Vesicular hand eczema transcriptome analysis provides insights into its pathophysiology

Angelique N. Voorberg1 | Hanna Niehues2 | Jart A. F. Oosterhaven1 | Geertruida L. E. Romeijn1 | Ivonne M. J. J. van Vlijmen-Willems2 | Piet E. J. van Erp2 | Thomas H. A. Ederveen3 | Patrick L. J. M. Zeeuwen2 | Marie L. A. Schuttelaar1

1Department of Dermatology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
2Department of Dermatology, Radboud Institute for Molecular Life Sciences (RIMLS), Radboud University Nijmegen Medical Center (Radboudumc), Nijmegen, The Netherlands
3Center for Molecular and Biomolecular Informatics, RIMLS, Radboudumc, Nijmegen, The Netherlands

Correspondence
Marie L. A. Schuttelaar, Department of Dermatology, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands. Email: m.l.a.schuttelaar@umcg.nl

Funding information
University of Groningen – J. P. Naterfonds

Abstract
Hand eczema is a common inflammatory skin condition of the hands whose pathogenesis is largely unknown. More insight and knowledge of the disease on a more fundamental level might lead to a better understanding of the biological processes involved, which could provide possible new treatment strategies. We aimed to profile the transcriptome of lesional palmar epidermal skin of patients suffering from vesicular hand eczema using RNA-sequencing. RNA-sequencing was performed to identify differentially expressed genes in lesional vs. non-lesional palmar epidermal skin from a group of patients with vesicular hand eczema compared to healthy controls. Comprehensive real-time quantitative PCR analyses and immunohistochemistry were used for validation of candidate genes and protein profiles for vesicular hand eczema. Overall, a significant and high expression of genes/proteins involved in keratinocyte host defense and inflammation was found in lesional vs. non-lesional palmar epidermal skin from a group of patients with vesicular hand eczema compared to healthy controls. Non-lesional patient skin showed no significant differences compared to healthy control skin. Lesional vesicular hand eczema skin shows a distinct expression profile compared to non-lesional skin and healthy control skin. Notably, the overall results indicate a large overlap between vesicular hand eczema and earlier reported atopic dermatitis lesional transcriptome profiles, which suggests that treatments for atopic dermatitis could also be effective in (vesicular) hand eczema.

KEYWORDS
barrier function, hand eczema, pathophysiology, RNA sequencing, transcriptomics
1 | INTRODUCTION

Hand eczema (HE) is a common skin disease, with a 1-year prevalence of up to 10% of the general population.1 Since HE has a big socioeconomic impact,2-3 so far, studies have focused on the treatment of this disease.4-7 However, hitherto limited research concentrated on the pathogenesis of HE. Publications are mainly restricted to epidemiological association studies and genetic investigations closely linked to atopic dermatitis (AD).8 For example, it was shown that mutations in the filaggrin gene (FLG) predict a persistent form of HE in patients with AD9,10 and others reported no association between FLG mutations and HE in individuals without AD.9,11

Hand eczema is a multifactorial skin disorder in which both endogenous and exogenous factors seem to play a role.12 Based on aetiology, HE can be classified into allergic contact dermatitis, irritant contact dermatitis, atopic HE, unclassified HE, protein contact dermatitis (PCD) and a combination of these. Morphologic classification of HE includes pulpitis, recurrent vesicular HE (VHE), hyperkeratotic HE, chronic fissured HE and nummular HE.12,13 VHE, which can be present without any known aetiological factors, is morphologically well characterized by frequent eruptions of vesicles on the palms, palmar or lateral aspects of the digits.13,14 Its episodic nature grants the opportunity to follow the development of disease morphology.

In this study, we performed RNA-sequencing (RNA-seq)15 to determine the transcriptome of vesicular lesional epidermis and non-lesional epidermis of VHE patients compared to epidermis from healthy controls. For a large set of either biologically relevant or highly up and downregulated genes according to RNA-seq, we validated mRNA expression by real time quantitative PCR (RT-qPCR). Additionally, for a subset of these candidate genes we studied protein level expression using immunohistochemistry (IHC). We here present the first transcriptome analysis of VHE lesion skin, which reveals a large overlap with the AD transcriptome based on previously reported studies. Non-lesional VHE skin, however, is not significantly different from healthy controls as is the case with non-lesional AD skin.16

2 | METHODS

2.1 Study design and participants

Skin biopsies were collected from 10 adult patients diagnosed with VHE, as defined by the Danish Contact Dermatitis Group and current guidelines.13,14 Exclusion criteria were usage of topical corticosteroids on the hands 2 days before taking biopsies; systemic treatment in the last 2 weeks; ultraviolet radiation therapy in the last 4 weeks; active bacterial, fungal or viral infection of the hands; other skin diseases of the hands; proven contact sensitization with clinical relevance to the hands, in which exposure to allergens is not avoided. To compare lesional (L) and non-lesional (NL) VHE skin samples with controls (C), biopsies from normal palmar skin were collected from 10 healthy adult subjects. Furthermore, patient and disease characteristics were collected. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee (Medical Ethical Review Board of the University Medical Center Groningen, reference: METc 2018/018) and with the Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all participants included in this study, including usage of photographs of the hands.

2.2 Biopsies

Biopsy locations were marked and photographed in advance. Two punch biopsies of 5 mm were taken from patients with VHE. The first biopsy was taken from lesional skin with visible vesicles, preferably with minimal other signs of inflammation. The second biopsy was taken from NL skin of the hands, defined as palmar skin without any vesicles and none or minimal other signs (e.g. erythema, squamae, infiltration). Both biopsies were taken with a minimum distance of 1 cm from each other. From healthy controls (C), the biopsy was taken from the hypothenar region of the non-dominant hand. Biopsies were cut in half; one half was formalin-fixed and embedded in paraffin for histological analysis. The epidermis of the second half of the biopsies was separated from the dermis by disperse (Roche) treatment for 24 h at 4°C as previously described,17 and the epidermis was subsequently frozen at −80°C until further use (for RNA isolation).

