

Molecular diagnostics of psoriasis, atopic dermatitis, allergic contact dermatitis and irritant contact dermatitis

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Summary

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Conflicts of interest

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Background Microarray studies on the epidermal transcriptome in psoriasis and atopic dermatitis (AD) have revealed genes with disease-specific expression in keratinocytes of lesional epidermis. These genes are possible candidates for disease-specific pathogenetic changes, but could also provide a tool for molecular diagnostics of inflammatory skin conditions in general.

Objectives To analyse if gene expression signatures as found in purified epidermal cells from AD are also present in other eczematous conditions such as allergic and irritant contact dermatitis.

Methods We used real-time quantitative polymerase chain reaction, immunohistochemistry and bioinformatics to investigate gene expression in different forms of eczema. Normal epidermis and psoriatic epidermis were analysed for comparison.

Results Carbonic anhydrase II was highly induced in epidermis from all forms of eczema but not in psoriasis. Remarkably, the presumed neuron-specific Nel-like protein 2 showed a strong induction only in AD epidermis. Interleukin-1F9, elafin, β -defensin-2 and vanin-3 were strongly induced in psoriasis, but not in any type of eczema. High levels of the chemokines CCL17 and CXCL10 were predominantly found in epidermis of allergic contact dermatitis. The chemokine CXCL8 was highly expressed in psoriasis, AD and allergic contact dermatitis, but not in irritant contact dermatitis. Cluster analysis or multinomial logistic regression indicated that expression levels of a set of seven genes are a strong predictor of the type of inflammatory response.

Conclusions These observations contribute to molecular diagnostic criteria for inflammatory skin conditions.

Atopic dermatitis (AD) and psoriasis are two major chronic inflammatory skin diseases that show distinct differences with respect to clinical appearance of the lesions, itch, transepidermal water loss, surface pH and bacterial colonization/infection.¹ Several nonoverlapping genetic associations have recently been established for both diseases, including genes involved in adaptive immunity, innate immunity and skin barrier function.^{2–5} Although there are some histopathological similarities such as epidermal hyperproliferation, acanthosis and a pronounced T-cell infiltrate, a number of differences at the cellular and molecular level can be noted. The immune responses are markedly polarized towards Th1 and Th17 cytokine profiles in psoriasis,^{6,7} and a Th2 profile in AD, particularly in the (sub)acute

phase.⁸ Using expression profiling by microarrays or large-scale real-time quantitative polymerase chain reaction (qPCR), several studies have found striking differences between expressed genes in the epidermis of lesional skin from patients with psoriasis and with AD, which appear to be determined both by the cytokine environment and cell-autonomous processes.^{9–11} The transcriptome of lesional psoriasis skin is very similar to that of cultured keratinocytes stimulated with proinflammatory cytokines [interleukin (IL)-1 or IL-1/tumour necrosis factor (TNF)- α /interferon (IFN)- γ] as demonstrated by several groups.^{11,12} In contrast, it was shown that the transcriptome of AD skin is quite distinct and is similar to that of Th2 cytokine-stimulated cultured keratinocytes. Large differences between epidermal gene

