity.

Table 3. Cytokine and chemokine protein concentrations in feline serum samples

Cytokine	Healthy (n = 17)	Allergic dermatitis (n = 18)	Asthma (n = 18)
sFAS	129.55 ± 161.70 (94%)	138.37 ± 240.94 (88%)	130.18 ± 162.34 (72%)
Flt-3 ligand	150.02 ± 76.93 (94%)	168.97 ± 90.27 (100%)	155.07 ± 60.71 (100%)
GM-CSF	31.19 ± 40.20 (59%)	28.84 ± 50.32 (61%)	24.66 ± 31.89 (56%)
IFN-γ	575.70 ± 723.76 (94%)	416.63 ± 872.03 (94%)	452.29 ± 780.99 (100%)
IL-1β	136.47 ± 143.47 (88%)	85.74 ± 140.26 (67%)	90.61 ± 129.04 (100%)
IL-2	86.55 ± 95.23 (88%)	65.84 ± 128.76 (72%)	80.45 ± 115.95 (67%)
IL-4	2,727.49 ± 3288.60 (94%)	2,005.09 ± 3961.02 (94%)	2,065.38 ± 3509.26 (100%)
IL-6	1,052.31 ± 1232.64 (94%)	708.60 ± 1300.65 (94%)	697.59 ± 1113.55 (88%)
IL-8	96.01 ± 78.50 (100%)	71.75 ± 88.69 (100%)	74.19 ± 81.21 (100%)
IL-12 p40	433.30 ± 296.19 (100%)	581.99 ± 627.33 (100%)	400.45 ± 206.74 (100%)
IL-13	66.48 ± 58.374 (94%)	60.92 ± 68.40 (100%)	60.57 ± 54.06 (100%)
IL-18	1,609.11 ± 1642.23 (94%)	1,127.64 ± 1819.76 (88%)	969.81 ± 1249.90 (100%)
IL-31	0.73 ± 1.95 (20%)	1.50 ± 3.14 (56%)	21.57 ± 84.01 (22%)
KC	16.61 ± 26.06 (82%)	36.91 ± 110.35 (83%)	38.80 ± 115.54 (94%)
MCP-1	6,890.39 ± 5640.70 (100%)	5,109.12 ± 5782.25 (78%)	5,321.13 ± 5603.20 (83%)
PDGF-BB	1,560.81 ± 2558.87 (94%)	843.81 ± 1368.12 (78%)	630.08 ± 510.57 (83%)
RANTES	94.90 ± 71.49 (100%)	104.05 ± 74.47 (100%)	79.12 ± 65.87 (100%)
SCF	338.53 ± 258.43 (100%)	277.15 ± 295.54 (100%)	318.60 ± 262.67 (100%)
SDF-1	3,459.99 ± 1685.02 (100%)	2,797.61 ± 1516.32 (100%)	2,909.02 ± 1213.19 (100%)
TNF-α	488.48 ± 644.46 (53%)	307.26 ± 687.22 (50%)	318.51 ± 596.99 (61%)

Older, C.E. et.al. *Vet Derm* Vol. 32, (2021)
No cytokines had significantly different levels between the healthy & allergic cats, but notable differences in the number of samples where IL31 could be measured (ultrasensitive assay)



Jebbawi,F., et.al. *Front. Immunol* Vol 15 (2024)
Gene expression of IL-31 from skin biopsies increased in Mild to Mod/Severe IBH

Skin Barrier & TARC (CCL17)

Renert-Yuval Y, et. al. *J Allergy Clin Immunol*. 2021;147(4).

Many of the potential biomarkers assessed are familiar cytokines, chemokines, and skin barrier molecules that play key roles in the **interactions of keratinocytes** with either the nervous system and/or immune cell populations.

Grading of Recommendations, Assessment, Development, and Evaluation(GRADE):

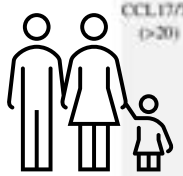
- Evaluated the strength of data for each potential biomarker across the spectrum of pediatric and adult cases, both at baseline and during topical and systemic treatments

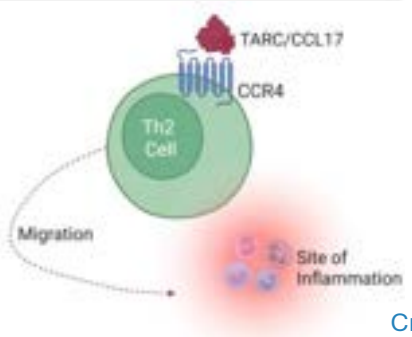
The molecule with the greatest evidence as a biomarker in human AD is thymus and activation-regulated chemokine (TARC) with a score of >20 publications.