2.3 RNA isolation, RNA-sequencing and data analysis

RNA isolation was performed as described earlier.18 RNA quality control, Illumina TruSeq strand-specific mRNA polyA library preparation, whole transcriptome sequencing, sample demultiplexing and basic data analysis was performed by BaseClear B.V. (Leiden, The Netherlands). Sequencing was performed on an Illumina NovaSeq system with paired-end 150 cycles protocol and indexing. RNA-sequencing reads were adapter trimmed and quality-checked using Trim Galore version 0.6.2.19 Next, RNA-seq reads were mapped to the human reference genome using STAR aligner version 2.7.1.20 The aligner identifies splice junctions between exons based on the mapping results. Assignment of mapped reads to genomic features (counting) was performed with featureCounts version 1.6.3.21 The normalized counts per sample were calculated using the TPM (Transcripts Per Kilobase Million) method that takes into account the gene length and the sequencing library size.

2.4 Real-time quantitative PCR

RT-qPCR analysis was performed as described earlier.18 All primers were designed and used as described previously.22 Target
gene expression was normalized to the expression of the housekeeping gene human acidic ribosomal phosphoprotein P0 (RPLP0). The ΔΔCt method was used to calculate relative mRNA expression levels. The primers used in this study are listed in Table S1.

2.5 | Morphological and immunohistochemical analysis

Tissue sections of 6 μm of hand skin biopsies were stained with hematoxylin and eosin (Sigma-Aldrich, St. Louis, MO) or processed for IHC analysis. Sections were stained with antibodies using an indirect immunoperoxidase technique ( Vectastain, Vector Laboratories, Burlingame, CA) as previously described. An overview of all used antibodies in this study is presented in Table S2.

2.6 | Quantification of protein expression in IHC biopsy sections

Image acquisition of stained IHC biopsy sections was performed by a ZEISS Axiocam 105 colour Digital Camera and a 10× or 20× objective. The ZEISS Axiocam 105 colour is a compact 5 megapixel camera (2560 × 1920 pixels) for high-resolution images with a 1/2.5'' sensor. The images were stored in CZI format. The images were analysed with the cell image analysis software CellProfiler (Broad Institute). In CellProfiler different algorithms for image analysis are available as individual modules that can be modified and placed in sequential order to form a pipeline that can be used to identify and measure biological objects and features in images. Pipelines for Ki-67, loricrin, filaggrin and pan-LCE3 analysis were created (available on request). Data visualization and statistical analysis were performed using Instant Clue Software.

2.7 | Statistical analysis

Differential gene expression analysis was performed with DESeq2, with a two-factor design for paired samples (i.e. patient L vs. patient NL) and with a single-factor design for all other comparisons (i.e. healthy control vs. patient). The p-values for statistical differences between the contrasts were Benjamini-Hochberg multiple-testing corrected (FDR).

The fold-change in gene expression is expressed as $\log_2$ ratio in average expression for a gene between the sample groups. Quantitative PCR ΔCt values were analysed using one-way ANOVA Kruskal-Wallis analysis followed by Dunn’s post-hoc analysis to test for significant differences between L, NL and C skin. For quantification of protein expression data a Kruskal-Wallis test was conducted to examine the differences in cell counts and protein expression. For all tests, $p < 0.05$ was considered statistically significant.

| TABLE 1 | Patient characteristics |
| --- | --- |
| Cases | Age | Sex | Disease duration, years | Contact sensitization | Severity (photoguide) | Atopic comorbidities | Atopy | Contributing aetiological factors |
| **Cases** | **Age** | **Sex** | **Disease duration, years** | **Contact sensitization** | **Severity (photoguide)** | **Atopic comorbidities** | **Atopy** | **Contributing aetiological factors** |
| 1 | 26 | F | 1 | - | Moderate | - | - | - |
| 2 | 52 | M | 2 | - | Very severe | - | - | - |
| 3 | 56 | M | 6 | - | Moderate | - | - | - |
| 4 | 21 | F | 5 | - | Severe | - | - | - |
| 5 | 64 | M | 24 | - | Very severe | - | - | - |
| 6 | 29 | M | 1 | - | Moderate | - | - | - |
| 7 | 65 | F | 3 | - | Severe | - | - | - |
| 8 | 46 | F | 1 | - | Very severe | - | - | - |
| 9 | 65 | F | 15 | - | Severe | - | - | - |
| 10 | 64 | F | 3 | - | Very severe | - | - | - |
| Abbreviations: AR/AC, allergic rhinitis/conjunctivitis, based on the ARIA criteria; F, female; ICD, irritant contact dermatitis; M, male; pAD, previous atopic dermatitis, based on the UK criteria. |
| a | Atopy based on specific IgE inhalant allergens > 0.99 kU/L. |
| b | ≥ 1 positive patch test reaction to allergens from the European Baseline Series. |
| c | Based on the GINA guideline. |
| d | Based on the GINA guideline. |
3 | RESULTS

3.1 | Descriptive characteristics of the study volunteers

The study included 10 VHE patients and 10 healthy controls. All participants were Caucasian and the healthy controls were sex-matched with the patient group, 7 females and 3 males. The mean age was 48.8 years (range 21–65 years) and 55.2 years (range 40–64 years) for the patient group and controls, respectively. Patients had a mean age of onset of 42.4 years (range 16–62 years) with a mean disease duration of 6.4 years (range 1–24 years). Patient characteristics are shown in Table 1, and information of the control group can be found in Table S3, respectively. All patients showed moderate to very severe vesicular eruptions on the palms and palmar or lateral sides of the fingers, combined with erythema.