expression in psoriasis and AD were found for antimicrobial proteins such as human β -defensin (hBD)-2, hBD-3, S100 proteins and elafin, for chemokines such as CCL18, CCL27, CCL13 and CXCL8, and for genes that do not have obvious functions in inflammation such as vanin-3 (VNN3), Nel-like 2 (NELL2) and carbonic anhydrase II (CAII).^{9–11,13–16} It was demonstrated that induction of these genes is likely to be under control of the specific psoriasis and AD cytokine environments.^{11,17,18} Antimicrobial proteins, many chemokines and VNN3 were found to be induced in cultured keratinocytes by psoriasis-associated cytokines (e.g. TNF- α , IL-1, IL-6, IL-17 and IL-22) whereas the AD-specific genes CAII and NELL2 were induced by Th2 cytokines (IL-4 and IL-13). Low expression levels of antimicrobial proteins in AD have been interpreted to contribute to the high prevalence of skin infections in AD compared with psoriasis.^{10,11,13} It remains to be investigated to what extent other highly differential genes, notably the psoriasis-associated vanin genes (VNN1 and VNN3), and the AD-associated genes NELL2 and CAII, contribute to the pathogenesis of these skin conditions. Based on the disease-specific patterns and the biological properties, there is sufficient reason to suspect that differentially expressed genes detected in the aforementioned studies are not merely innocent bystanders, but could have a causal relationship with the disease process. For this reason we wanted to investigate if AD-associated epidermal gene expression was specific for AD or could also be demonstrated in other eczematous conditions such as allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). ACD and ICD represent two common skin conditions that are mechanistically different but share clinical and histopathological similarities with AD such as itch, spongiosis and increased trans-epidermal water loss due to skin barrier disruption.^{19,20} Here we have compared gene expression patterns in lesional skin of patients with psoriasis and AD with experimentally induced eczematous lesions of ACD and ICD. Our results indicate that psoriasis is molecularly quite distinct from AD, ACD and ICD. The eczematous conditions, however, show similar expression patterns for some but not for all genes. Our results indicate that, using a set of only seven genes, a molecular distinction can be made between epidermis from normal skin, psoriasis, AD, ACD and ICD.

Materials and methods

Patients and healthy volunteers

Patients and healthy volunteers were recruited by the Dermatology clinic of the Radboud University Nijmegen Medical Centre. All diagnoses were made by a dermatologist. The study was approved by the local medical ethical committee (Commissie Mensgebonden Onderzoek Arnhem–Nijmegen) and conducted according to the Declaration of Helsinki principles. Written informed consent was obtained from each volunteer. Patients with chronic AD, diagnosed according to the criteria of Hanifin and Rajka,²¹ were in an active phase of disease for which they were hospitalized and biopsies were taken from subacute lesions. Care was taken to exclude vesicles or scratched skin.

All patients with psoriasis had moderate to severe plaque-type psoriasis. To induce ACD, patients with a known allergy were exposed for 48 h to nickel or perfume I (European standard series) on the lower back. Seventy-two hours after exposure 3-mm biopsies were taken from these patients with ACD, and healthy skin was used as a control. To induce ICD, 400 μ L of 5% sodium dodecyl sulphate (SDS) solution was applied on a 2 $\times$ 2 cm patch of absorbent nonwoven fabric (Medicomp; Hartmann, Heidenheim, Germany), which was subsequently placed on the lower back of healthy volunteers and covered with Tegaderm (3M Health Care, Neuss, Germany) and Soft Cloth Surgical Tape (3M Health Care) as described previously. After 4 or 8 h the patch with SDS was removed. Forty-eight hours after exposure, 3-mm biopsies were taken from these patients with ICD, and healthy skin was used as a control. Skin reactions to SDS application were found to be variable. Biopsies were taken only from individuals who showed a clear response as witnessed by strong erythema. Some individuals showed severe epidermal damage and a subsequent wound-healing response as assessed by histological analysis. These samples were excluded from the analysis.

Isolation of epidermal sheets and preparation of cells for flow cytometry

Isolation of epidermal sheets for mRNA isolation was performed as previously described.²² In short, a 3-mm punch biopsy was incubated for 4 h at 4 °C in phosphate-buffered saline (PBS) with $\text{Ca}^{2+}/\text{Mg}^{2+}$, containing 12 mg mL^{-1} Dispase (Roche Diagnostics, Basel, Switzerland) and 5 $\mu\text{g mL}^{-1}$ Actinomycin-D (Sigma-Aldrich, St Louis, MO, U.S.A.). After incubation, the epidermis was removed with tweezers and stored in Trizol (Invitrogen, Groningen, the Netherlands) at –80 °C until use. Cell suspensions for flow cytometry were prepared as described before.²³ Biopsies were incubated overnight in PBS containing 2.5 mg mL^{-1} trypsin (trypsin 250; Difco Laboratories, Detroit, MI, U.S.A.) at 4 °C. Ten per cent fetal calf serum (Harlan Sera-Lab, Loughborough, U.K.) was added and the epidermis and dermis were separated with tweezers. The epidermal side of the dermis was gently scraped to ensure detachment of basal cells. Both the epidermis and dermis were mixed on a vortex to allow detachment of all keratinocytes. Subsequently, the stratum corneum and dermis were removed and the remaining cells were pelleted and fixed by slowly adding 1 mL cold ethanol while vortexing at low speed. Cell suspensions were stored at –80 °C for at least 2 h.