- **Note, it may not be specific enough for a diagnostic.**
- **Strongly correlates with clinical severity and therefore monitor biomarker potential**

TABLE III. Potential biomarkers reported to strongly and significantly correlate with clinical severity indices of AD (correlation coefficient ≥0.4, $P < .05$)

Biomarker (no. of publications)	Serum: Author	Lab method	Corr method	Year	Cohort (n)	Skin: Author	Lab method	Corr method	Year	Cohort (n)
CCL17/TARC (>20)	Kakinuma et al[20]	E	S	2001	40	Morita et al[21]* (LS-TS)	IF	S	2010	33
	Horikawa et al[22]	E	P	2002	52	McAleer et al[23]† (NL-TS)	ECL	S	2019	66
	Fujisawa et al[24]	E	S	2002	29	He et al[25] (LS-B)	PCR	P	2020	61
	Leung et al[26]†	E	S	2003	20					
	Hijnen et al[27]‡	E	S	2004	177					
	Jahnz-Rozyk et al[28]	E	P	2005	43					
	Song et al[29]†	E	S	2006	157					
	Nakazato et al[30]†	E	S	2008	34					
	Fujisawa et al[31]†	E	S	2009	27					
	van Velsen et al[32]†	E	P/S	2010	60					
	Morita et al[21]	E	S	2010	33					
	Kou et al[33]	E	S	2012	121					
	Machura et al[34]†	E	S	2012	26					
	Furue et al[35]§	E	S	2012	61					
	Mizawa et al[14]	NA	S	2013	30					
	Kataoka[36]§	NA	NA	2014	96					
	Landheer et al[37]‡	E	S	2014	320					
	Ahrens et al[38]†	E	S	2015	128					
	Gu et al[39]	E	S	2015	73					
	Hulshof et al[40]†	L	S	2018	41					





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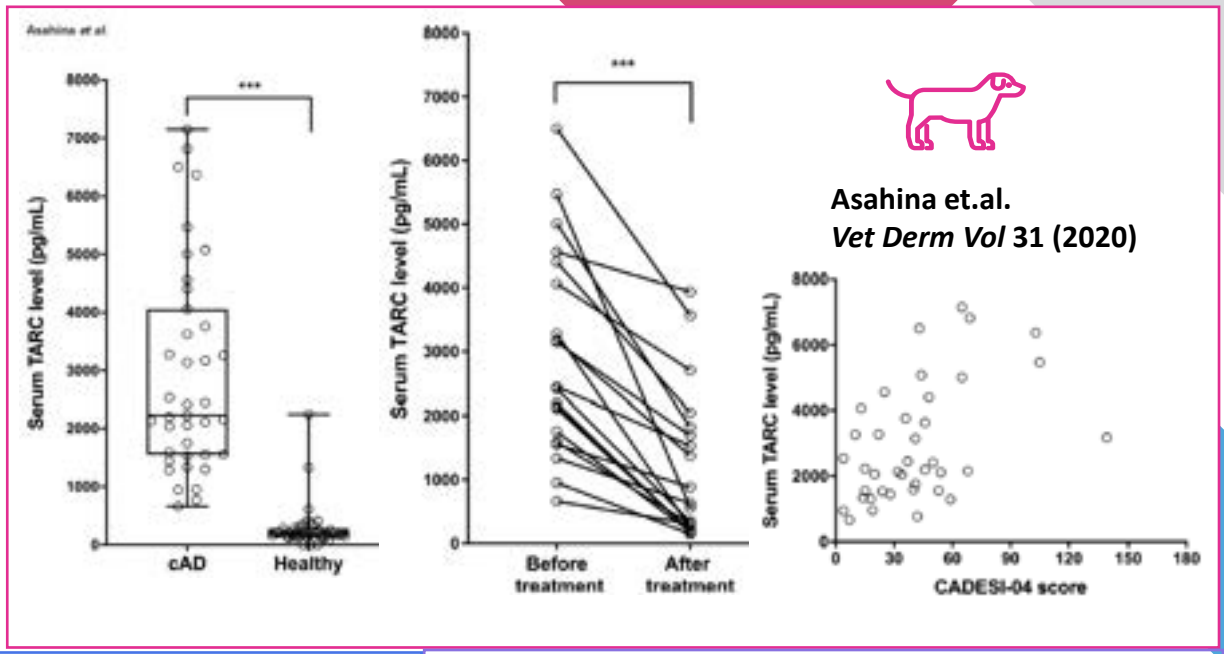
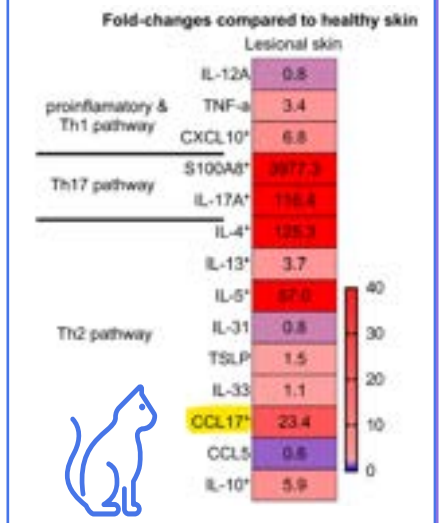
- TARC is a chemokine primarily associated with attracting Th2 cells
- Believed to play a significant role in allergic diseases like atopic dermatitis and asthma by recruiting immune cells to the site of inflammation
- TARC binds CCR4 receptors on Th2 to signal their migration and exacerbates inflammation that can impair skin barrier by affecting key proteins like filaggrin which maintain barrier integrity