3.2 | RNA-sequencing data analysis shows distinct gene expression profiles for lesional compared to non-lesional skin and healthy control skin

Quantity and quality of epidermal RNA was checked by the sequence provider (data not shown), and 1 µg of each sample was used for library preparation for RNA-seq. For a detailed overview of all normalized gene counts per study sample see File S1. RNA-seq analysis yielded between 9.8 and 16.9 million reads obtained per sample (12.4 million on average). A large part of the reads (92.7%) was unique and could be mapped to the human genome (see RNA-seq data statistics in File S2). Multivariate gene expression analysis by principal component analysis (PCA) shows a distinct expression profile for lesional VHE samples (Figure 1). This PCA reflects the distances between study samples (L, NL and C) based on their individual gene expression profiles for all genes measured in the total dataset, showing that L samples are separated from the NL and C samples. In total, 28 614 genes were analysed of which 52 were differentially expressed according to the predetermined stringent criteria (for a complete overview of differentially expressed genes see the volcano plots at Figure S1). These criteria can be adjusted in the File S1 to alter the list of differentially expressed candidate genes. Of these 52 genes, 41 are upregulated, while 11 are downregulated. In contrast to lesional skin, a large overlap was observed between C and NL transcriptomes (Figure 2). Lesional skin of VHE patients showed significant upregulation of several genes involved in keratinocyte host defense and inflammation (e.g. LTF, LYZ, LCN2, LCE3A, PI3, and genes belonging to the S100 gene family: S100A7A, S100A7, S100A8 and S100A9), epidermal proliferation and differentiation (e.g. SPRR2A, SPRR2B, SPRR2D, SERPINA3, SERPINB3, SERPINB4, and keratin family members KRT6, KRT16 and KRT17), and immune signaling (MMP12, CHI3L2, CCL22, IL4R). A relative small number of genes was significantly downregulated in lesional skin, most notably LOR (epidermal differentiation), IL37 (immune signaling), and LCE1D/E (host defense). S100A7A was the most differentially expressed gene (fold-changes of 13.98 and 11.14 for L vs. NL and C, respectively). S100A7, S100A8 and S100A9 were also represented at the top of this list of most upregulated genes in lesional skin (Figure 2). In addition, we found a set of upregulated genes in lesional skin with a relationship to HE and AD (e.g. HAS3, CDH3, TNC, DPP4, C1R, C1S, CD207, TMEM173, CD1A), and also a number of upregulated genes that have not yet been described to be expressed in skin or related to HE or AD (e.g. HEPHL1, BIRC3, PRSS53, LCP1, LAPTMS, TYM, PARP9). In deeper downstream bioinformatics analysis of the transcriptomics data, Gene Set Enrichment Analysis (GSEA) based on GO terms hinted towards the involvement of adaptive immune system processes in lesional VHE skin, as a strong and significant enrichment of upregulated genes belonging to this GO:0002250 gene set was detected in comparison to matching non-lesional VHE skin samples (normalized enrichment score of 3.0 for NL to L, Gene Ratio of 0.5, FDR p = 0.0025, see Figure S2, and File S3 for more details).

3.3 | Real-time quantitative PCR for validation of RNA-seq data

To validate our RNA-seq results, we selected a number of genes that were found to be upregulated in lesional skin for replication with RT-qPCR. We also included genes that were reported to be up or downregulated in the existing HE literature, for example FLG, FLG-2, HRNR, KLF5, KLF7, CST6, KRT10, CLDN1, MKI67, and a set of cytokeratins.27–29 In addition we added three immune signalling genes that are known to be involved in AD (CCL17, TSLP and IL32). For description of all genes see Table S1. The same RNA samples included in the RNA-seq dataset were used for validation by RT-qPCR. For 13 candidate genes (including the household gene RPLP0), RNA yield was sufficient to perform validation on the complete set of samples (n = 10), while for the remaining set of 25 genes we used less samples in the C and NL group (n = 6) as shown in Table S4A–B.

RT-qPCR analyses confirmed that most of the investigated genes involved in epidermal differentiation were significantly upregulated (KRT6A, KRT14, KRT16, KRT17, SPRR2D, SERPINA3) in lesional VHE skin, in correspondence to the RNA-seq data (Figure 3A). Four epidermal differentiation genes (LOR, KRT2, FLG, FLG-2) were found to be significantly downregulated according to RT-qPCR analysis (Figure 3A), as well as in our RNA-seq analysis (fold-changes are respectively ~3.16, ~2.97, ~1.41, and ~2.24) as shown in Figure 2 or calculated from File S1. We found no significant differential expression for MKI67, HRNR, KRT9, KRT10, CLDN1, and CST6, by RT-qPCR analysis. The significant differential expression of keratinocyte host defense and inflammation genes in the RNA-seq dataset (contrast: C vs. L) was also confirmed by RT-qPCR analysis (Figure 3B). This set of genes is not only differentially expressed but is also the most abundant set of genes in lesional palmar skin as shown in the right columns of Figure 2 (gene expression) and in Table S4. The late cornified envelope genes (LCE3A, LCE1D and LCE1E), which were found to be significantly up or downregulated in the RNA-seq dataset (with
fold-changes of 5.80, −3.38 and −2.91, respectively), could not be reproduced by RT-qPCR.

The last group of genes that we validated concerns molecules involved in immune signalling: MMP12, CHI3L2, IL4R, and CCL22. RT-qPCR analysis confirmed their significant upregulation in lesional skin (Figure 3C). IL32 and CCL17, two genes that were not included in the RNA-seq data list of 52 differentially expressed genes (as they did not reach the threshold of >500 averaged normalized counts between the groups and, thus, are only very lowly expressed), were found to be significantly upregulated in lesional skin (Figure 3C). From File S1 fold-changes of 5.17 and 6.99 were calculated for both genes. Finally, in line with the RNA-seq results, IL37 was found to be downregulated in RT-qPCR analysis, and the kallikreins KLK5 and KLK7 showed no significant differences according to both methods (both genes showed a slight downregulation in the RNA-seq dataset with fold-changes of −1.86 and −0.57).

3.4 Immunohistochemical analysis confirms transcriptome data

To confirm the gene expression data at the protein level, we performed IHC analyses of all samples in our cohort (n = 10, for each of each study group). An example of the clinical manifestation of lesional VHE skin and the location of the biopsy punch is shown in Figure 4A (the location of a biopsy in non-lesional patient skin is shown in Figure S3). The clinically characteristic vesicles of lesional skin are clearly visible in the H&E staining (Figure 4B upper left). Large vesicles with infiltrate are present in the highly acanthotic epidermis. Epidermal thickness is significantly increased in L compared to C and NL skin, but no difference was observed between C and NL skin, as shown in Figure S4A. We also determined the targeted expression of specific proliferation, differentiation, host defense and immune signalling proteins (Figure 4B).