RNA extraction and real-time quantitative polymerase chain reaction

Epidermal sheets were used for RNA extraction, and a DNase I treatment was performed following the manufacturer's protocol (Invitrogen, Carlsbad, CA, U.S.A.). Reverse transcriptase reactions were performed with 1 μg RNA as described previously.¹⁰ The reverse transcriptase reaction products were used for qPCR, which was performed with the MyiQTM

Single-Color Real-Time Detection System for quantification with SYBR Green Supermix (Biorad, Richmond, CA, U.S.A.) and melting curve analysis, as described previously.²³ DNA was PCR-amplified under the following conditions: 4.5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C, with data collection in the last 30 s. All primer concentrations were 300 nmol L⁻¹ in a total reaction volume of 25 µL. The amount of mRNA for a given gene in each sample was normalized to the amount of mRNA of the human ribosomal phosphoprotein P0 (RPLP0) reference gene in the same sample. The RPLP0 gene is, at least for skin biopsies, more suitable than commonly used genes such as actin or GAPDH, as RPLP0 correlates far better with 28S ribosomal RNA. Primers for qPCR were accepted only if their efficiency was 100 ± 10%, which means that the PCR product was doubled at each cycle and the threshold cycle (C_t) will be linear with the log of the initial concentration. Corrections were made for primer efficiency.

For graphical representation of relative mRNA expression levels we used the $\Delta\Delta C_t$ method.²⁴ Primer sequences are shown in Table 1. All primers were obtained from Biolegio (Nijmegen, the Netherlands).

Immunohistochemistry

Skin biopsies were immediately fixed in a 10% formalin solution (Baker Mallinckrodt, Deventer, the Netherlands) for 4 h and subsequently embedded in paraffin. Before staining with antibodies that recognize CD45 (Dako, Glostrup, Denmark), Ki67 (Immunotech, Fullerton, CA, U.S.A.) and keratin 16 (KRT16) (Monosan, Uden, the Netherlands), a citrate treatment was performed: sections were boiled for 10 min in a

microwave oven in a solution containing 1.8 mmol L⁻¹ citric acid and 8.2 mmol L⁻¹ sodium citrate. Six-micrometre sections were blocked for 15 min with PBS with 20% normal rabbit serum (for hBD-2), normal goat serum (for elafin) or normal horse serum (for CD45, Ki67 and KRT16) and subsequently incubated with anti-hBD-2 (Abcam, Cambridge, U.K.) in a 1 : 100 dilution, anti-elafin in a 1 : 500 dilution, anti-CD45 in a 1 : 100 dilution, anti-Ki67 in a 1 : 50 dilution, and anti-KRT16 in a 1 : 50 dilution for 1 h at room temperature. After washing in PBS, sections were incubated for 1 h with a secondary antibody (biotinylated rabbit antigoat, biotinylated goat antirabbit or biotinylated horse antimouse in PBS containing 1% bovine serum albumin; Vector Laboratories, Burlingame, CA, U.S.A.). After 30 min incubation with avidin-biotin complex (Vector Laboratories), sections were treated with 3-amino-9-ethylcarbazole (Calbiochem, San Diego, CA, U.S.A.) for 10 min. Nuclei were stained with Mayer's haematoxylin (Sigma-Aldrich), and sections were subsequently mounted in glycerol gelatin (Sigma-Aldrich).