TARC (CCL17)

- TARC has strong correlation with human AD clinical severity at baseline and during therapy. **Serum** TARC concentrations were significantly (>10-fold) ⇧ in dogs with canine AD as compared to healthy controls & **serum** TARC correlated with CADESI-04 severity in dogs.
- **Serum** TARC has been evaluated as a monitoring biomarker for response to JAK inhibitors in both human and canine AD with promising results
- Several studies show TARC ⇧ serum and/or tissues in various human <u>eosinophilic</u> conditions
- In a small study, **transcriptomics** from **biopsies** of eosinophilic plaques from four cats with feline atopic skin syndrome (FASS) showed ⇧ TARC, when compared to healthy tissue control samples.
- In horses with IBH however, TARC was not significantly differentially expressed in lesion samples compared to healthy horse **skin**, however other Th2 cytokines and chemokines are



Asahina et.al.
***Vet Derm Vol* 31 (2020)**



Vargo et.al. *Vet Derm Vol* 34 (2023) median fold changes of qRT-PCR in eosinophilic plaque biopsies vs healthy

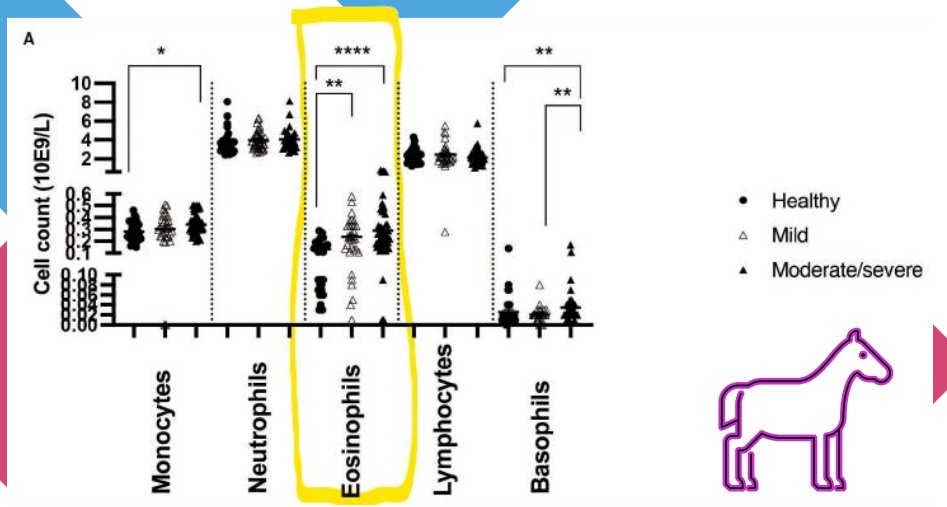
Genes/comparison	(A) IBH vs healthy	(B) IBH vs healthy (lesion mean score)		
		Mild vs healthy	Moderate/severe vs healthy	Moderate/severe vs. mild
IL-4	115.01	0.18	86.32	460.25
IL-5	7.58	1.79	9.03	5.04
IL-13	5.55	1.04	5.84	5.60
IFN-γ	12.82	0.04	15.96	368.69
IL-12b	17.74	0.5	22.02	43.89
IL-10	23.66	1.45	29.25	20.16
IL-31	29.45	88.88	19.89	0.22
IL-31Ra	3.42	0.03	4.24	134.79
IL-5Ra	29.68	0.23	38.94	162.92

IL4, IL5, IL13, IFN-γ, IL-12b, IL-10, IL-31, IL31Ra, IL-5Ra

Jebbawi,F., et.al. *Front. Immunol* Vol 15 (2024) mRNA fold-change in cytokines / chemokines associated with T-cells in IBH vs healthy & with increasing severity by lesion mean score

Other Biomarkers with Strong Evidence for Correlation with Disease Severity in Human AD

1. General Inflammation Markers:
   - Serum lactate dehydrogenase (**LDH**)
   - C-reactive protein (**CRP**)
2. Allergy-Related Markers:
   - peripheral **eosinophil** counts
3. Th2-Related Cytokines and Chemokines:
   - **IL-13**
   - CCL26/eosinophil-attracting chemokine (**eotaxin-3**)
   - CCL27/cutaneous T-cell–attracting chemokine (**CTACK**)
   - CCL18/pulmonary and activation-regulated chemokine (**PARC**)
   - CCL22/macrophage-derived chemokine (**MDC**)
4. Th22-Related Cytokine:
   - **IL-22**
5. Periostin (treatment effects)



Jebbawi,F., et.al. *Front. Immunol* Vol 15 (2024)
Differential blood counts from healthy and IBH horses in relation to disease severity.