Proliferation of keratinocytes is similar for VHE lesional skin and healthy control skin (Figure S4B). The epidermal differentiation- and skin barrier-associated proteins filaggrin, hornerin and loricrin show decreased expression levels in lesional skin of VHE patients (Figure 4B). This is also reflected by the disruption of the granular epidermal layer, which is likely affected by the vesicle formation within the epidermis. We further quantified IHC images for protein expression of filaggrin, loricrin and LCE3, which were all three strongly reduced in the L group (Figure S4C–E). Moreover, we found that based on these three characteristic keratinocyte proteins, NL skin is significantly different from L skin, but closely resembles C skin (Figure S4C–E). Cystatin M/E protein was absent in all palm biopsies. We also stained for three keratin proteins as some keratins were differentially expressed (up or downregulated) on mRNA level as described above (see also Figure 3 and Table S4). Total amount of keratin 10 protein might be elevated in L skin, due to epidermal acanthosis, but its expression profile in NL is comparable to C skin (data not shown). In contrast, lesional skin shows strong reduction of keratin 2 protein compared to C skin, not restricted to the vesicular area. This nicely reflects its gene expression pattern we found in the transcriptome analyses (fold-change = −2.97) and qPCR analysis (see Figure 3). Keratin 16 is clearly present in lesional skin only. The H&E staining showed infiltrating cells in the dermis, the epidermis and in the vesicles of lesional skin (Figure 4). We additionally stained CD45, a leukocyte marker, to reveal infiltration, inflammation and migration of T-cells from the dermis up to the epidermis in lesional skin. In inflammatory skin diseases like AD, the level of antimicrobial proteins (AMP) is elevated in the skin lesions. Therefore, we determined the expression of psoriasin, S100-A8, SKALP and LCE3 (Figure 4). For all of these AMP, except for LCE3, we found that expression was absent in C and NL skin and only induced in the lesional skin samples. The amount of protein was especially high for psoriasin and S100-A8, and notably, most suprabasal keratinocytes expressed these two proteins. Total LCE3 antimicrobial protein per biopsy is expected to be greater in lesional skin, as the epidermis is substantially thicker, however quantification reveals that the percentage of LCE3 protein is lower in L compared to NL and C epidermis (Figure S4E). In contrast to elevated mRNA expression for LCE3A (Figures 2 and 3), total LCE3 protein (LCE3A-E) seems to be reduced in lesional skin.
| Gene     | Fold-change | Gene Expression |
|----------|-------------|-----------------|
|          | C vs. NL    | NL vs. L        | C vs. L    | C     | NL     | L     |
|          |             |                 |            |       |        |       |
| S100A7   | 5.79        | 11.14           | 13.98      | 1     | 736    | 12579 |
| S100A9   | 8.05        | 7.86            | 11.23      | 78    | 20706  | 195308|
| S100A8   | 6.91        | 7.52            | 10.26      | 135   | 16280  | 172181|
| SPRR2A   | 6.61        | 6.41            | 9.40       | 10    | 980    | 7089  |
| SERPINB4 | 3.81        | 7.99            | 10.12      | 17    | 904    | 20373 |
| MMP12    | 5.50        | 7.60            | 8.79       | 7     | 315    | 3207  |
| LTF      | 4.36        | 8.19            | 9.00       | 8     | 1256   | 4431  |
| S100A7   | 6.64        | 4.34            | 8.53       | 338   | 33638  | 130507|
| HEPHL1   | 3.05        | 6.44            | 8.35       | 5     | 155    | 1841  |
| CHI3L2   | 5.16        | 4.95            | 7.27       | 74    | 2637   | 11859 |
| SPRR2B   | 5.93        | 3.76            | 7.39       | 37    | 2275   | 6479  |
| LYZ      | 3.54        | 4.64            | 6.49       | 33    | 382    | 3087  |
| SERPINA3 | 4.17        | 3.68            | 6.79       | 33    | 587    | 3751  |
| SPINK6   | 2.65        | 5.38            | 6.08       | 34    | 212    | 2366  |
| KRT6G    | 2.36        | 4.75            | 6.70       | 786   | 4036   | 84840 |
| LCN2     | 4.79        | 2.80            | 5.87       | 17    | 476    | 1049  |
| BIRC3    | 3.83        | 4.15            | 5.44       | 27    | 386    | 1241  |
| LCE3A    | 3.25        | 4.19            | 5.80       | 51    | 489    | 2971  |
| KRT6A    | 2.49        | 4.16            | 5.78       | 5951  | 33438  | 340387|
| PRSS53   | 3.15        | 3.78            | 5.49       | 37    | 330    | 1743  |
| KRT6B    | 1.21        | 4.44            | 5.48       | 1671  | 3864   | 77361 |
| SPRR2D   | 3.07        | 2.79            | 4.84       | 444   | 3718   | 13221 |
| CCL22    | 2.35        | 3.60            | 4.72       | 84    | 431    | 2308  |
| PI3      | 2.04        | 3.12            | 4.94       | 208   | 857    | 6640  |
| CLS      | 1.52        | 3.88            | 4.34       | 74    | 213    | 1564  |
| LCPI     | 1.99        | 3.10            | 4.21       | 205   | 814    | 3935  |
| KRT16    | 0.87        | 3.94            | 4.41       | 12731 | 23289  | 280551|
| IL4R     | 1.82        | 2.91            | 4.02       | 98    | 347    | 1659  |
| CD1A     | 2.04        | 2.14            | 3.66       | 110   | 454    | 1455  |
| TMEM173  | 1.20        | 2.93            | 3.70       | 96    | 221    | 1302  |
| CD207    | 1.91        | 2.23            | 3.28       | 208   | 782    | 2109  |
| LAPTM5   | 1.55        | 2.38            | 3.37       | 118   | 345    | 1263  |
| C1R      | 1.16        | 2.81            | 3.30       | 278   | 622    | 2840  |
| TYMP     | 0.90        | 3.01            | 3.32       | 265   | 496    | 2760  |
| HAS3     | 0.96        | 2.81            | 3.46       | 177   | 343    | 2020  |
| PARP9    | 1.38        | 2.47            | 3.29       | 122   | 318    | 1240  |
| DPP4     | 0.94        | 2.76            | 3.37       | 111   | 211    | 1184  |
| SERPINB3 | 0.99        | 2.54            | 3.47       | 2375  | 4730   | 27302 |
| TNC      | 0.21        | 3.24            | 3.24       | 332   | 383    | 3249  |
| CDH3     | 0.50        | 2.72            | 3.06       | 878   | 1241   | 7584  |
| KRT17    | -1.14       | 4.09            | 2.85       | 6409  | 2513   | 48150 |

( between contrasts ) ( for each group )
4 | DISCUSSION

Here we report the first RNA-seq analysis of the VHE transcriptome. Overall, high expression of genes/proteins involved in keratinocyte host defense and inflammation was found in lesional VHE skin, with statistically significantly differential expression compared to healthy skin or internal controls for many of these candidates. Furthermore, molecules involved in epidermal differentiation were specifically up or downregulated, while immune signalling genes were upregulated in lesional skin, albeit with relatively low expression levels.

