

The transcriptome of hand eczema assessed by tape stripping

Julie B. K. Sølberg , Anna S. Quaade, Stine B. Jacobsen, Jeppe D. Andersen, Marie-Louise Kampmann, Niels Morling, Thomas Litman, Jacob P. Thyssen, Jeanne D. Johansen

First published: 23 November 2021

<https://doi-org.bibliosan.clas.cineca.it/10.1111/cod.14015>

Funding information: LEO Fondet, Grant/Award Number: 114

Abstract

Background

No biomarkers have been identified that can classify subtypes of hand eczema (HE). Although skin biopsies represent the gold standard for investigations of the skin, the invasive technique is not favorable when investigating skin from sensitive areas. Recent advances in the use of skin-tape strips for molecular investigations enable noninvasive investigations of HE.

Objective

By using whole transcriptome sequencing (WTS), the molecular profile of HE according to different localizations on the hands, etiologies, and clinical/morphological subtypes was investigated.

Methods

Thirty adult, Danish HE patients, 12 with and 18 without concurrent atopic dermatitis (AD), as well as 16 controls were included. Tape strip samples were collected from lesional, nonlesional, and healthy skin. Total RNA was extracted and WTS was performed.

Results

The largest molecular difference of HE patients with and without AD was found in nonlesional skin areas and included a downregulation of *CXCL8* for HE patients without AD. Differences between allergic and irritant contact dermatitis included promising epidermal biomarkers such as *EPHA1*.

Conclusion

Skin tape strip samples could be used to assess the gene expression profile of HE on

different localizations of the hands. The skin tape strip method identified new molecular markers that showed promising result for the identification of HE subtypes.

Abbreviations

ACD

allergic contact dermatitis

AD

atopic dermatitis

DEG

differentially expressed gene

GO

gene ontology

HECSI

hand eczema severity index

HE^{+AD}

hand eczema with atopic dermatitis

HE^{-AD}

hand eczema without atopic dermatitis

HE

hand eczema

ICD

irritant contact dermatitis

WTS

whole transcriptome sequencing

1 INTRODUCTION

Hand eczema (HE) is a prevalent disease with a 1-year prevalence of 9% in the general population.¹ It may affect quality of life, impact work ability,^{2, 3} and require treatment periodically or continuously, depending on severity and chronicity.⁴

HE may be a result of different etiologies,⁵ which complicates both effective treatment and prevention. HE can be a part of atopic dermatitis (AD), and/or environmental factors such as exposure to allergens and/or irritants may result in allergic contact dermatitis (ACD) and/or irritant contact dermatitis (ICD) on the hands. The skin impairment of AD also makes the skin more vulnerable to irritants, sometimes leading to a mixed pattern of AD and ICD.

Diagnostic criteria exist for the different etiological subtypes. ACD can be diagnosed by a combination of patch testing and exposure analysis⁵; AD has distinct disease characteristics, such as early onset, and can be diagnosed by fulfilment of the Hanifin Rajka criteria⁶; whereas ICD depends on exclusion of ACD and exposure analysis demonstrating significant exposures to irritants. ACD is driven by a type IV hypersensitivity reaction to one or more contact allergens by allergen-specific T cells releasing pro-inflammatory cytokines. T_H1 cells

play a key role in the response. It is believed that danger signals induced by the allergen or co-exposures to irritants are important for initiation of the reaction. ICD is caused by agents that cause direct damage to the skin barrier, thereby initiating the innate immune system. The state of the skin barrier is important for the risk of ICD, and persons with current or previous AD in general have an increased risk of developing ICD, especially if filaggrin mutations are present.⁷ The immunology of AD is very complex, reflecting the heterogeneity of the disease, but in the classical understanding the disease is driven by a T_H2 response in the acute phase, that changes toward a T_H1 -driven response in the chronic phase. Although the molecular profiles related to the various etiologies differ, there are currently no biomarkers in use for supporting the diagnosis of these etiological subtypes in the routine investigation of patients with HE. In addition, subtypes exist, with so far unknown etiology. Some have distinct morphology including palmar hyperkeratotic HE and recurrent vesicular HE, whereas other have no apparent detectable causal factors.⁵ In addition,, anatomical patterns exist, so that some patients primarily have a dorsal and others a palmar pattern.⁸

Recent advances in the use of skin tape strips to obtain valid skin samples now enable research within immunotypes and specific biomarkers.⁹⁻¹³ In a previous study, we found that the stratum corneum transcriptome in AD could be assessed by tape stripping of the skin.¹¹ Based on a paired comparison with skin biopsies from the same patients, we identified established molecular markers of AD in the tape samples. In the current study, we are taking the next step by investigating if differences in the transcriptome can be detected by tape stripping of HE patients with different anatomical patterns, of different etiology, and main morphologies.

2 MATERIALS AND METHODS

2.1 Study population

Thirty adult, Danish patients with HE were recruited from the Department of Dermatology and Allergy, Herlev-Gentofte hospital, Denmark, between March 2019 and September 2020. The patients included eight patients with current AD, four with a history of AD (HE^{+AD}), and 18 with HE and no history of AD (HE^{-AD}).

Furthermore, 16 age-matched adult controls with no history of inflammatory skin diseases or other atopic diseases (asthma or hay fever) were included. All participants were asked to abstain from use of topical anti-inflammatory treatment and emollients for 24 hours before inclusion. Patients did not use any systemic therapies for their HE or AD. Other exclusion criteria included ongoing infections, pregnancy, lactation, or use of antibiotics, phototherapy, or self-tanners within 4 weeks of sample collection.

HE^{+AD} included patients with current AD and historical AD. Current AD was diagnosed by the treating physician at the department of dermatology, and all these patients fulfilled the Hanifin Rajka criteria.⁶ Historical AD was based on self-reports from the patients. HE^{-AD}

included patients with current HE and no history of AD. Current or previous AD had been ruled out by the treating physician in all HE^{-AD} cases. Clinical severity scores included the Hand Eczema Severity Index (HECSI)¹⁴ for all patients and the Eczema Area and Severity Index (EASI)¹⁵ for patients with concomitant active AD. Furthermore, clinical-morphological subtypes of HE were evaluated according to the classification by Menné et al.⁵ All participants answered a detailed questionnaire including questions on use of emollients, and for the patients, the duration of their HE.

Health care data relevant to the patients' etiological classification of HE was extracted from electronic health records (EPIC). The etiological classification of HE included irritant contact dermatitis (ICD), allergic contact dermatitis (ACD), atopic HE (AHE), and protein contact dermatitis (PCD). If the HE could not be classified etiologically according to these four sub-diagnoses or had more than one sub-diagnosis, the HE was grouped as “etiologically unclassifiable” or “mixed etiology,” respectively. The ACD sub-diagnosis was given if the patient had at least one clinically relevant type IV allergy. The ICD sub-diagnosis was given if the patient had a clinically relevant exposure to irritants.⁵ In [Data S1](#), an overview of the exposures to irritants and/or allergens relevant for the HE can be seen. No patients were diagnosed with PCD.

The characteristics of the study population can be seen in [Table 1](#). Based on the questionnaire, 67% of the HE^{+AD} patients reported that their HE started before the age of 2 years, whereas 89% of the HE^{-AD} patients reported that their HE started when they were 18 years or older. A majority of patients reported that the eczema was present almost all the time (HE^{-AD} 92% and HE^{+AD} 75%), and more than half of the patients reported that the eczema had been present all the time within the last year (HE^{-AD} 56% and HE^{+AD} 58%). A full overview of the self-reported duration of HE can be seen in [Data S2](#).

TABLE 1. Characteristics of study participants

	All patients n = 30	HE ^{+AD} n = 12	HE ^{-AD} n = 18	Controls n = 16
Sex, female (% female)	21 (70.0)	12 (100.0)	9 (50.0)	10 (62.5)
Age, median [IQR]	59.0 [44.3; 66.0]	59.0 [37.0; 66.0]	57.5 [48.5; 64.0]	59 [49.0; 64.0]
HECSI, median [IQR]	56.0 [34.0; 136.5]	58.5 [37.5; 144.5]	56.0 [33.0; 129.0]	-
EASI, median [IQR]	-	14.1 [6.7; 22.4]	-	-
<i>Etiologies of HE</i>				
Atopic HE	6	6	0	-
Irritant contact HE	5	0	5	-

	All patients	HE ^{+AD}	HE ^{-AD}	Controls
	n = 30	n = 12	n = 18	n = 16
Allergic contact HE	5	1	4	-
Mixed etiology	7	5	2	-
Etiologically unclassifiable	7	0	7	-

Note: The study included 12 hand eczema (HE) patients with a history of AD (HE^{+AD}), 18 HE patients with no history of AD (HE^{-AD}), and 16 age matched controls. Severity of the eczema was measured by the Hand Eczema Severity Index (HECSI) and the Eczema Area and Severity Index (EASI).

The study was approved by the local ethics committee (H-16050507) and the Danish Data Protection Agency (HGH-2017-073), oral and written consent was gathered before inclusion, and the study followed the Declaration of Helsinki.

2.2 Skin samples

Skin samples of both lesional and nonlesional stratum corneum were taken from the patients by tape stripping.¹⁶ The lesional tape strips were taken from a lesion on the most affected area of the hands (palmar or dorsal aspects). The nonlesional skin sample was taken from the upper arm of all patients. From the controls, a healthy skin sample was taken from the hands (palmar or dorsal aspects). From each sample site, two consecutive standard D-squame tape strips (Monaderm, Monaco, France) were collected applying uniform pressure as described previously.^{11, 16} For RNA extraction, both tapes were used. The samples were stored and shipped at room temperature, and RNA was extracted within 3 days of sampling.

2.3 RNA extraction and whole transcriptome sequencing

RNA was extracted with the miRNeasy Micro Kit from Qiagen (Qiagen Denmark, Copenhagen, Denmark) following the manufacturer's instruction. RNA from the two tapes was pooled directly on the column after phase separation.