Top 5 Causes of Eosinophilia in Cats

- Internal & External Parasitism
- **Feline Allergic Dermatitis**
- **Feline Asthma**
- **Eosinophilic Gastrointestinal Disease**
- Hypereosinophilic Syndrome, Neoplasia, & Paraneoplastic Syndrome

Monitoring Biomarkers
(Correlate with Disease Severity)

Diagnostics & Predictive Biomarkers

- Diagnosis of AD is based on clinical criteria involving combinations of clinical signs & history
- **Continued molecular characterization and correlation to clinical disease are critical first steps in identifying novel biomarkers**
- Still early in its research stages in human health, are efforts to distinguish between endotypes of hAD → → → **Precision Medicine**

Common Signatures Across Species
Prominent Th2 Signatures: IL-4, IL-5, IL-13 etc.

Equine Insect Bite Hypersensitivity:
Eosinophilic chemokines & receptors:
- IP10 (CXCL10),CCL11, CCL26
- IL-5RA, CCR5

Feline Atopic Skin Syndrome:
Th17 Signatures:
- S100A8
- IL17A
- IP10 (CXCL10)

Atopic Dermatitis

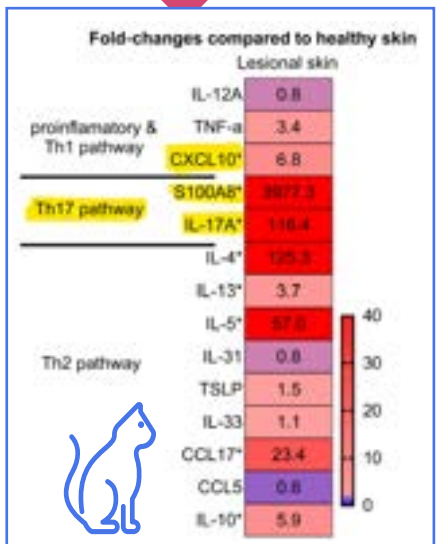
- **SKIN**: Matrix Metalloproteinases (MMP8/9)

Psoriasis

- **SKIN**: Nitric Oxide Synthase (NOS2/iNOS)
- **SKIN** & **BLOOD**: Beta-defensin 2 (hBD2)

Parameter		(A) IBH vs healthy
Functionnal group	Genes/comparison	
Type I allergy	IGHE	3.03
Inflammation	CXCL8	6982.98
	IL-1α	14.14
	IL-1β	33.32
	CCL2	212.57
	TNF	39.41
Eosinophils	CXCL10	16
	CCR3	3.83
	CCL11	8.81
	CCL24	11.33
	CCL26	34.78
T cells	IL-4	115.01
	IL-5	7.58
	IL-13	5.55
	IFN-γ	12.82
	IL-12b	17.74
	IL-10	23.66
	IL-31	29.45
	IL-31Ra	3.42
	IL-5Ra	29.68
Histamine receptors	HRH2	15.18
	HRH4	44.71
Adaptive and innate immune cells	CCR5	78.03
	IL-33	17.59
Epithelial cells	TSLP	12.28

Jebbawi,F., et.al. *Front. Immunol* Vol 15 (2024) mRNA fold-change in cytokines / chemokines associated with T-cells in IBH vs healthy & with increasing severity by lesion mean score



Vargo et.al. *Vet Derm Vol* 34 (2023) median fold changes of qRT-PCR in eosinophilic plaque biopsies vs healthy