Previously, proteome analysis of lesional palmar skin of patients with chronic HE and healthy controls revealed that several skin barrier proteins, most notably filaggrin, filaggrin-2 and hornerin, were downregulated in diseased skin compared to unaffected healthy skin, suggesting that skin barrier dysfunction plays a role in the pathogenesis of chronic HE. In another study, using a targeted approach on six candidate skin barrier genes in patients diagnosed with chronic HE, normalization (upregulation) was found for the mRNA expression levels of FLG, LOR and KRT10 after treatment with alitretinoin, which is indicated for treatment of severe chronic HE. Although both studies are limited in size and only focus on a small predefined set of genes and proteins, the expression levels of the studied skin barrier molecules are in agreement with our data. In contrast to both studies, we could not confirm upregulation of TSLP in lesional skin, nor downregulation of the cysteine protease inhibitor CST6 and the serine proteases KLK5 and KLK7. Moreover, we observed complete absence of cystatin M/E protein expression in the hand palm biopsies, which is remarkable as this protein is strongly expressed in the stratum granulosum of keratinocytes at other skin locations. Proportion of keratinocytes was similar between groups, as demonstrated by the MKI67/Ki-67 gene and protein expression levels. This is in contrast to a recent study, in which epidermal hyperproliferation and an increased number of Ki-67 positive cells was found in hyperkeratotic HE. This might be explained by the studied HE subtype that is characterized by hyperkeratosis and desquamation. To identify chronic HE phenotypes, the mRNA expression of a set of genes involved in skin barrier function was recently studied using qPCR. Genes involved in filaggrin degradation, natural moisturizing factor synthesis, and structural barrier genes were downregulated suggesting that skin barrier dysfunction is an important parameter to discriminate chronic HE patients. However, a comparison with our data is difficult, since Tauber et al. analysed lesional dorsal skin, whereas we studied lesional palmar skin. Furthermore, this study did not compare lesional HE skin with non-lesional and healthy control skin.

The most differentially upregulated gene in lesional VHE skin, S100A7A, has previously been identified in inflamed hyperplastic psoriatic skin and is being expressed throughout the epidermis of chronic AD. Other S100-family members (S100A7, S100A8 and S100A9), which we also found to be highly expressed in lesional VHE skin, are known to be involved in the regulation of a broad range of cellular processes, and show antimicrobial properties as well. It has been suggested that these S100 calcium binding proteins promote cytokine production, which is critical in the inflammatory response, and lead to decreased filaggrin and loricrin expression levels in the epidermis. Enhanced antimicrobial and innate defense responses attenuates skin barrier function. Our RNA-seq data also shows highly increased expression levels of host defense genes such as PI3, LCN2, LTF, and LYZ, which may contribute to an impaired skin barrier function in VHE.

The most downregulated gene in the VHE transcriptome is c5orf46, also known as skin and saliva secreted protein 1 (SBSIP1), which is located on chromosome 5q32 in between the family of SPINK genes. Its function is still unknown, but it is hypothesized that c5orf46 interacts with TMBIM6, which modulates endoplasmic reticulum calcium homeostasis. Calcium homeostasis might play an important role in the pathogenesis for multiple skin disorders, including AD, since it is considered to be a central regulator for keratinocyte differentiation.

As we have successfully validated the reliability of our RNA-seq data by RT-qPCR and IHC, we believe it’s valid to discuss and evaluate genes from this dataset that have not been analysed by RT-qPCR. One such example is FABP7, a lipid-associated gene which is significantly downregulated in VHE. Decreased expression of FABP7 mRNA has recently also been reported for psoriasis and for a form of autosomal recessive congenital ichthyosis. Fatty acid binding proteins (FABP) are involved in long-chain fatty acid metabolism, by facilitating their delivery to intracellular sites. The upregulation of family member FABP5 in AD and psoriasis is thought to be a response to the abnormal differentiation and proliferation of keratinocytes. Therefore, the decreased expression levels of FABP7 might suggest that an impaired lipid metabolism is a contributing factor in HE pathogenesis as well.

In addition, we found DPP4 to be upregulated in lesional VHE. DPP4 is an intrinsic membrane glycoprotein, which was found to be inducted by type 2 cytokines like IL-4 and IL-13 in type 2 inflammation disorders, including AD, asthma and chronic rhinosinusitis, and has been opted to be a biomarker in type 2 inflammation. One gene that was strongly upregulated in the VHE transcriptome, SPINK6, may also play an important role in skin barrier function. The protein encoding this gene, serine peptidase inhibitor Kazal-type 6, has been reported to control the protease activity of kallikreins (KLK5, KLK7 and KLK14) that regulate skin desquamation by cleaving corneodesmosomal components.
FIGURE 3 Gene expression of VHE skin. RT-qPCR data represents fold differences in mRNA expression of NL and L skin compared to C (set to 1) of (A) epidermal proliferation and differentiation genes, (B) keratinocyte host defense genes and (C) immune signalling related genes. p-values of one-way ANOVA statistical analysis comparing all three contrasts are indicated with *p < 0.05, **p < 0.001, and ***p < 0.0001. Asterisks above L plots only represent significance of these contrasts compared to C (we found no significant differences between NL and C). Significance between NL and L are indicated by asterisks above the line.