For whole transcriptome sequencing (WTS), the library build was performed with the SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian kit (Takara Bio Europe, Saint-Germain-en-Laye, France) following the manufacturer's recommendation without fragmentation.

Paired-end sequencing (2 × 100 bp) was performed on a NovaSeq 6000 (Illumina, California USA) using the NovaSeq 6000 S2 Reagent Kit v1 (Illumina, California USA). The sequencing

resulted in an average sequencing depth of 82.7 M reads per case.

Quality assessment and alignment of sequencing reads were performed as described previously.¹¹

2.4 Normalization and differential expression analysis

Gene counts were normalized and analyzed by the Bioconductor package DESeq2 (version 1.30.1) using “apeglm” for LFC shrinkage.¹⁷ A gene was considered as a differentially expressed gene (DEG) if an observed difference in normalized read counts between two experimental conditions had a Benjamini-Hochberg adjusted *P*-value <.1 and a log2 fold change >1 or <-1. For the visual examination, data were transformed by the variance stabilizing transformation (vst) function of DESeq2, with “blind” set to “FALSE.”

Cluster, gene ontology (GO), and keyword analysis were conducted with STRING (Version 11.0, <https://version-11-0.string-db.org/>).

Data visualization including principal component analysis (PCA), heatmaps, and unsupervised hierarchical clustering were performed in Qlucore Omics Explorer v. 3.6 (Qlucore AB, Lund, Sweden).

2.5 Statistics

The statistical analyses were conducted in R (R core team, version 4.0.4, <http://www.R-project.org/>).

Differential analyses were performed between skin areas in patients (lesional, nonlesional) and compared with controls (healthy skin), anatomical localizations on the hands (palm and dorsum), AD status (HE^{-AD} and HE^{+AD}), HE etiologies (ACD, ICD and AD), and clinical HE subtypes.

For the investigation of the gene expression differences between the etiologies, patients with a single etiology were included (ACD *n* = 5, ICD *n* = 5, and AD *n* = 6) (Table 1). Patients grouped as “etiologically unclassifiable” or having a mixed etiology were not included in this data analysis.

The investigation of clinical HE subtypes included groups with at least three patients and only one clinical subtype, which was only fulfilled by: chronic fissured hand eczema and recurrent vesicular hand eczema.

For the statistical test between RNA parameters and individual biomarker levels, Wilcoxon rank sum test was performed.

3 RESULTS

3.1 Whole transcriptome sequencing on RNA from tape strips from

the hands

WTS was performed in a total of 76 tape strip samples (a lesional and nonlesional sample from each HE patient and a healthy sample from each control) (Figure 1A). Seven samples were subsequently excluded due to quality issues as assessed by the FastQC reports (four lesional and three nonlesional samples). With the removal of these, a total of 69 samples were included in the subsequent analyses (26 lesional, 27 nonlesional, and 16 healthy samples).

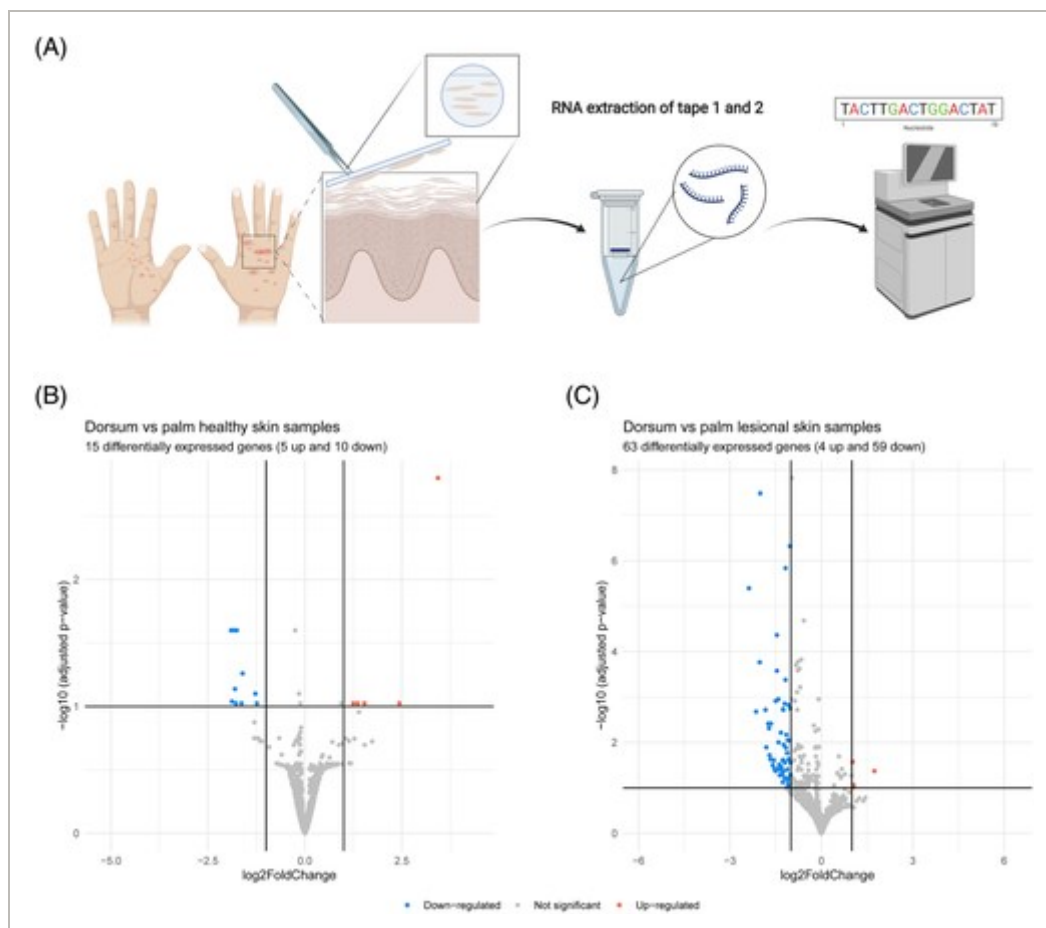


FIGURE 1

[Open in figure viewer](#) | [PowerPoint](#)

A, Tape strip samples were taken from the hands (a lesional from the patients and a healthy from the controls). Furthermore, a nonlesional tape strip sample was taken from the upper arm of the patients. Whole transcriptome sequencing was performed on RNA extracted from the first two tapes, shipped, and stored at room temperature. B, Differential analysis of dorsum vs palm of healthy skin samples resulted in 15 differentially expressed genes (5 up and 10 down) (adjusted P -value $< .1$, fold change > 2). C, Differential analysis of dorsum vs palm of lesional skin samples resulted in 63 differentially expressed genes (4 up and 59 down) (adjusted P -value < 0.1 , fold change > 2). Created with Biorender

The mean number of protein coding read counts (having an open reading frame) was 2.7 million for all samples (median [IQR]: 0.7 million [0.4-3.2 million]), with no statistically

significant difference ($P > .05$) between the skin areas (average; lesional: 2.2 million, non-lesional: 3.1 million, healthy: 1.1 million) ([Data S4](#), Figure [S1A](#)).

The average input read length was the same across skin sites; however, the average mapped read length showed a statistically significant decrease ($P < .05$) from lesional to nonlesional and healthy skin sites, respectively ([Data S4](#), Figure [S1B,C](#)). No difference was observed between the number of intronic reads according to skin areas ([Data S4](#), Figure [S1D](#)).

3.2 Transcriptional differences between the dorsum and palm were higher for eczema patients

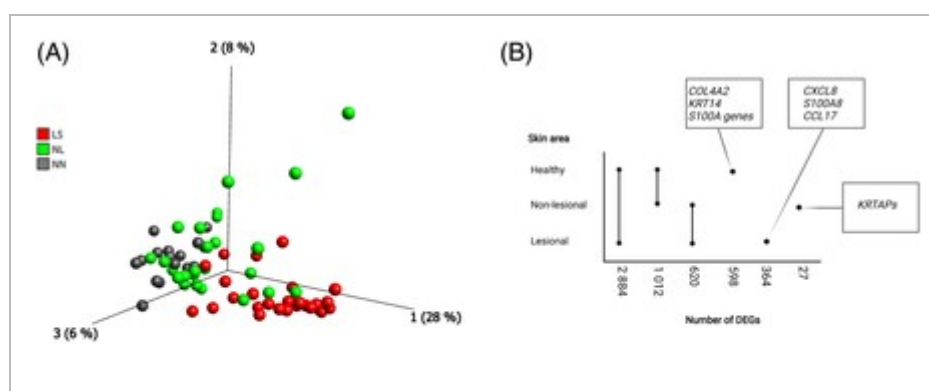
The 16 control samples included 6 samples from the dorsum and 10 from the palm. The 26 lesional samples included 7 samples from the dorsum and 19 from the palm (HE^{+AD}: 6 dorsal and 5 palmar; HE^{-AD}: 1 dorsal and 14 palmar).