Susceptibility & Prognostic Biomarkers

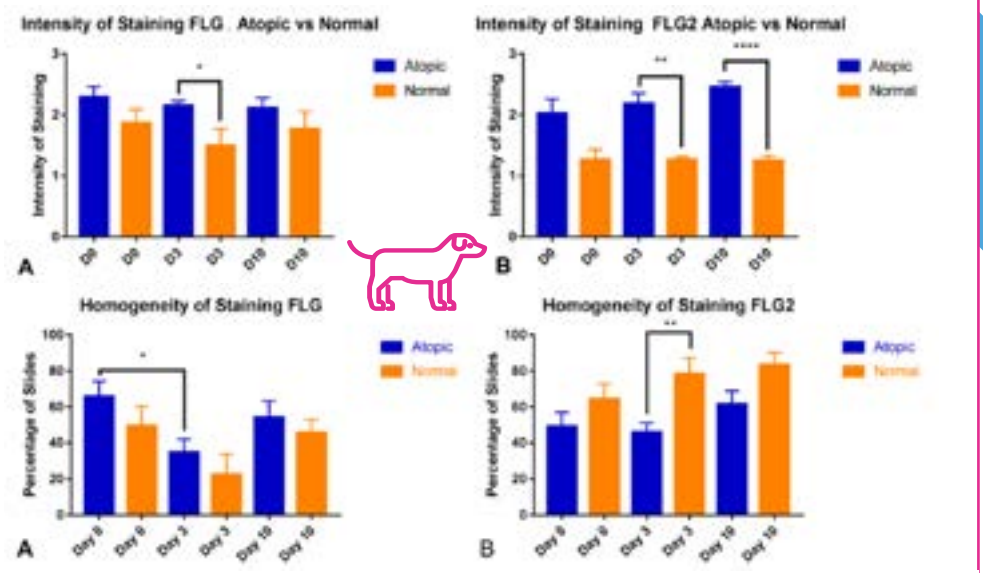
Filaggrin type proteins are crucial for epidermal barrier integrity; genetic variants in filaggrin affect the terminal differentiation of keratinocytes and therefore **impair the epithelial barrier**, making it more permeable for different allergens.

Susceptibility (risk): Clinical disease not present

- Loss-of-function variants in the **filaggrin** gene are significant predisposing factors for developing hAD

Prognostic: Clinical disease present

- **filaggrin** gene mutations correlate with the severity and early onset of persistent hAD into adulthood



Marsella, et.al. *Animals* Vol 14 (2024)
In laboratory beagles sensitization to house dust mite (HDM), serial biopsies were investigated for Filiggrin protein and gene expression compared to normal dogs when exposed to HDM.

Filaggrin status in Insect Bite Hypersensitivity in Horses

No differential expression in keratinocyte terminal differentiation proteins like filaggrin in SKIN	Altered Phospholipid profile in SERUM and profiles change according to clinical status	Sphingomyelin appears to have an active role during equine IBH disease

Very limited knowledge of the skin barrier in feline patients exists. No data on potential filaggrin and lipid abnormalities in the skin of allergic cats.

NAVDF 2025

Advancements in Molecular Biology

MicroRNA & Allergic Dermatitis

MicroRNAs regulate gene expression through mRNA degradation or translational repression through specific binding

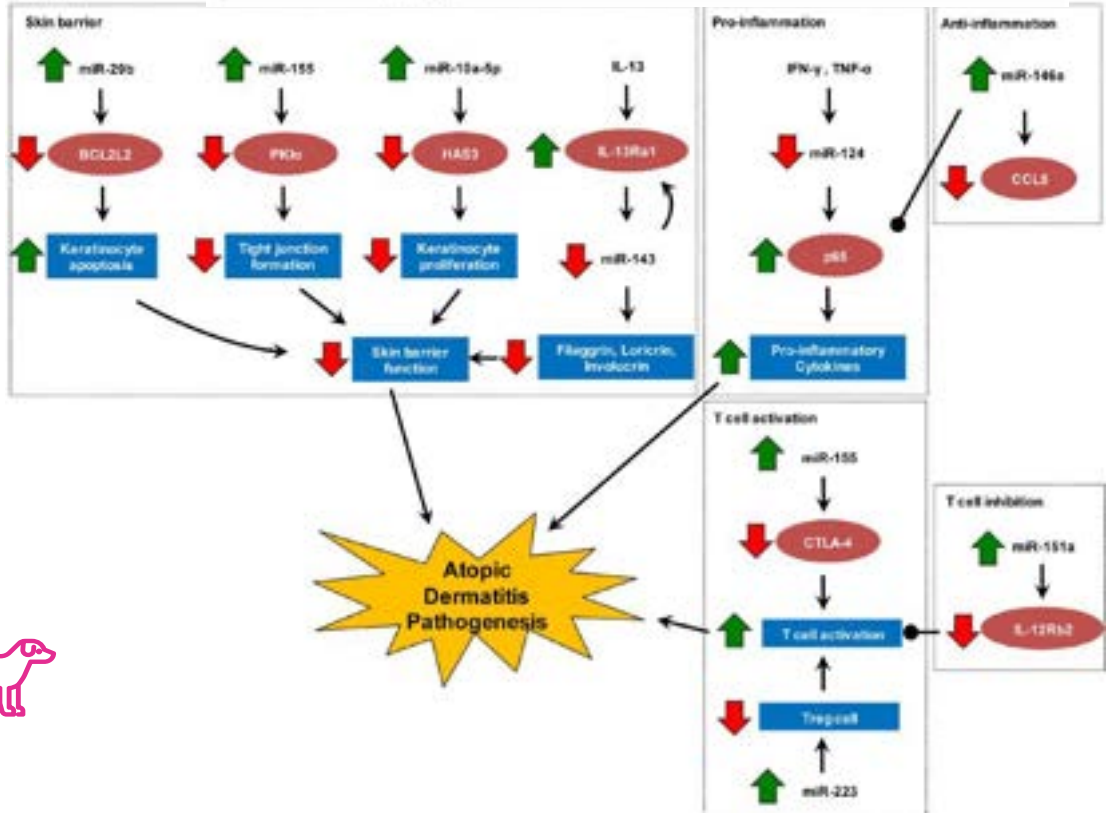
miRNAs	Mechanism of action	Target cells	Target mRNA
miR-10a-5p	Inhibition of keratinocyte proliferation	Epidermal keratinocytes	HAS3
miR-29b	Promotion of INF-γ-induced keratinocyte apoptosis	Epidermal keratinocytes	BCL2L2
miR-124	Inhibition of inflammatory responses	Epidermal keratinocytes	RELA (p65 subunit of NF-κB)
miR-143	Suppression of IL-13-induced dysregulation of skin barrier proteins	Epidermal keratinocytes	IL-13Rα1
miR-146a	Suppressing the expression of many pro-inflammatory factors	Epidermal keratinocytes	IRAK1, CARD10, CCL5
miR-151a	Inhibition of IL-12 signalling	T helper cells	IL12RB2
miR-155	Promotion of Th17 differentiation	T cells	CTLA-4
	Inhibition of tight junction formation	Epidermal keratinocytes	PKIα
miR-223	Positive correlation with Treg cell number	Not specified	Not specified