Data are represented as mean ± SD
between enzyme activity of serine proteases and their inhibitors might be an contributing factor in the pathogenesis of HE. However, SPINK6 expression is reported to be decreased in AD lesional skin. Theories on HE pathogenesis are mostly based on AD literature, which mainly focuses on skin barrier defects. The link between HE and AD is still largely unknown, but AD is certainly a risk factor for developing HE. Our data on the VHE skin transcriptome are largely comparable to published gene expression data of AD lesional skin (Table S5). Both are predominantly characterized by the upregulation of keratinocyte host defense and inflammation genes, immune signalling genes, and the downregulation of several skin barrier related genes. The similarities in gene expression may indicate that new treatments for AD might also be effective for HE, as current treatment options for severe HE are limited. Most of the novel therapies for AD are focusing on pathway-targeted therapies, for example dupilumab, a monoclonal antibody that inhibits signalling of the hallmark AD cytokines IL-4 and IL-13. In the RNA-seq data set, IL4R was found to be highly upregulated in lesional HE skin vs. healthy control skin. Noteworthy, dupilumab has been found to be effective in HE patients with AD, and in patients with isolated hand eczema.54 Furthermore, JAK3 was also found to be upregulated in lesional HE skin vs. healthy control skin. However, this gene did not reach the threshold of >500 averaged normalized counts between the groups and, thus, is only very lowly expressed. This finding still may be promising for pan-Janus Kinase (JAK) inhibitors, e.g. gusacitinib and the topic JAK-inhibitor delgocitinib. Future research should focus on similar pathways in HE.

In AD lesional skin, KRT6, KRT16 and KRT17 are highly expressed, while KRT1 and KRT10 are downregulated, most likely due to suppression by IL-4 and IL-13. In HE, we also found high expression...
levels of KRT6, KRT16 and KRT17, but we did not observe downregulation of KRT10 gene expression, which we confirmed by protein expression analysis. On the other hand, we observed a very significant downregulation of KRT2 gene and protein expression, which has not been reported in AD literature. Absence of KRT2 might lead to disturbance of the epidermal integrity of plantar skin, which suggests that this protein has an important role in HE pathogenesis.

It is well known that non-lesional AD skin significantly differs from healthy skin, including delayed and intermittent expression of barrier genes and their proteins (e.g. LOR, FLG) and increased expression of multiple immune-related genes (e.g. IL-13, CCL17, CCL22, S100A7). In the NL skin transcriptome of VHE, no significant differences with healthy skin were found, which is striking as it has been hypothesized that a primary dysfunctional epidermal barrier is one of the key pathways for developing HE.

One of the limitations of our study is the small sample size of ten HE patients vs. ten healthy controls. Secondly, it is important to note that we used stringent criteria for the RNA-seq data as presented in Figure 2 (fold-change ≤−3 or ≥3, average normalized counts). We could not to compare our study to other HE studies in literature, since these reports included other subtypes or non-specified subtypes of HE, and different techniques such as immunofluorescence staining.

In conclusion, we have identified a significantly different gene expression profile in palmar lesional skin of VHE patients, compared to non-lesional skin and skin of healthy controls. The large overlap between the transcriptome of VHE and AD lesional skin suggests that treatments for AD might be also effective in HE, at least in VHE. Transcriptome analysis of HE subtypes other than VHE could provide insight into specific differences between HE subtypes, which may lead to a new classification of HE based on endophenotypes, as suggested for AD.

ACKNOWLEDGEMENTS
We thank all patients and volunteers that have participated in this study. This study was partially funded by the University of Groningen - J. P. Natterfonds. This research did not receive any other grants from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST
Dr. Schutteelaar received a research grant from Sanofi Genzyme; received consultancy fees from Sanofi Genzyme and Regeneron Pharmaceuticals; and is advisory board member for Sanofi Genzyme, Regeneron Pharmaceuticals, Pfizer, Abbvie, LEO Pharma, Lilly. The other authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION
All authors have read and approved the final manuscript. Conceptualization: M.L.A.S., P.L.J.M.Z., A.N.V., H.N. (equal). Methodology: M.L.A.S. (lead), P.L.J.M.Z. (lead), A.N.V. (lead), H.N. (lead), T.H.A.E. (supporting). Software: T.H.A.E. (lead). Validation: all authors (equal). Analysis: H.N. (lead), P.L.J.M.Z. (supporting), T.H.A.E. (supporting), Investigation: all authors (equal). Resources: all authors (equal). Data curation: all authors (equal). Writing – original draft: A.N.V. (lead), H.N. (lead), M.L.A.S. (supporting), P.L.J.M.Z. (supporting). Writing – review & editing: M.L.A.S. (lead), P.L.J.M.Z. (lead), all other authors (supporting). Visualization: all authors (equal). Supervision: M.L.A.S., P.L.J.M.Z. (equal). Project administration: all authors (equal). Funding acquisition: A.N.V., M.L.A.S. (equal).

ORCID
Angélique N. Voorberg https://orcid.org/0000-0001-5061-894X
Hanna Niehues https://orcid.org/0000-0002-6954-6955
Jari A. F. Oosterhaven https://orcid.org/0000-0001-7214-7528
Geertruida L. E. Romeijn https://orcid.org/0000-0003-4408-8596
Ivonne M. J. J. van Vlijmen-Willems https://orcid.org/0000-0002-3522-2573
Piet E. J. van Erp https://orcid.org/0000-0002-6955-8817
Thomas H. A. Ederveen https://orcid.org/0000-0003-0068-1275
Patrick L. J. M. Zeeuwen https://orcid.org/0000-0002-6878-2438
Marie L. A. Schutteelaar https://orcid.org/0000-0002-0766-4382