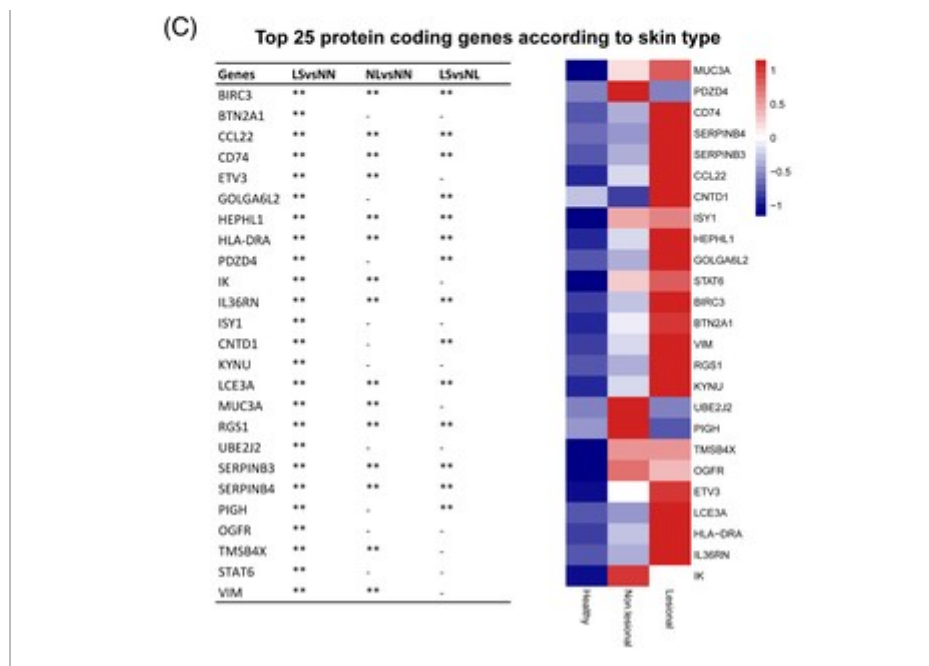
For the healthy skin samples, no statistically significant difference in the number of protein coding reads between the localizations (dorsal and palmar aspects) on the hands were observed ($P = .64$); however, for the lesional samples, a statistically significant higher number of protein coding reads were observed for the dorsal aspects of the hands ($P = .035$) ([Data S4](#), Figure [S2](#)).

Only a few DEGs were detected between the dorsum and palm (15 for healthy skin samples and 63 for lesional skin samples) (Figure [1B and C](#), respectively), and the genes did not show enrichment for specific biological processes (data not shown). In addition, PCA did not show apparent clustering related to the dorsal or palmar aspects of the hands for either skin area ([Data S4](#), Figure [S3A and B](#), respectively).

3.3 Tape strip samples show the immunology of lesions on the hands

The main transcriptomic differences between skin areas (healthy, nonlesional, and lesional) were found between lesional and nonlesional as well as healthy skin (PC1: 28%), whereas the nonlesional and healthy skin samples had similar overall gene expression profiles (Figure [2A](#), and [Data S4](#), Figure [S4](#)).



**FIGURE 2**[Open in figure viewer](#) | [PowerPoint](#)

A, The principal component analysis (PCA) shows that the largest difference (PC1) between skin areas (lesional [LS], nonlesional [NL], and healthy [NN] skin) is found between lesional and healthy/nonlesional skin. B, A total of 2884 genes are differentially expressed between lesional and healthy skin, 1012 genes between healthy and nonlesional, and 620 genes between nonlesional and lesional skin areas. The diagram also shows that 364 genes were related to lesional skin, 598 to healthy skin, and 27 to nonlesional skin areas. C, The heatmap shows the mean normalized count of the different skin areas for the top 25 genes differentially expressed between lesional and healthy skin (defined by the smallest Benjamini-Hochberg corrected *P*-values and a fold change >2) scaled by gene. The table shows the adjusted *P*-values (** = <.001). Ten of the 25 genes are differentially expressed between all skin area differences. Created with Biorender

When investigating the number of DEGs between skin areas, the largest difference in gene expression—based on the numbers of DEGs between the skin areas—was found between lesional and healthy skin areas (2884 DEGs, 1355 up and 1529 down in lesional skin). The second largest difference was observed between nonlesional and healthy skin areas (1012 DEGs, 697 up and 315 down in nonlesional skin). The difference between patients' lesional and nonlesional skin areas included 620 DEGs (273 up and 347 down in lesional skin) (Figure 2B). Figure 2C shows the top 25 DEGs between healthy and lesional skin areas. Of these 25 genes, 10 were differentially expressed between all skin contrasts (healthy vs nonlesional, healthy vs lesional, and nonlesional vs lesional). The 10 genes enriched for gene ontologies important for inflammation including “cytokine activity” (GO:0005125) and “antigen processing and presentation of exogenous peptide antigen via MHC class II” (GO:0019886).

When looking at the genes common to the specific skin areas, the genes associated with

healthy skin included *COL4A2*, *KRT14*, and *S100A* ([Data S1](#)), genes associated with nonlesional skin included hair genes (*KRTAPs*) and *IL18* ([Data S2](#)), and genes associated with lesional skin areas included *CXCL8*, *S100A8*, and *CCL17* ([Data S3](#)) (Figure [2B](#) and [Data S4](#), Figure [S5](#)).

3.4 The most prominent difference between HE^{+AD} and HE^{-AD} was at the nonlesional skin sites

Looking at the number of DEGs between HE^{+AD} and HE^{-AD} showed that the greatest difference was observed for the nonlesional skin sites (211 DEGs) as compared to the lesional skin site (28 DEGs) (Figure [3A](#)). The difference between the nonlesional skin of HE^{+AD} and HE^{-AD} showed an increase in several inflammatory markers including *S100A12*, *MMP9*, *CXCL8*, and *IL1B*, whereas lesional skin in HE^{+AD} patients showed a decreased expression of *PRLR* and *PIBP1* related to the “activation of Janus kinase activity” (GO:0042976) compared to lesional skin of HE^{-AD} patients.

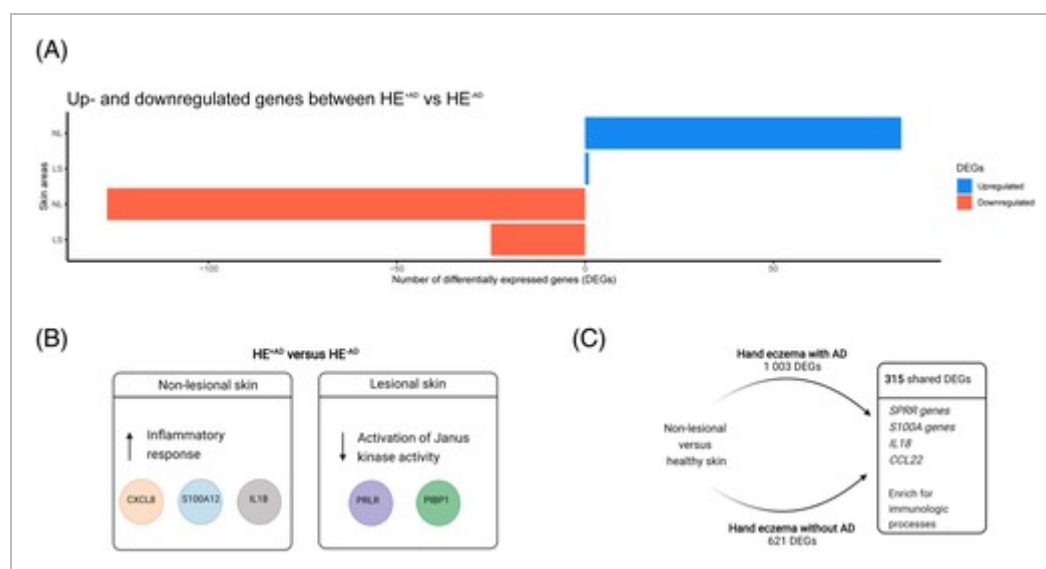


FIGURE 3

[Open in figure viewer](#) | [PowerPoint](#)

A, Upregulated and downregulated genes of lesional and nonlesional skin of hand eczema with AD (HE^{+AD}) and hand eczema without AD (HE^{-AD}), respectively. B, Diagram showing the difference between HE^{+AD} and HE^{-AD} for nonlesional and lesional skin, respectively. C, Schematic of the difference between nonlesional and healthy skin for HE^{+AD} and HE^{-AD} , respectively, as well as the common differentially expressed genes (DEGs). Created with Biorender

Next we investigated if the overall transcriptomic difference between nonlesional and healthy skin was driven by the HE^{+AD} patients ([Data S4](#), Figure [S6](#)). In general, the genes specific for the difference between nonlesional and healthy skin for HE^{+AD} and HE^{-AD} , respectively, did not enrich for immunologic pathways (data not shown); however, the specific genes for the HE^{+AD} nonlesional and healthy skin difference included known markers of AD and general inflammation such as *MMP12*, *CCL17*, *CCL27*, and several *S100A*

genes.^{18, 19} The 315 genes common to the nonlesional and healthy skin difference of HE^{+AD} and HE^{-AD} included several genes important for the immunology of eczema lesions including *SPRR* and *S100A* genes, *IL18*, and *CCL22*.²⁰ Furthermore, the common genes enriched for several immunological processes included interferon-gamma-mediated signaling pathway (GO:0060333) and antigen processing and presentation of exogenous antigen (GO:0019884).

3.5 The transcriptome differs between atopic hand eczema and irritant contact dermatitis

When investigating the difference between lesional skin sites according to the etiologies of AD, ACD, and ICD only, we saw that the greatest difference was found between AD and ICD (32 DEGs), followed by the difference between ACD and ICD (six DEGs). Only one (*NRK*), and not a skin relevant gene, was differentially expressed between AD and ACD.

Of the 32 DEGs between AD and ICD, 25 genes were upregulated, and seven genes were downregulated in AD ([Data S3](#)). When investigating the gene ontologies related to the 25 upregulated genes, the top three GO enrichment were related to response to interferon-alpha and mast cell activation (cellular response to interferon-alpha; GO:0035457, response to interferon-alpha; GO:0035455, and mast cell activation; GO:0045576). For the two other differences, too few genes were differentially expressed to conduct gene enrichment analysis.

When looking at the six genes differentially expressed in our study between ACD and ICD we found markers that could have potential for the distinction between ACD and ICD, including *EPHA1* and *ACTN3*. The genes, however, did not distinguish, for example AC,D from AD and the mixed etiologies ([Data S4](#), [Figure S7](#)).

3.6 The tape strip samples detect gene expression differences between clinical subtypes of hand eczema

Next, we investigated if the tape strip samples detected gene expression differences according to the clinical subtypes of HE.