Several miRNAs have been found to be implicated in the crosstalk between inflammatory cells and keratinocytes in human patients affected by atopic dermatitis

Yu, et.al. *J Cell Mol Med*. Vol 24 (2020). Systematic review sought to summarize our current understanding regarding the role of microRNAs in AD development [73 PubMed items, 117 items from Web of Science found with 25 original studies investigating miRNAs in AD included.

- miR-155 most significantly upregulated in lesioned SKIN
- Needed: mechanistic insight into the roles of miRNAs across species
- Like cytokines / chemokines miRNA profiles or “signatures” could be used as biomarkers.
- They are easily detectable in body fluids and more stable than mRNA
- **Increased expression of miR-142, miR-146a, miR-155, and miR-21 was detected in the lesional skin of Labrador and Golden Retrievers with AD compared to healthy controls**

Morlang et.al. *Vet Dermatol* Vol 32 (2021)

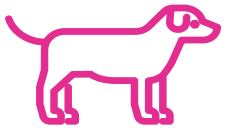


MicroRNA & Allergic Dermatitis

Vet Dermatol 2021; **32**: 331–e92 DOI: 10.1111/vde.12971

Cutaneous microRNA expression in healthy Labrador and Golden retrievers and retrievers with allergic and inflammatory skin diseases

Marie Isabel Morlang*·[1], Karin Weber*, Wolf vonBomhardt and Ralf S. Mueller*



Cutaneous expression of 18 target miRNAs were investigated in healthy, allergic & nonallergic inflamed skin (FFPE) samples from Labrador and Golden Retrievers:

Table 2. Significantly up- and downregulated micro (mi)RNAs of allergic and nonallergic dermatitis specimens compared to healthy skin

Allergic dermatitis versus healthy			Nonallergic skin disease versus healthy		
miRNA	Fold-change	*P*-value	miRNA	Fold-change	*P*-value
Upregulated miR-142	3.40	0.0116	miR-142	7.36	0.0012
miR-146a	2.51	0.0002	miR-146a	4.08	0.0004
miR-155	2.07	0.0049	miR-155	3.44	0.0052
miR-21	4.42	0.0013	miR-21	9.08	0.0000
			miR-18b	2.64	0.0081
			miR-223	4.38	0.0095
			miR-409	2.15	0.0129
Downregulated					
			miR-193b	0.43	0.0142

- Allergic and Non-allergic showed similar expression patterns
- Unable to differentiate allergic dermatitis from other inflammatory diseases
- Further work is needed to understand miRNA signatures to predict disease phenotypes and utility as Biomarkers