REFERENCES
1. Thyssen JP, Johansen JD, Linneberg A, Menné T. The epidemiology of hand eczema in the general population - prevalence and main findings. Contact Dermatitis. 2010;62:75-87.
2. Politiek K, Oosterhaven JA, Vermeulen KM, Schutteelaar ML. Systematic review of cost-of-illness studies in hand eczema. Contact Dermatitis. 2016;75(2):67-76.
3. Agner T, Elsner P. Hand eczema: epidemiology, prognosis and prevention. J Eur Acad Dermatol Venereol. 2020;34(S1):4-12.
4. Diepgen TL, Andersen KE, Chosidow O, et al. Guidelines for diagnosis, prevention and treatment of hand eczema. JDDG J der Dtsch Dermatologischen Gesellschaft. 2015;13(1):e1-e22.
5. Lee GR, Maarouf M, Hendricks AK, Lee DE, Shi VY. Current and emerging therapies for hand eczema. Dermatol Ther. 2019;32(3):e12840.
6. Christoffers WA, Coenraads P-J, Svensson Å, et al. Interventions for hand eczema. Cochrane Database Syst Rev. 2019;4:CD004055.
7. Elsner P, Agner T. Hand eczema: treatment. J Eur Acad Dermatol Venereol. 2020;34(S1):13-21.
8. Ruff SM, 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.
9. Heede NG, Thyssen JP, Thuesen BH, Linneberg A, Johansen JD. Anatomical patterns of dermatitis in adult filaggrin mutation carriers. J Am Acad Dermatol. 2015;72(3):440-448.
10. de Jongh CM, Khenrova L, Verberk MM, et al. Loss-of-function polymorphisms in the filaggrin gene are associated with an increased susceptibility to chronic irritant contact dermatitis: a case-control study. Br J Dermatol. 2008;159(3):621-627.
11. Lerbaek A, Bisgaard H, Agner T, Ohm Kyvik K, Palmer CN, Menné T. Filaggrin null alleles are not associated with hand eczema or contact allergy. Br J Dermatol. 2007;157(6):1199-1204.
12. Coenraads PJ. Hand eczema. N Engl J Med. 2012;367(19):1829-1837.
13. 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.
14. Agner T, Aalto-Korte K, Andersen KE, et al. Classification of hand eczema. J Eur Acad Dermatol Venereol. 2015;29(12):2417-2422.
15. Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. Genome Res. 2008;18(9):1509-1517.

16. 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.

17. van Ruisen F, Jansen BJ, de Jongh GJ, Zeeuwen PL, Schalkwijk J. A partial transcriptome of human epidermis. Genomics. 2002;79(5):671-678.

18. Smits JP, Edeerveen TH, Rikken G, et al. Targeting the cutaneous microbiota in atopic dermatitis by coal via AHR-dependent induction of antimicrobial peptides. J Invest Dermatol. 2020;140(2):415-424.e10.

19. TrimGalore! www.bioinformatics.babraham.ac.uk/projects/trim-galore/. Accessed June 2, 2020.

20. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2012;29(1):15-21.

21. Liao Y, Smyth GK, Shi W. FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 2014;30(12):1923-90.

22. Zeeuwen PL, de Jongh GJ, Rodijk-Olthuis D, et al. Genetically programmed differences in epidermal host defense between psoriasis and atopic dermatitis patients. PLoS One. 2008;3(6):e2301.

23. Livak KJ, Schmittgen TD. Analysis of relative gene expression. Methods. 2001;25(4):402-408.

24. McQuin C, Goodman A, Chernyshev V, et al. Cell Profiler 3.0: next-generation image processing for biology. PLoS Biol. 2018;16(7):e2005970.

25. Nolte H, MacVicar TD, Tellkamp F, Krüger M. Instant clue: a software suite for interactive data visualization and analysis. Sci Rep. 2018;8(1):12648.

26. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.

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

28. Kumari V, Timm K, Kühl AA, Heine G, Worm M. Impact of systemic allertinoin treatment on skin barrier gene and protein expression in patients with chronic hand eczema. Br J Dermatol. 2016;175(6):1243-1250.

29. Politiek K, Loman L, Pas HH, et al. Hyperkeratotic hand eczema: eczema or not? Contact Dermatitis. 2020;83(3):196-205.

30. Suárez-Fariñas M, Ungar B, Correa da Rosa J, et al. RNA sequencing of atopic dermatitis transcriptome profiling provides insights into novel disease mechanisms with potential therapeutic implications. J Allergy Clin Immunol. 2015;135(5):1218-1227.

31. Wallmeyer L, Dietert K, Sochorová M, et al. TSLP is a direct trigger for T cell migration in filaggrin-deficient skin equivalents. Sci Rep. 2017;7(1):774.

32. Meyer N, Zimmermann M, Bürgler S, et al. IL-32 is expressed by human primary keratinocytes and modulates keratinocyte apoptosis in atopic dermatitis. J Allergy Clin Immunol. 2010;125(4):858-865.e10.

33. Cheer SM, Foster RH. Allertinoin. Am J Clin Dermatol. 2000;1(5):306-307.

34. Zeeuwen PL, van Vlijmen-Willems IM, Jansen BJ, et al. Cystatin M/E expression is restricted to differentiated epidermal keratinocytes and sweat glands: a new skin-specific proteinase inhibitor that is a target for cross-linking by transglutaminase. J Invest Dermatol. 2001;116(5):693-701.

35. Tauber M, Bérand E, Lourari S, et al. Latent class analysis categorizes chronic hand eczema patients according to skin barrier impairment. J Eur Acad Dermatol Venereol. 2020;34(7):1529-1535.

36. Wolf R, Ruzicka T, Yuspa SH. Novel S100A7 (psoriasin)/S100A15 (koebergserin) subfamily: highly homologous but distinct in regulation and function. Amino Acids. 2011;41(4):789-796.

37. Wolf R, Lewerenz V, Büchau AS, Walz M, Ruzicka T. Human S100A15 splice variants are differentially expressed in inflammatory skin diseases and regulated through Th1 cytokines and calcium. Exp Dermatol. 2007;16(8):685-691.

38. Gross SR, Sin CG, Barraclough R, Rudland PS. Joining S100 proteins and migration: for better or for worse, in sickness and in health. Cell Mol Life Sci. 2014;71(9):1551-1579.

39. Kim MJ, Im MA, Lee J-S, et al. Effect of S100A8 and S100A9 on expressions of cytokine and skin barrier protein in human keratinocytes. Mol Med Rep. 2019;20(3):2476-2483.

40. Lee Y, Jang S, Min J-K, et al. S100A8 and S100A9 are messengers in the crosstalk between epidermis and dermis modulating a psoriatic milieu in human skin. Biochem Biophys Res Commun. 2012;423(4):647-653.

41. Egawa G, Kabashima K. Multifactorial skin barrier deficiency and atopic dermatitis: essential topics to prevent the atopic march. J Allergy Clin Immunol. 2016;138(2):350-358.e1.

42. C5orf46 (UNQ472/PRO839) result summary | BioGRID. https://thebiogrid.org/133099. Accessed June 2, 2020.

43. Elsholz F, Harteneck C, Muller W, Friedland K. Calcium – a central regulator of keratinocyte differentiation in health and disease. Eur J Dermatol. 2014;24(6):650-661.