A total of 248 DEGs were detected between chronic fissured and vesicular eczema (240 upregulated and 8 downregulated in chronic fissured eczema) (data not shown). The 240 upregulated genes did not correlate with any gene enrichment; however, the 8 downregulated genes enriched for epidermis development (GO:0008544), cornification (GO:0070268), and tissue development (GO:0009888), mainly due to the involvement of *KRT4*, *KRT78*, *RPTN*, and *EMP1*.

4 DISCUSSION

Tape strip skin samples can be used to assess the gene expression profile of different localizations on the hands. We showed that the most notable differences between HE^{-AD} and

HE^{+AD} were found in nonlesional skin areas. The tape strip skin samples detected transcriptomic differences between etiologies of HE as well as some clinical-morphological subtypes. There seems to be a potential use of noninvasive tape strip samples for detecting new biomarkers to facilitate the molecular investigation of the different subtypes of HE.

To our knowledge, the transcriptomic differences according to localizations on the hands have not yet been investigated despite apparent differences such as the epidermis of the palm being thicker than at other localizations on the hands.²¹ We saw no difference in the number of protein coding reads from the tape strip samples of healthy skin from different localizations on the hands (palm and dorsum). In contrast, for lesional samples, a statistically significant higher number of protein coding reads was detected from the dorsal samples, but the transcriptomic profile of the palm as compared to the dorsum did not show differences related to specific biological processes from either skin area. This shows that the tape strip samples can be used to investigate the transcriptome of the hands independent of the localization.

When investigating the molecular markers related to the different skin areas (lesional, nonlesional, and healthy) we found that *S100A* genes were expressed in both lesional and healthy skin. *S100A* genes are known to be important for the inflammatory process of AD,²²⁻²⁴ and have also been shown to be increased in lesions of HE²⁵; however, they are also expressed in the epidermis of healthy skin.²⁶ The expression of various *S100A* genes of both lesional and healthy skin on the hands suggests that these genes play a role not only in the pathogenesis of HE, but also in the healthy skin of the hands. Therefore, further studies are needed to establish the role of different *S100A* genes in healthy and diseased skin of the hands.

The genes related to lesional skin also included *CCL17*, one of the most reliable biomarkers of AD.^{18, 24} Though *CCL17* has also been shown to correlate to the severity of AD in tape strip samples,²⁷ we did not see a correlation between *CCL17* and *HECSI* in this study (data not shown). Whether this is due to the scoring system or the biomarker level of *CCL17* from the hands needs further investigation.

A recent study by Kumari et al. investigated the protein expression of selected molecular markers of HE and their relation to treatment with alitretinoin.²⁸ In that study, they found *CLDN1*, *LOR*, *FLG*, *KRT10*, and *TSLP* to be related to the lesional skin of HE. In our study, we only rediscovered a difference between healthy and lesional skin for *CLDN1*. If this is due to the difference in study population, sample type (biopsy vs tape stripping) or proteins vs mRNA is not known. It should be noted that differences in *LOR* and *FLG* expression between lesional AD skin and healthy skin has previously been shown by tape strip samples, and therefore the differences are most likely not due to the sampling method.¹³

The largest difference between HE with and without AD was found at the nonlesional skin sites. Nonlesional skin areas of AD patients have been shown previously to have a distinct

molecular pattern as compared to healthy skin.^{29, 30} We therefore tested if the difference between nonlesional and healthy skin was greater for the HE^{+AD} group. When investigating the difference between nonlesional and healthy skin for the two HE subtypes we could see that the HE^{+AD} group indeed showed common markers of AD; however, the genes showing enrichment for inflammatory processes were shared between the two HE subtypes. This indicates that in the HE^{-AD} patients, a general skin inflammation was observed even at skin areas distant from the active eczema on the hands.

In this study, HE^{+AD} was defined as HE in patients with current or previous AD.³¹ This definition includes historic AD in the HE^{+AD} group, although it might not be the cause of their current HE. When considering the etiology of the current HE lesion, only current AD was considered; however, for only two HE^{+AD} patients their AD was not considered relevant for their HE lesion, supporting the importance of the impaired skin barrier of AD patients.³²

When investigating the patients' current etiologies, we found that the largest transcriptomic difference was between AD only and ICD only. This difference included an upregulation of genes that enriched for the biological process of response to interferon-alpha and mast cell activation, both important for an inflammatory process. This finding suggests that AD^{+HE} patients have a larger activation of mast cells as well as interferon-alpha.

A recent study investigated if machine-learning-driven biomarker discovery could differentiate ACD from ICD.³³ The study was based on applications of known allergens or irritants and was therefore not based on samples from a clinical setting. Furthermore, the skin samples were obtained by skin biopsies, and therefore included the deeper layers of epidermis and dermis. The study identified 21 genes that in combination made up 28 gene sets that were able to distinguish the two etiologies. In our study we did not rediscover transcriptomic differences between the biomarkers identified in this study ([Data S4](#)). This could be due to the differences between gene expression of full biopsy samples vs stratum corneum tape samples where a major global difference is expected.¹¹

Six genes were differentially expressed between ACD and ICD. These genes included *EPHA1*, which has been found to be an important marker for a normal epidermal differentiation.^{34, 35} Although the role of *EPHA1* in contact dermatitis is not known, the results may have importance for the distinction between ACD and ICD. A study by Tam et al. found *LOR* as a candidate gene to distinguish ACD from ICD. The study, however, were performed on patch test samples and not on a hand eczema lesion as in our study.³⁶ It should be noted, however, that the biomarkers found in this study had difficulty distinguishing ACD and ICD to the mixed etiologies. Therefore, to study this in detail, larger, more homogenous patient groups should be included to validate this based on the specific etiologies, as well as PCD, which was not included in the current study.

There are some limitations to our study; first, sample size, even though we aimed at fairly large groups for a study of this nature, the mixed etiologies and morphologies meant that

the number of patients with some pure forms were limited. The HE^{+AD} group included patients with a history of AD (both current and historical AD). However, only patients with active AD received the AHE etiological sub-diagnosis. We took the nonlesional sample distant from the lesional samples, this may have caused confounding effects; however, it can be difficult to be sure that samples are truly nonlesional if they are obtained from skin areas adjacent to the inflammatory area on the hands. Patients were asked to abstain from topical anti-inflammatory treatment and emollients for 24 hours before inclusion in the study. A longer washout period would have been preferable; however, many patients are not willing to abstain from topicals for longer periods and therefore compliance might have been lower with a longer washout period.

This study shows that the tape strip method is a valuable tool for investigating the transcriptomic differences between HE with and without AD independent of the localization of the lesion. The use of noninvasive skin sampling of the hands enables large-scale studies of HE with no harm to the patients. Furthermore, the noninvasive tape strip samples show potential for the investigation of the molecular profile related to different etiologies as well as clinical subtypes of HE.

ACKNOWLEDGEMENTS

We acknowledge Nina Heede Ulrich for her great work in the preparation and planning of the study, nurse Anne Marie Topp for her assistance with the patients and sampling in the clinic, and lab technician Helle Byrgesen for her excellent work in the laboratory. This work was financially supported by the LEO Foundation (GRANT ID no 114). Jacob Thyssen was supported by an unrestricted research grant from the Lundbeck Foundation.

AUTHOR CONTRIBUTIONS

Julie Sølberg: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (lead); software (equal); validation (lead); visualization (lead). **Anna Sophie Quaade:** Conceptualization (equal); investigation (supporting); resources (supporting). **Stine Bøttcher Jacobsen:** Formal analysis (supporting); investigation (supporting); methodology (supporting); software (supporting); visualization (supporting). **Jeppe Dyrberg Andersen:** Conceptualization (supporting); formal analysis (supporting); investigation (supporting); methodology (supporting); software (supporting); supervision (supporting); visualization (supporting). **Marie-Louise Kampmann:** Formal analysis (supporting); investigation (supporting); software (supporting). **Niels Morling:** Resources (equal); supervision (supporting). **Thomas Litman:** Formal analysis (supporting); investigation (supporting); methodology (supporting); software (supporting); validation (equal); visualization (supporting). **Jacob Pontoppidan Thyssen:** Conceptualization (equal); investigation (supporting); methodology (supporting); supervision (equal); validation (supporting). **Jeanne Duus Johansen:** Conceptualization (equal); formal analysis (supporting); funding acquisition (lead); project administration

(supporting); supervision (lead).

CONFLICTS OF INTEREST

Thomas Litman is funded by LEO Pharma A/S. All other authors declare no conflicts of interest.

Open Research

DATA AVAILABILITY STATEMENT

Research data are not shared.

Supporting Information

Filename	Description
cod14015-sup-0001-DatasetS1.csv xls, 8.9 KB	Data S1. Dataset S1
cod14015-sup-0002-DatasetS2.csv xls, 393 B	Data S2. Dataset S2
cod14015-sup-0003-DatasetS3.csv xls, 5.5 KB	Data S3. Dataset S3
cod14015-sup-0004-SupinfoS1.docx docx, 1.1 MB	Data S4. Supporting Information

Please note: The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing content) should be directed to the corresponding author for the article.

REFERENCES

1

Quaade AS, Simonsen AB, Halling AS, et al. Prevalence, incidence, and severity of hand eczema in the general population – a systematic review and meta-analysis. *Contact Dermatitis*. 2021; **84**(6): 361- 374. doi:[10.1111/cod.13804](https://doi.org/10.1111/cod.13804)
[Wiley Online Library](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

2

Diepgen TL, Purwins S, Posthumus J, et al. Cost-of-illness analysis of patients with chronic hand eczema in routine care in germany: focus on the impact of occupational disease. *Acta Derm*

Venereol. 2013; **93**(5): 538- 543.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

3 Agner T, Andersen KE, Brandao FM, et al. Hand eczema severity and quality of life: a cross-sectional, multicentre study of hand eczema patients. *Contact Dermatitis*. 2008; **59**(1): 43- 47.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

4 Thyssen JP, Silverberg JI, Guttman-Yassky E. Chronic hand eczema understanding has ramifications on clinical management. *J Eur Acad Dermatol Venereol*. 2020; **34**(8): e429- e430.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

5 Menné T, Johansen JD, Sommerlund M, Veien NK. Hand eczema guidelines based on the Danish guidelines for the diagnosis and treatment of hand eczema. *Contact Dermatitis*. 2011; **65**(1): 3- 12.