Table 1. Patient data of retrievers included in the study

	Case	Breed	Age (years)	Sex	Skin disease
Healthy	Healthy dogs				
	C1	GR	7	M	None
	C2	GR	10	M	None
	C3	LR	10	MC	None
	C4	GR	7	FS	None
	C5	LR	3	FS	None
	C6	LR	8	MC	None
	C7	GR	4	M	None
Allergic	Allergic dogs				
	A1	GR	7	M	Cutaneous allergies
	A2	GR	10	M	Cutaneous allergies
	A3	GR	6	M	Cutaneous allergies
	A4	GR	8	MC	Cutaneous allergies
	A5	LR	6	M	Cutaneous allergies
	A6	LR	6	MC	Cutaneous allergies
	A7	LR	4	M	Cutaneous allergies
Other Skin	Dogs with nonallergic skin diseases				
	I1	LR	3	M	Mycosis
	I2	LR	9	M	Mycosis
	I3	LR	10	M	Demodicosis
	I4	LR	5	F	Mast cell tumour
	I5	GR	10	F	Mast cell tumour

LR, Labrador retriever; GR, golden retriever; M, male; F, female; MC, male castrated; FS, female spayed

*MCT had to be excluded

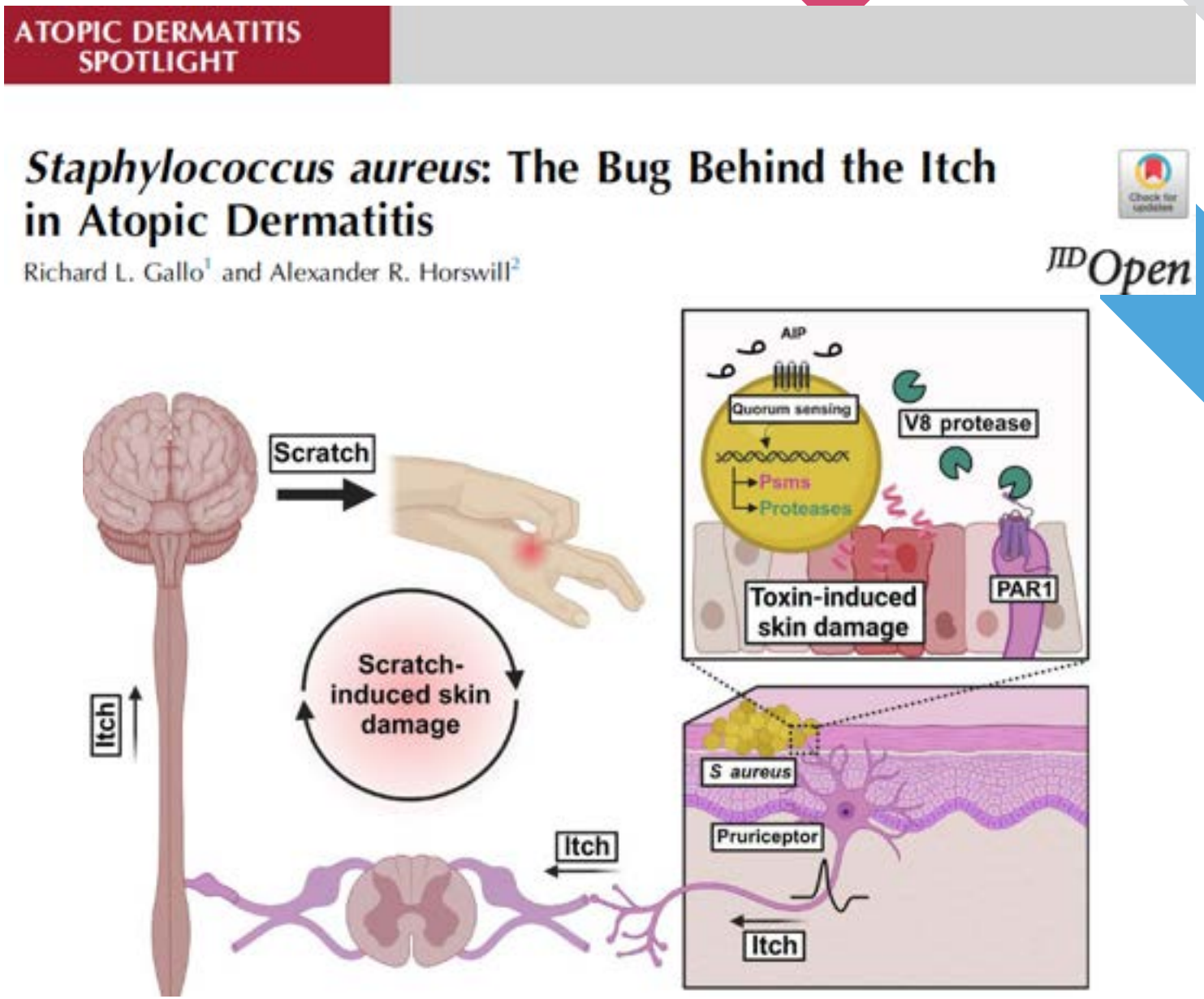
miR-21

- One of the first mammalian miRNAs identified, highly conserved
- Most frequently upregulated in solid tumors (B cell lymphomas, etc.)
- Circulating biomarker of various Carcinomas
- Possible role in Cardiac Dz