Flow cytometry

Ethanol-fixed cells were washed with PBS/1% fetal calf serum and subsequently labelled with anti-CAII (Abcam) in a 1 : 500 dilution. After 30 min incubation at 4 °C, cells were washed and incubated with Alexa Fluor 488 goat antirabbit secondary antibody (Invitrogen). After 30 min incubation cells were washed, and resuspended in propidium iodide and 0.1% RNase (Sigma-Aldrich). The labelled cell suspensions were analysed on an EPICS Elite flow cytometer (Coulter, Luton, U.K.). A bandpass filter of 515–535 nm (green, fluorescein isothiocyanate) was used to measure emitted light, while a

Table 1 Primer sequences of all genes examined by real-time quantitative polymerase chain reaction

HUGO gene symbol	Description of gene/protein. In parentheses: name used in text	Forward primer	Reverse primer
CA2	Carbonic anhydrase II (CAII)	AACAATGGTCATGCTTTCAACG	TGTCCATCAAGTGAACCCAG
CCL17	Chemokine (C-C motif) 17, TARC, ABCD-2 (CCL17)	CAGGGATGCCATCGTTTTTG	ACTGCATTCTTCACTCTCTTGTTGTT
PTPRC	CD45 (CD45)	GGAACAAGCATCACAAGAGTACA	TGTTTCCGCACTTCTTAAGAGATT
IL8	Interleukin 8, IL-8, CXCL8 (CXCL8)	CTTGGCAGCCTTCCTGATTT	TTCTTTAGCACTCTTGGCAAAA
IL18	Interleukin 18 (IL-18)	ATCGCTTCCTCTCGCAACA	CATTGCCACAAAGTTGATGCA
CXCL10	IP-10, chemokine (C-X-C motif) ligand 10 (CXCL10)	TTCTGCAAGCCAATTTTGTC	TCTTCTCACCCCTCTTTTTCATTGT
KRT6A	Keratin 6A (KRT6)	AGAGAATGAATTTGTGACTCTGAAGAAG	TACAAGGCTCTCAGGAAGTTGATCT
DEFB4	Human β -defensin-2, hBD-2 (hBD-2)	GATGCCTCTCCAGGTGTTTTT	GGATGACATATGGCTCCACTCTT
IL1B	Interleukin 1 β , IL-1 β (IL-1 β)	AATCTGTACCTGTCTGCGTGTT	TGGGTAATTTTGGGATCTACACTCT
IL1F9	Interleukin 1 family member 9, IL-1F9 (IL-1F9)	AGGAAGGGCCGTCTATCAATC	GAACTGCCACAAGGTTCTGAC
NELL2	Nel-like 2 (NELL2)	TAAGGGTATAATGCAAGATGTCCAATT	AGATCTGGGCACTGAGCAATAAA
RPLP0	Ribosomal phosphoprotein P0 (RPLP0)	CACCATTGAAATCCTGAGTGATGT	TGACCAGCCCAAGGAGAAG
PI3	SKALP, elafin (elafin)	CATGAGGGCCAGCAGCTT	TTTAACAGGAACCTCCGTGACA
VNN1	Vanin-1 (VNN1)	GAACCCAGTATGTCTTTCTT	CATACAACCTCCCAACAGA
VNN3	Vanin-3 (VNN3)	GATCATTTCTAAGTGGGAGTCA	CGTCCATCTCTTGAAATCTCA

gate in the right angle scatter (a measure of regularity) vs. the forward scatter (a measure of size) was used to exclude debris. To test for CAII specificity, the CAII antibody was pre-incubated with excess CAII protein and added to epidermal cells instead of the primary antibody alone. Only a very few CAII-positive cells could now be detected by flow cytometry (not shown), indicating a high specificity of the CAII antibody.

Cluster analysis and statistics

All data were analysed with the Statistica software package v. 7.0 (StatSoft Inc., College Station, TX, U.S.A.) or SPSS v. 16.0 (SPSS Inc., Chicago, IL, U.S.A.). For the qPCR experiments, statistical analysis by ANOVA was performed on ΔC_t values corrected for primer efficiency. ΔC_t is the difference between the C_t of the target gene and the reference gene. These values are normally distributed. A Bonferroni *post-hoc* test was used to test the significance of the difference between each of the groups. Note that for graphical representation of the data (Fig. 1) we used the relative expression levels as described by Livak and Schmittgen.²⁴ Cluster analysis was performed on corrected ΔC_t values of seven selected genes (CAII, NELL2, hBD-2, IL-1F9, CXCL8, CXCL10 and CCL17). The values were subjected to a Z-transformation, where $Z = (x - \mu)/\sigma$. This will yield a matrix with a row mean of 0 and an SD of 1. After this transformation, the Euclidean distance is used as the dissimilarity measure. Samples were clustered by Ward's amalgamation rule. Polytomous logistic regression analysis, including forward selection procedure, was used to predict the diagnostic group based on the expression levels of the above-mentioned seven selected genes. Leave-one-out cross-validation was applied to obtain an estimate of the error rate that is not biased by overfitting.