[Wiley Online Library](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

6 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol*. 1980; **60**(92): 44- 47.

[Google Scholar](#)

7 Visser MJ, Landeck L, Campbell LE, et al. Impact of atopic dermatitis and loss-of-function mutations in the filaggrin gene on the development of occupational irritant contact dermatitis. *Br J Dermatol*. 2013; **168**(2): 326- 332.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

8 Simpson EL, Thompson MM, Hanifin JM. Prevalence and morphology of hand eczema in patients with atopic dermatitis. *Dermatitis*. 2006; **17**(3): 123- 127.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

9 Dyjack N, Goleva E, Rios C, et al. Minimally invasive skin tape strip RNA sequencing identifies novel characteristics of the type 2–high atopic dermatitis disease endotype. *J Allergy Clin Immunol*. 2018; **141**(4): 1298- 1309. doi:[10.1016/j.jaci.2017.10.046](https://doi.org/10.1016/j.jaci.2017.10.046)

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

10 Leung DYM, Calatroni A, Zaramela LS, et al. The nonlesional skin surface distinguishes atopic dermatitis with food allergy as a unique endotype. *Sci Transl Med*. 2019; **11**(480):eaav2685. doi:[10.1126/scitranslmed.aav2685](https://doi.org/10.1126/scitranslmed.aav2685)

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

11 Sølberg J, Jacobsen SB, Andersen JD, et al. The stratum corneum transcriptome in atopic dermatitis can be assessed by tape stripping. *J Dermatol Sci*. 2021; **101**(1): 14- 21.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

12 Benson NR. Tape stripping methods for analysis of skin disease and pathological skin state. 2007.

[Google Scholar](#)

13 Guttman-Yassky E, Diaz A, Pavel AB, et al. Use of tape strips to detect immune and barrier abnormalities in the skin of children with early-onset atopic dermatitis. *JAMA Dermatol.* 2019; **155**(12): 1358- 1370. doi:[10.1001/jamadermatol.2019.2983](https://doi.org/10.1001/jamadermatol.2019.2983)
[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

14 Held E, Skoet R, Johansen JD, Agner T. The hand eczema severity index (HECSI): a scoring system for clinical assessment of hand eczema. A study of inter- and intraobserver reliability. *Br J Dermatol.* 2005; **152**(2): 302- 307.
[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

15 Tofte S, Graeber M, Cherill R, et al. Eczema area and severity index (EASI): a new tool to evaluate atopic dermatitis. *J Eur Acad Dermatol Venereol.* 1998; **10**(1): S197.
[Crossref](#) | [Google Scholar](#)

16 Sølberg J, Ulrich NH, Krustup D, et al. Skin tape stripping: which layers of the epidermis are removed? *Contact Dermatitis.* 2018; **80**(5): 319- 321.
[Web of Science®](#) | [Google Scholar](#)

17 Zhu A, Ibrahim JG, Love MI. Heavy-tailed prior distributions for sequence count data: removing the noise and preserving large differences. *Bioinformatics.* 2019; **35**(12): 2084- 2092.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

18 Saeki H, Tamaki K. Role of TARC/CCL17 and CTACK/CCL27 in the pathogenesis of atopic dermatitis. *Curr Immunol Rev.* 2008; **4**(4): 221- 229.
[Crossref](#) | [CAS](#) | [Google Scholar](#)

19 Mansouri Y, Guttman-Yassky E. Immune pathways in atopic dermatitis, and definition of biomarkers through broad and targeted therapeutics. *J Clin Med.* 2015; **4**(5): 858- 873.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

20 Carregaro F, Stefanini ACB, Henrique T, Tajara EH. Study of small proline-rich proteins (SPRRs) in health and disease: a review of the literature. *Arch Dermatol Res.* 2013; **305**(10): 857- 866.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

21 Olsen LO, Takiwaki H, Serup J. High-frequency ultrasound characterization of normal skin. Skin thickness and echographic density of 22 anatomical sites. *Skin Res Technol.* 1995; **1**(2): 74- 80.
[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Google Scholar](#)

22 Gittler JK, Shemer A, Suárez-Fariñas M, et al. Progressive activation of TH2/TH22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol.* 2012; **130**(6): 1344- 1354.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

23 Suárez-Fariñas M, Dhingra N, Gittler J, et al. Intrinsic atopic dermatitis shows similar TH2 and higher T H17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol.*

2013; **132**(2): 361- 370.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

24 Renert-Yuval Y, Thyssen JP, Bissonnette R, et al. Biomarkers in atopic dermatitis—a review on behalf of the International Eczema Council. *J Allergy Clin Immunol*. 2021; **147**(4): 1174- 1190.e1.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

25 Molin S, Merl J, Dietrich KA, et al. The hand eczema proteome: imbalance of epidermal barrier proteins. *Br J Dermatol*. 2015; **172**(4): 994- 1001.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

26 Eckert RL, Broome AM, Ruse M, et al. S100 proteins in the epidermis. *J Invest Dermatol*. 2004; **123**(1): 23- 33.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

27 Morita E, Takahashi H, Niihara H, et al. Stratum corneum TARC level is a new indicator of lesional skin inflammation in atopic dermatitis. *Allergy*. 2010; **65**(9): 1166- 1172.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

28 Kumari V, Timm K, Köhl AA, et al. Impact of systemic alitretinoin treatment on skin barrier gene and protein expression in patients with chronic hand eczema. *Br J Dermatol*. 2016; **175**(6): 1243- 1250.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

29 Suárez-Fariñas M, Tintle SJ, Shemer A, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol*. 2011; **127**(4): 954- 964.e1-4.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

30 Holm JG, Clausen ML, Agner T, et al. Epidermal biomarker levels differentiate lesional from non-lesional skin and show variation across anatomical locations in patients with atopic dermatitis. *J Eur Acad Dermatol Venereol*. 2020; **35**(5): e325- e327.

[Web of Science®](#) | [Google Scholar](#)

31 Agner T, Elsner P. Hand eczema: epidemiology, prognosis and prevention. *J Eur Acad Dermatol Venereol*. 2020; **34**(suppl 1): 4- 12.

[Wiley Online Library](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

32 Ruff SMD, Engebretsen KA, Zachariae C, et al. The association between atopic dermatitis and hand eczema: a systematic review and meta-analysis. *Br J Dermatol*. 2018; **178**(4): 879- 888.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

33 Fortino V, Wisgrill L, Werner P, et al. Machine-learning–driven biomarker discovery for the discrimination between allergic and irritant contact dermatitis. *Proc Natl Acad Sci U S A*. 2021; **117**(52): 33474- 33485.

[Crossref](#) | [Web of Science®](#) | [Google Scholar](#)

34 Lin S, Wang B, Getsios S. Eph/ephrin signaling in epidermal differentiation and disease. *Semin Cell Dev Biol.* 2012; **23**(1): 92- 101.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

35 Hafner C, Becker B, Landthaler M, Vogt T. Expression profile of Eph receptors and ephrin ligands in human skin and downregulation of EphA1 in nonmelanoma skin cancer. *Mod Pathol.* 2006; **19**(10): 1369- 1377.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

36 Tam I, Hill KR, Park JM, Yu J. Skin tape stripping identifies gene transcript signature associated with allergic contact dermatitis. *Contact Dermatitis.* 2021; **84**(5): 308.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

[Download PDF](#)

About Wiley Online Library

Privacy Policy

Terms of Use

Cookies

Accessibility

Publishing Policies

Help & Support

Contact Us

Training and Support

DMCA & Reporting Piracy

Opportunities

Subscription Agents

Advertisers & Corporate Partners

Connect with Wiley

The Wiley Network

Wiley Press Room

Copyright © 1999-2021 John Wiley & Sons, Inc. All rights reserved

Appendices

Supplementary tables

Supplementary table 1. Known relevant type IV allergies and irritants as well as exposures. ACD= allergic contact dermatitis, ICD= irritant contact dermatitis.

Etiology	Relevant type IV allergy	Relevant exposure to irritants
ACD	MCI/MI Methylchloroisthiazolinone/Methylisothiazolinone	NA
ACD	Fragrance mix 1, fragrance mix2, tree moss, citral, and linalool	NA
ICD	NA	Gloves at work
ACD	Fragrance mix I, fragrance mix II, Hydroxycitronellal	NA
ICD	NA	Rubber gloves and hand sanitizers at work
ACD	Colophonium, Asteraceae (sesquiterpene)	NA
ACD	Sesquiterpenelactone mix, and alpha-methylene-γ-butyrolactone-mix	NA
ICD	NA	Occupational: Gardener
ICD	NA	Wet hands and use of gloves
ACD	Sesquiterpene, chrome, and nickel	NA
ICD	NA	Frequent domestic hand wash

Supplementary table 2. Questionnaire about age of onset and duration of hand eczema. HE^{-AD} ($n=18$) refers to hand eczema without atopic dermatitis and HE^{+AD} ($n=12$) refers to atopic hand eczema.