44. Rinnerthaler M, Streubel MK, Bishof J, Richter K. Skin aging, gene expression and calcium. Exp Gerontol. 2015;68:59-65.

45. Yu Z, Gong Y, Cui L, et al. High-throughput transcriptome and pathogenesis analysis of clinical psoriasis. J Dermatol Sci. 2020;98(2):109-118.

46. Murase Y, Takeichi T, Kawamoto A, et al. Reduced stratum corneum acylceramides in autosomal recessive congenital ichthyosis with a NIPAL4 mutation. J Dermatol Sci. 2020;97(1):50-56.

47. Lin M-H, Khynkyn D. Fatty acid transporters in skin development, function and disease. Biochim Biophys Acta. 2014;1841(3):362-368.

48. Siegenthaler G, Hotz R, Chatellardgruaz D, Jaconi S, Saurat JH. Characterization and expression of a novel human fatty acid-binding protein: the epidermal type (E-FABP). Biochim Biophys Res Commun. 1993;190(2):482-487.

49. Emson C, Pham T-H, Manetz S, Newbold P. Periostin and dipeptidyl peptidase-4: potential biomarkers of interleukin 13 pathway activation in asthma and allergy. Immunol Allergy Clin North Am. 2018;38(4):611-628.

50. Meyer-Hoffert U, Wu Z, Kantyka T, et al. Isolation of SPINK6 in human skin: selective inhibitor of kallikrein-related peptidases. J Biol Chem. 2010;285(42):32174-32181.

51. Lee N, Chipalkatti N, Zancanaro P, Kachuk C, Dumont N, Rosmarin M. Joining S100 proteins and atopic dermatitis expression and function. PLoS One. 2014;285(4):32164.

52. Beck LA, Thaçi D, Hamilton JD, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. N Engl J Med. 2014;371(2):130-139.

53. Guttmann-Yassky E, Bissonnette R, Ungar B, et al. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. J Allergy Clin Immunol. 2019;143(1):155-172.

54. Lee N, Chipalkatti N, Zancanaro P, Kachuk C, Dumont N, Rosmarin M. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. J Allergy Clin Immunol. 2019;143(1):155-172.

55. Lee N, Chipalkatti N, Zancanaro P, Kachuk C, Dumont N, Rosmarin M. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. J Allergy Clin Immunol. 2019;143(1):155-172.

56. Meyer-Hoffert U, Wu Z, Kantyka T, et al. Isolation of SPINK6 in human skin: selective inhibitor of kallikrein-related peptidases. J Biol Chem. 2010;285(42):32174-32181.
57. Totsuka A, Omori-Miyake M, Kawashima M, Yagi J, Tsunemi Y. Expression of keratin 1, keratin 10, desmoglein 1 and desmocollin 1 in the epidermis: possible downregulation by interleukin-4 and interleukin-13 in atopic dermatitis. *Eur J Dermatol*. 2017;27(3):247-253.

58. Fischer H, Langbein L, Reichelt J, Buchberger M, Tschachler E, Eckhart L. Keratins K2 and K10 are essential for the epidermal integrity of plantar skin. *J Dermatol Sci*. 2016;81(1):10-16.

59. Lan C-C, Tu H-P, Wu C-S, et al. Distinct SPINK5 and IL-31 polymorphisms are associated with atopic eczema and non-atopic hand dermatitis in Taiwanese nursing population. *Exp Dermatol*. 2011;20(12):975-979.

60. Wang X, Ye L, Lai Q, et al. Altered epidermal permeability barrier function in the uninvolved skin supports a role of epidermal dysfunction in the pathogenesis of occupational hand eczema. *Skin Pharmacol Physiol*. 2020;33(2):94-101.

61. Molin S, Vollmer S, Weiss EH, Ruzicka T, Prinz JC. Filaggrin mutations may confer susceptibility to chronic hand eczema characterized by combined allergic and irritant contact dermatitis. *Br J Dermatol*. 2009;161(4):801-807.

62. Molin S, Vollmer S, Weiss EH, Weisenseel P, Ruzicka T, Prinz JC. Deletion of the late cornified envelope genes LCE3B and LCE3C may promote chronic hand eczema with allergic contact dermatitis. *J Investig Allergol Clin Immunol*. 2011;21(6):472-479.

63. Bieber T, D’Erme AM, Aksis CA, et al. Clinical phenotypes and endophenotypes of atopic dermatitis: where are we, and where should we go? *J Allergy Clin Immunol*. 2017;139(4S):S58-S64.

64. Thijs JL, Strickland I, Bruijnzeel-Koomen CA, et al. Moving toward endotypes in atopic dermatitis: identification of patient clusters based on serum biomarker analysis. *J Allergy Clin Immunol*. 2017;140(3):730-737.

65. Bousquet J, Khaltava N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update in collaboration with the World Health Organization, GA2LEN and AllerGen. *Allergy Eur J Allergy Clin Immunol*. 2008;63:8-160.

66. Williams HC, Jburney PG, Pembroke AC, Hay RJ. The U.K. working party’s diagnostic criteria for atopic dermatitis. III. Independent hospital validation. *Br J Dermatol*. 1994;131(3):406-416.

67. Wilkinson M, Gonçalo M, Aerts O, et al. The European baseline series and recommended additions: 2019. *Contact Dermatitis*. 2019;80(1):1-4.

68. Global initiative for asthma. Global strategy for asthma management and prevention. www.ginasthma.org. Accessed June 30, 2020.

69. Ter Braak CJ, Smilauer P. *Canoco reference manual and user’s guide: software for ordination*, version 5.0. Ithaca, NY: Microcomputer Power; 2012.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

- App S1. Supplemental information
- File S1. RNA-seq results
- File S2. RNA-seq alignment statistics
- File S3. Supplementary table GSEA based on GO (BP)

How to cite this article: Voorberg AN, Niehues H, Oosterhaven JAF, et al. Vesicular hand eczema transcriptome analysis provides insights into its pathophysiology. *Exp Dermatol*. 2021;30:1775–1786. [https://doi.org/10.1111/exd.14428](https://doi.org/10.1111/exd.14428)