Results

mRNA expression profiles in psoriasis, atopic dermatitis, allergic contact dermatitis and irritant contact dermatitis

Previous studies have identified a number of genes that are associated with psoriasis and AD. Some of these studies used microarrays without confirmation by qPCR or protein expression. We therefore first tested a number of these proposed candidate genes by qPCR to confirm microarray data as published previously. We focused on genes that were reported to be differentially expressed in two different studies,^{9–11} and gave robust signals in qPCR assays (Data S1 and Table S1; see Supporting Information). The following inflammation-associated genes were selected for further study by qPCR: the antimicrobial proteins elafin and hBD-2; the cytokines IL-1F9, IL-1 β and IL-18; the chemokines CCL17, CXCL8 and CXCL10; and the hyperproliferation-associated keratin 6 (KRT6). Based on two microarray studies and subsequent qPCR confirmation we selected four additional genes not known to have a function in inflammatory processes. These genes NELL2, CAII, VNN3 and VNN1 were among the most highly differential

genes in studies that compared psoriasis and AD gene expression. The skin conditions studied here can be quite diverse in their clinical manifestations, but care was taken to include lesions of similar clinical severity with respect to erythema. Lesions of all skin conditions were from strongly inflamed skin. ICD and ACD were by definition in an acute phase as they were induced for that purpose, the AD skin was from erythematous skin of individuals with chronic eczema during a relapse, and psoriasis biopsies were taken from chronic plaques that were nevertheless clearly inflamed. As the skin conditions studied here are likely to have varying amounts of cellular infiltrate in the epidermal compartment, we measured CD45 mRNA expression as a semiquantitative marker for cells of haematopoietic origin. Figure 1 shows the results of these analyses for mRNA expression of the most informative genes in epidermal sheets from normal skin and from individuals with psoriasis, AD, ACD and ICD. Our results confirmed findings from previous studies on AD and psoriasis, showing a strong increase of antimicrobial genes (hBD-2, elafin), IL-1F9 and vanins in psoriasis, whereas CAII and NELL2 were strongly increased in AD. Remarkably, hBD-2, elafin, IL-1F9 and VNN3 were quite specific for psoriasis. Although some induction was found in eczematous conditions compared with normal skin, the expression levels in psoriasis were significantly higher. The induction of CAII, first observed for AD and not for psoriasis, was also found for ACD and ICD. High expression of NELL2 was found only for AD, and a small but significant increase compared with normal skin was found in ACD. The functional significance of this highly specific AD-associated molecule for the disease process remains to be investigated. Two chemokine genes, CCL17 and CXCL10, showed high expression levels only in ACD epidermis. Although the number of infiltrating T lymphocytes is also higher in ACD than in any other of the conditions studied (see qPCR data in Fig. 1 and immunohistochemistry in Fig. 2) we assume that high levels of CCL17 and CXCL10 are derived from keratinocytes as T lymphocytes are not known to be strong producers of these chemokines. None of the studied molecular markers was specifically upregulated in ICD as shown in Figure 1. IL-1 β and CXCL8 were found to discriminate between ICD and the other inflammatory conditions, as ICD was the only condition that did not show strong induction of these genes compared with normal skin. IL-18 and KRT6 expression did not significantly discriminate between the four inflammatory conditions (not shown).