How old were you when the hand eczema first appeared? (n)			How often have you had eczema on your hands since then? (n)			Have you, during the previous 12 months, had hand eczema on any occasion? (n)		
	HE^{-AD}	HE^{+AD}		HE^{-AD}	HE^{+AD}		HE^{-AD}	HE^{+AD}
Under 6 years	0	8	Only one time and in less than two weeks	0	0	Yes, all the time	10	7
Between 6 and 14 years	0	2	Only one time lasting for two weeks or more	4	0	Yes, more than half of the time	5	3
Between 15 and 18 years	2	0	Several times	3	3	Yes, approximately half of the time	2	1
Over 18 years	16	2	Nearly all the time	11	9	Yes, less than half of the time	1	1

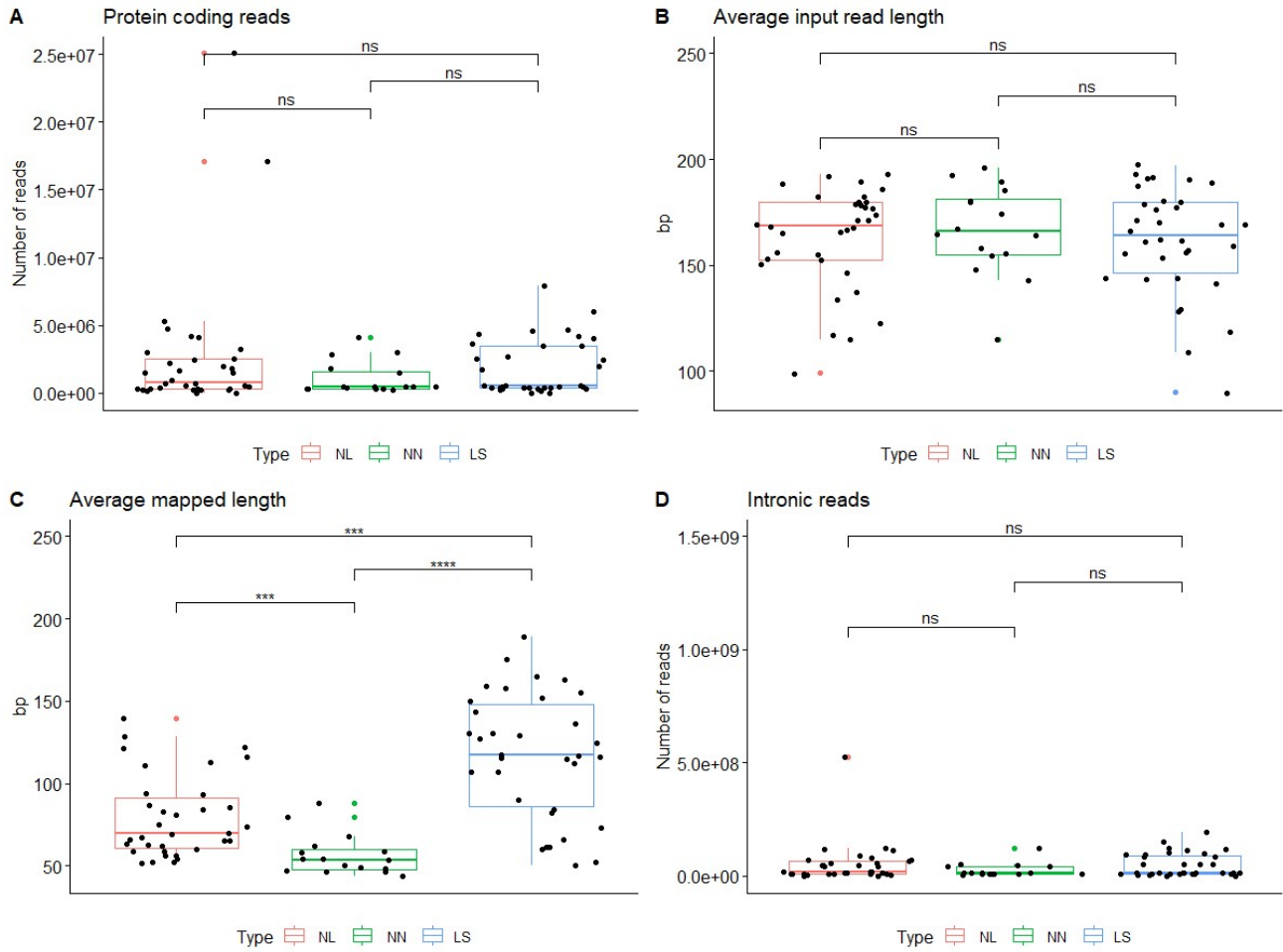
Supplementary table 3. Differentially expressed genes between atopic dermatitis and irritant contact dermatitis for lesional skin sites. A gene is considered differentially expressed if the adjusted p-value (Benjamini-Hochberg) is below 0.1 and the fold change is larger than 2.

Gene	baseMean	log2FoldChange	lfcSE	p-value	padj
ADGRA1	181.86	-2.97	0.70	8.35E-07	0.0015
ZNF787	109.39	-2.88	0.82	1.43E-05	0.0091
WISP1	81.20	-2.82	0.74	4.32E-06	0.0049
ATP6V1H	101.03	-1.90	0.60	4.48E-05	0.016
ACTN3	90.47	-1.39	0.41	2.71E-05	0.013
REEP2	74.52	-1.10	0.39	0.000149	0.029
FANCM	81.14	-1.00	0.46	0.00062	0.061
RSAD2	50.28	1.08	0.62	0.0018	0.099
UTP6	116.70	1.22	0.54	0.00068	0.062
GBP2	115.34	1.27	0.60	0.00093	0.073
FBXL5	51.13	1.33	0.59	0.00069	0.062
RHOH	87.52	1.38	0.51	0.00023	0.037
FFAR2	54.03	1.42	0.52	0.00018	0.034
LCP2	190.06	1.48	0.68	0.00072	0.063
SELL	45.030	1.54	0.76	0.0010	0.077
CSF3R	140.62	1.54	0.59	0.00026	0.040
TAGAP	73.69	1.55	0.87	0.0015	0.094
PLAUR	260.49	1.57	0.77	0.00094	0.073
RPTN	175.90	1.59	0.78	0.00089	0.072
SOD2	512.13	1.63	0.83	0.0011	0.078
FGF1	95.02	1.66	0.79	0.00086	0.071
AQP9	91.94	1.74	0.90	0.0012	0.082
DDX60L	130.88	1.82	0.51	1.55E-05	0.0093
IFIT2	51.52	1.88	0.83	0.00058	0.061
CASP4	49.59	1.95	0.69	0.00015	0.029
BCL2A1	83.61	1.97	1.01	0.0011	0.077
GBP1	88.07	1.97	0.89	0.00065	0.062
IFITM2	57.16	2.00	0.90	0.00067	0.062
GBP5	108.72	2.45	0.76	4.58E-05	0.016
IFIT3	51.070	2.49	0.82	7.66E-05	0.022
G0S2	356.53	2.53	0.90	0.00015	0.029
SEMG2	342.50	3.10	1.079	0.00011	0.028

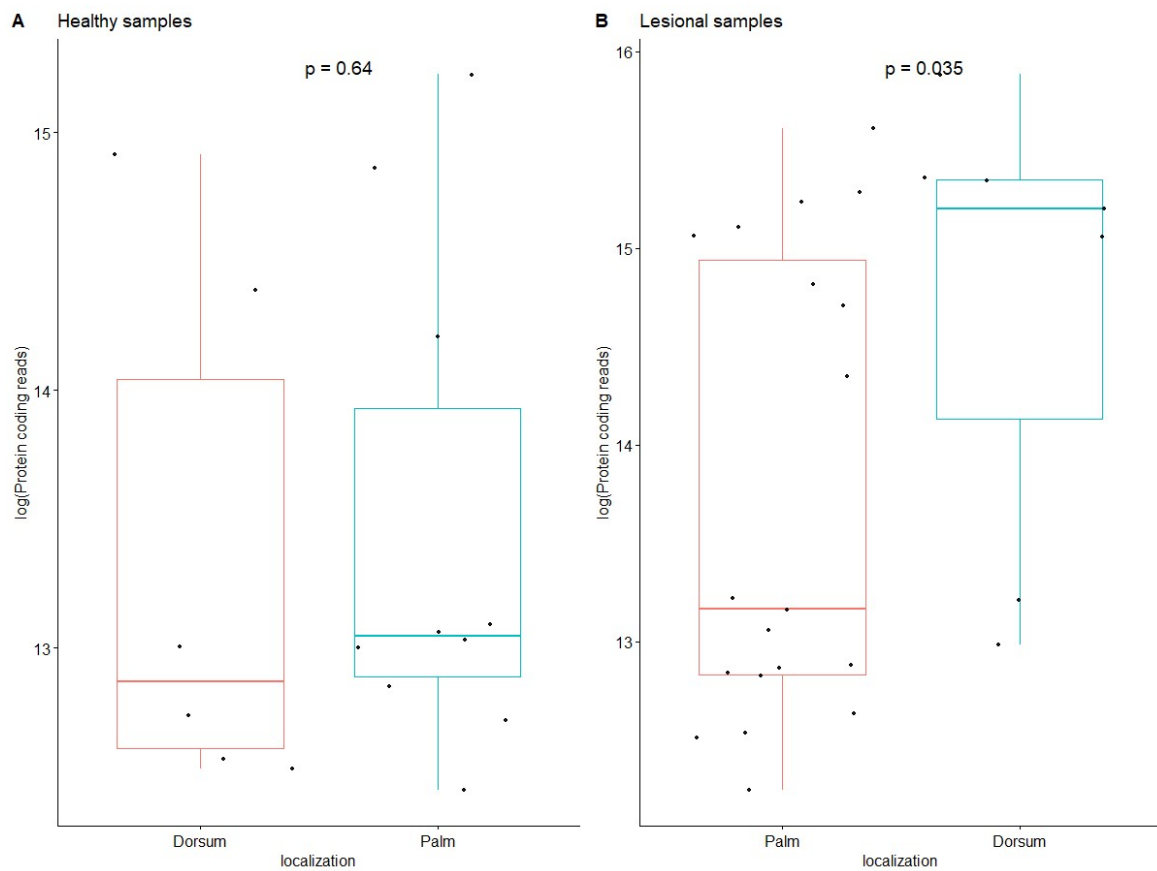
Supplementary table 4. Genes from V. Fortino, L. Wisgrill, P. Werner, S. Suomela, N. Linder, E. Jalonen, A. Suomalainen, V. Marwah, M. Kero, M. Pesonen, J. Lundin, A. Lauerma, K. Aalto-Korte, D. Greco, H. Alenius, N. Fyhrquist, Machine-learning-driven biomarker discovery for the discrimination between allergic and irritant contact dermatitis, *Proc. Natl. Acad. Sci. U. S. A.* 117 (2021) 33474–33485. <https://doi.org/10.1073/PNAS.2009192117>.