Microbiome & Allergic Dermatitis

Mechanistic Links:

- Increasing emphasis on the importance of the cutaneous microbiome in **managing AD**
- Shifts in the microbiome diversity (**dysbiosis**) in AD contributes to impaired **skin barrier function** & **immune cell dysfunction**
- Both the **cutaneous** and **gut** microbiota can influence the pathogenesis of atopic diseases



Microbiome & Allergic Dermatitis

If disruption of the microbiome is a trigger or consequence of disease, restoration or stabilization of the microbiome are logical goals to treat or prevent disease… clinical cure and microbiological cure are not the same … because the main causes of bacterial infections are the same organisms that are often found on healthy skin (Weese et.al., 2013)

Santoro et.al. *Vet Derm* Vol 35 (2024)

SKIN Dysbiosis in favor of ***Staph pseudintermedius***

Malassezia **dermatitis** and **otitis** are recurrent features of atopy: Dysbiosis in favor of ***M. pachydermatis***

The gut microbiota influences the **allergen tolerance** (Tregs or SCFA)

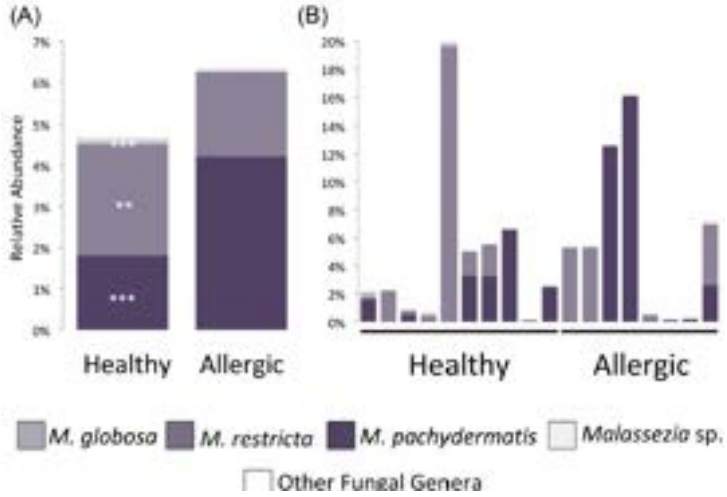
Dysbiotic gut microbiota (**lower alpha-diversity**) of dogs with AD

Rostaher et.al. *Animals* Vol 12 (2022)

M. globosa may be protective against Staph spp.; Allergic dysbiosis away from *M. globosa* and towards M. *pachydermatis*

Might the skin microbiome - microbiota & mycobiota - be used as a Monitoring Biomarker to assess restoration or stabilization?

Meason-Smith et.al. *Medical Mycology* Vol 58 (2020). Malassezia spp. relative abundances using NGS and qPCR by health status A) all samples B) across body sites.



Where to Next?

Clinical Utility & Application

Contents of a Biomarker Description:

- Added to the BEST glossary in 2020 to succinctly summarize key aspects necessary for universal “biomarker description”

1. The **biomarker identity**:
   a. name of the specific analyte, anatomic feature, or physiological characteristic as well as unique identifiers or commonly used acronyms.
   b. The specific source (e.g., urine, liver, biopsy, etc.).
   c. The type of biomarker (e.g., physiological, molecular, histological, etc.).
2. The **biologic plausibility:** summary of the biological, physiological, or pathological pathway for the association of biomarker with disease or condition. “Contextual linkage between biomarker and intended use”
3. **Measurement method:** used to measure, image, or otherwise quantify the biomarker with details to interpret the results (e.g., units)

Summary

- The stringency of experimental proof required for biomarker validation depends on its position between a research tool and a clinical endpoint. It is important to note that at this stage, despite robust research evidence, none of the candidate biomarkers in human AD have reached validation, and no single biomarker is routinely used in the clinical setting.
- In human health, the discovery of a novel, validated disease-related biomarker is demanding and requires multiple steps, from the first detection of the potential tissue-derived factor to the final confirmation and acceptance by regulatory organizations. Veterinary medicine lacks a formal regulatory process.
- Research, on endotypic biomarkers is ongoing to fine-tune the identification and stratification of human patients with AD. In the pursuit of breakthrough treatments for our veterinary patients, especially those without options other than steroids, exploration of biomarkers and their clinical application across the spectrum of allergy and dermatological diseases in the drug development pipeline might just be the key.
- Collaborations between academic consortia, clinical entities, and commercial organizations will be essential for the success of such endeavors.

Thank you

Nikki M. Thellman DVM, PhD

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