Gene	baseMean	log2FoldChange	lfcSE	p-value	padj
<i>CD47</i>	57.37	0.068	0.13	0.10	NA
<i>BATF</i>	26.03	0.0091	0.11	0.74	NA
<i>FASLG</i>	14.91	0.015	0.11	0.58	NA
<i>RGS16</i>	33.29	0.035	0.11	0.37	NA
<i>SYNPO</i>	130.44	-0.038	0.11	0.37	0.69
<i>SELE</i>	27.16	0.035	0.11	0.28	NA
<i>PTPN7</i>	73.21	-0.0030	0.102	0.93	NA
<i>WARS</i>	194.78	-0.019	0.11	0.20	0.57
<i>PRC1</i>	44.97	0.064	0.12	0.20	NA
<i>EXO1</i>	40.74	-0.010	0.10	0.78	NA
<i>RRM2</i>	39.40	0.0074	0.10	0.83	NA
<i>PBK</i>	13.22	0.0030	0.11	0.91	NA
<i>RAD54L</i>	28.68	-0.063	0.12	0.16	NA
<i>KIFC1</i>	3.17	0.0068	0.11	0.64	NA
<i>SPC25</i>	0.86	-0.0028	0.11	0.69	NA
<i>TPX2</i>	48.07	0.021	0.10	0.59	NA
<i>DLGAP5</i>	18.44	0.012	0.11	0.67	NA
<i>CH25H</i>	12.79	0.0086	0.11	0.70	NA
<i>IL37</i>	11.29	-0.0049	0.11	0.80	NA

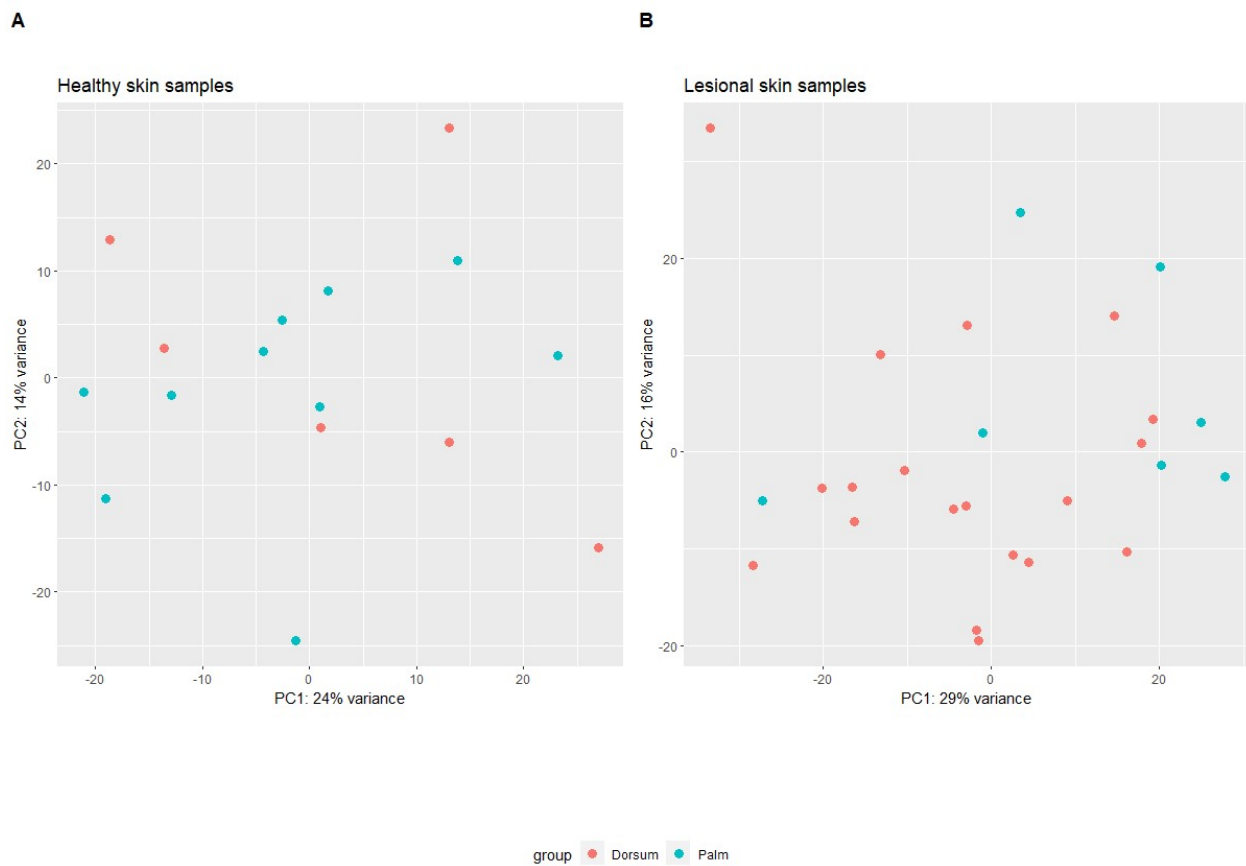
Supplementary figures



Supplementary figure 1. The figure shows A) The number of reads having an open reading frame between skin areas (lesional: LS, non-lesional: NL, and healthy: NN). B) The average input read length according to skin area. C) the STAR Average mapped length according to skin area. D) The number of intronic reads between skin areas. Significance values are calculated by a Wilcoxon sum rank test (non-significant: ns, $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$; ***, $p < 0.0001$; ****).

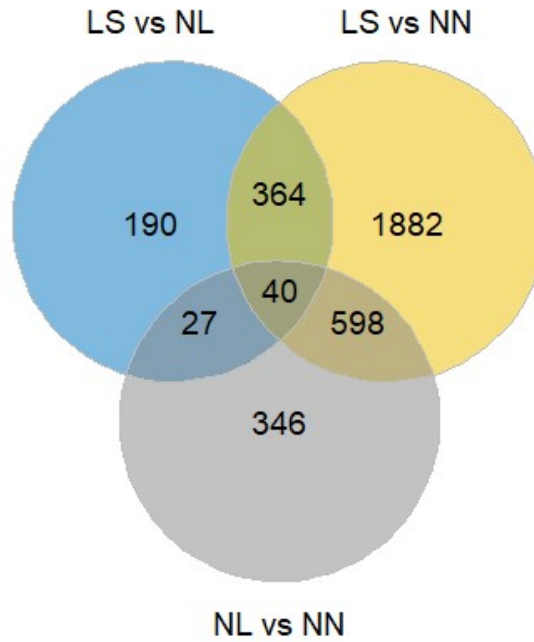


Supplementary figure 2. A) Number of reads in protein coding regions for healthy samples according to localization on the hands. No statistically significant difference (Wilcoxon sum rank test) was observed ($p=0.645$). B) Number of reads in protein coding regions for lesional samples according to localization on the hands. A statistically significant difference (Wilcoxon sum rank test) was observed ($p=0.035$)

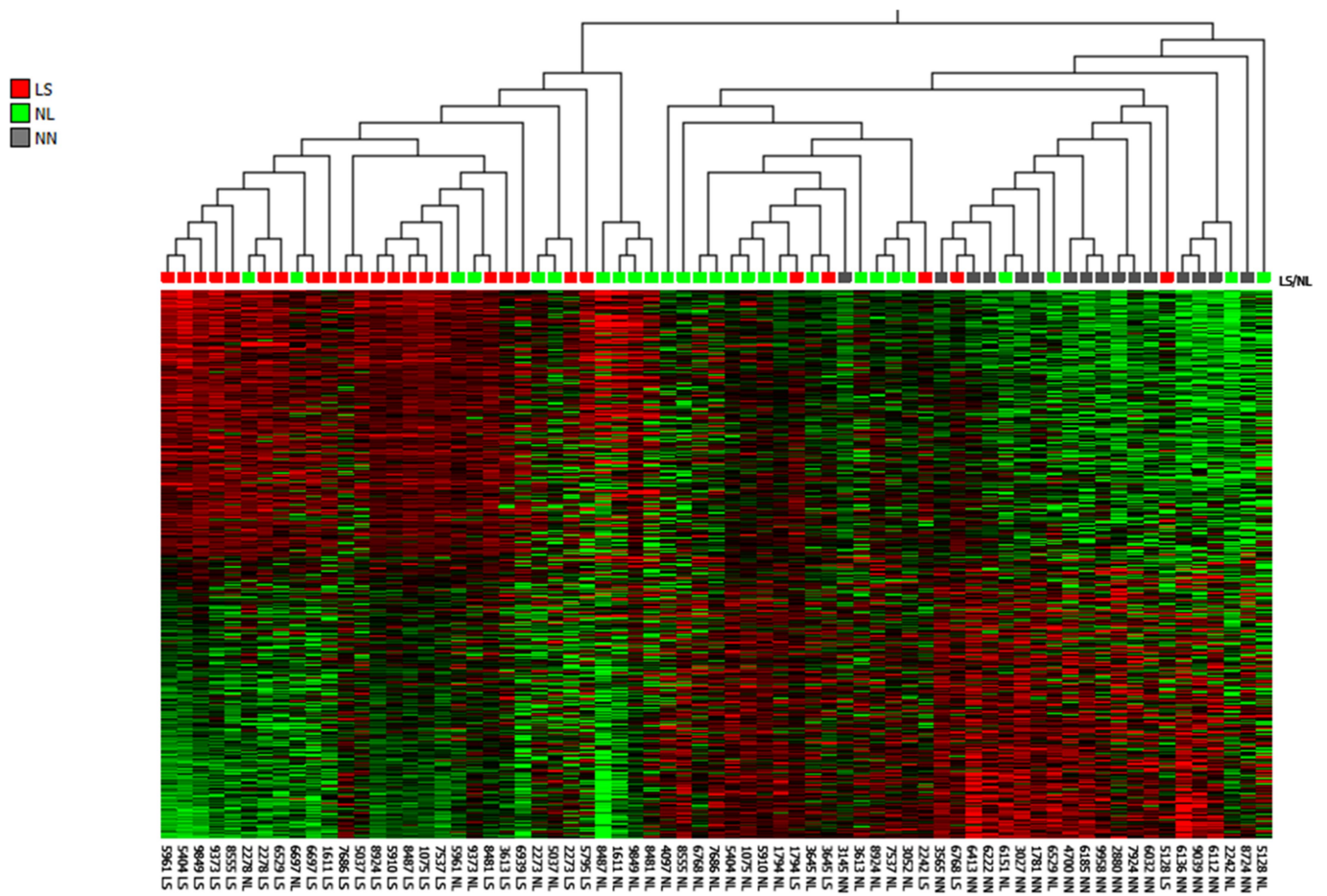


Supplementary figure 3. A) Principal component analysis (PCA) of the top 100 most differentially expressed genes of healthy skin on the hands do not show apparent clustering according to localization (dorsum and palm). B) PCA of the top 100 most differentially expressed genes of lesional skin on the hands do not show apparent clustering according to localization (dorsum and palm).

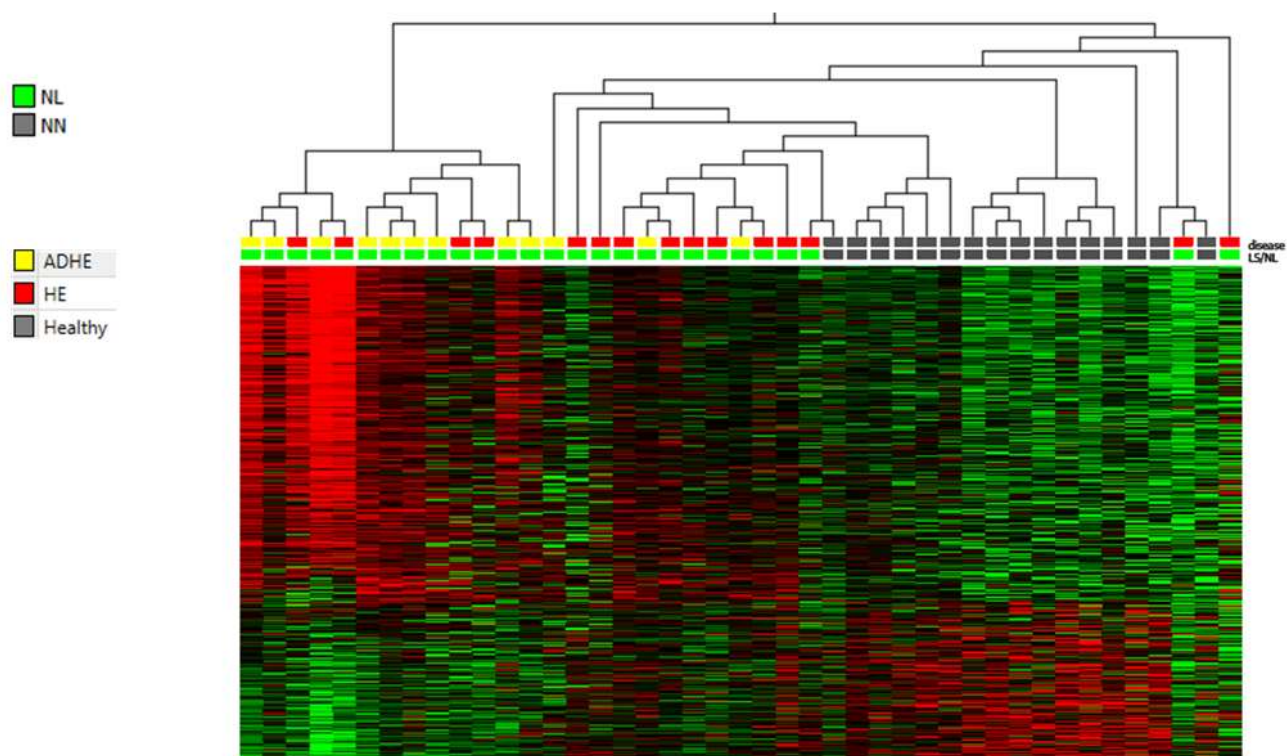
Up- and down-regulated DEGs



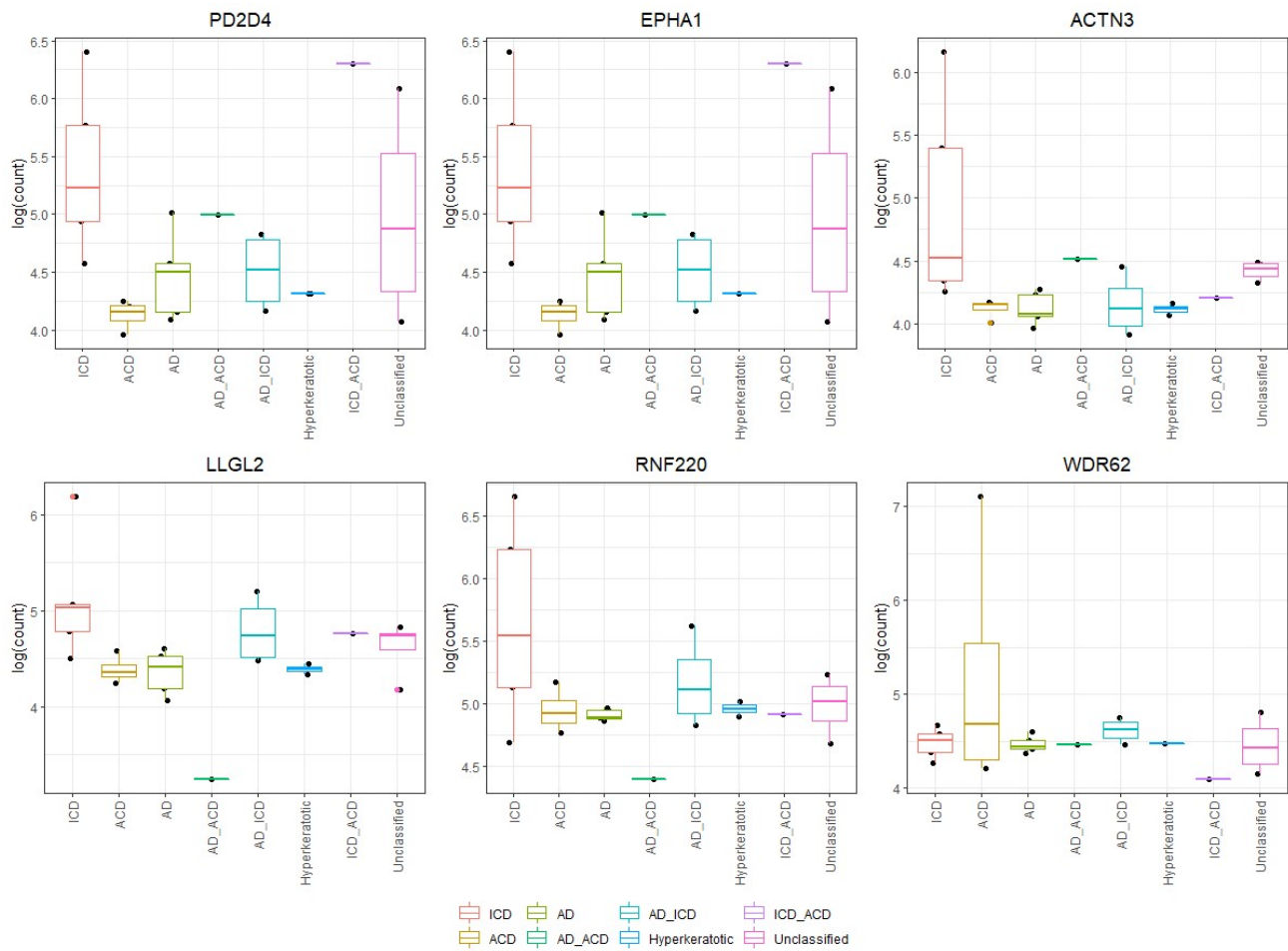
Supplementary figure 4. The figure shows A) A Venn diagram showing the number of differentially expressed genes between the different skin areas (lesional (LS), non-lesional (NL) and healthy (NN) skin). A total of 2 884 genes are differentially expressed between lesional and healthy skin, 1 012 genes between healthy and non-lesional and 620 genes between non-lesional and lesional skin areas. The diagram also shows that 364 genes were related to lesional skin, 598 to healthy skin and 27 to non-lesional skin areas



Supplementary figure 5 Unsupervised hierarchal clustering based on the 3441 differentially expressed genes between all skin areas (lesional (LS), non-lesional (NL), and healthy (NN)) ($FDR < 0.1$ and $FC > 2$).



Supplementary figure 6 Unsupervised hierarchal clustering based on the 1009 differentially expressed genes between HE with and without AD for non-lesional skin samples ($FDR < 0.1$, $FC > 2$).



Supplementary figure 7. The six differentially expressed genes between lesional skin areas of irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). Etiologies with an underscore refers to a mixed etiology.