Protein expression of selected genes in normal skin, psoriasis, atopic dermatitis, allergic contact dermatitis and irritant contact dermatitis

Although qPCR is by far the most sensitive and accurate method for gene expression quantitation, these findings do not necessarily reflect protein expression levels. We used immunohistochemistry and flow cytometry to analyse a number of markers for which reliable staining protocols were available. Staining of hBD-2 and elafin confirmed the known

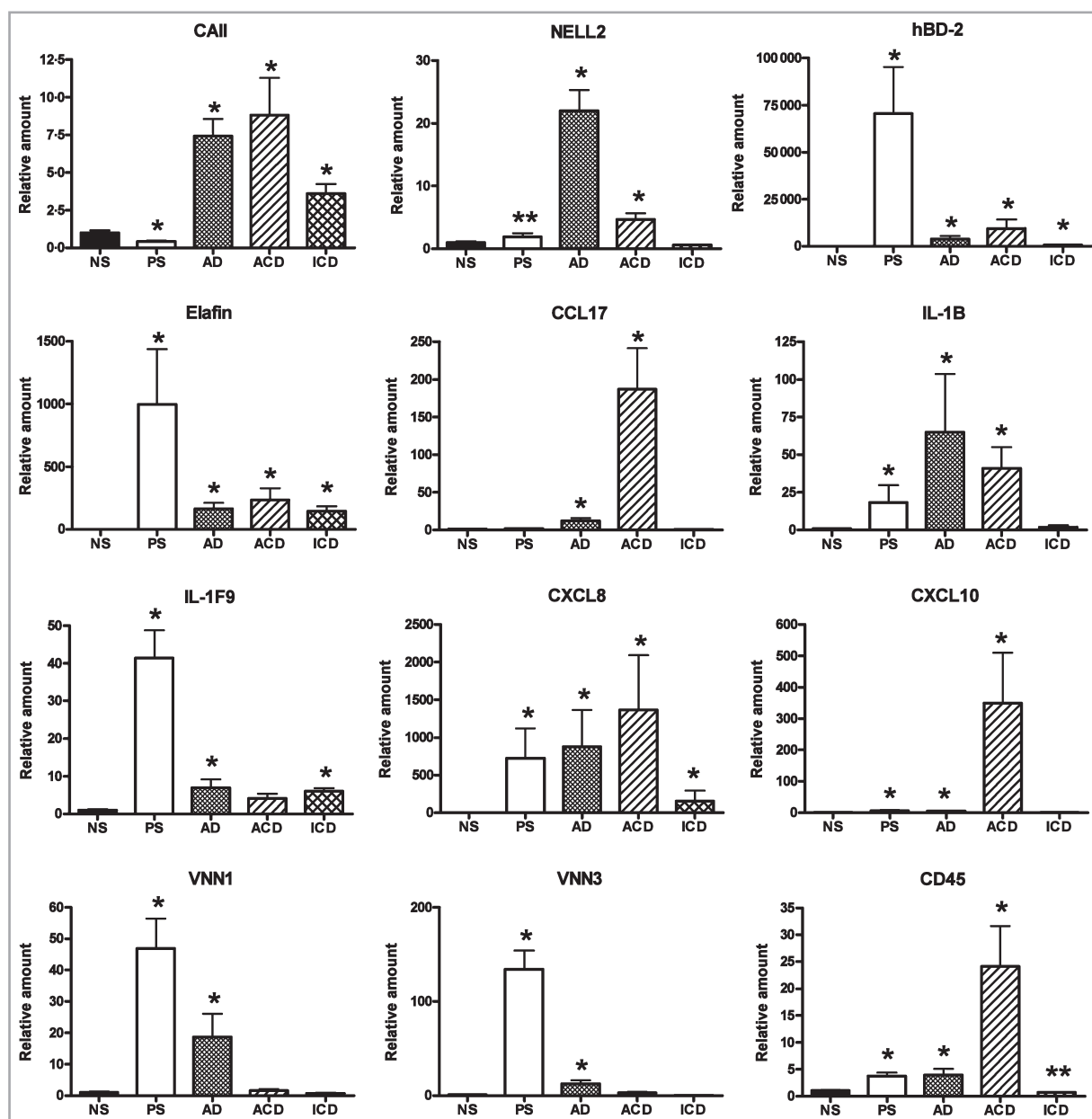


Fig 1. Real-time quantitative polymerase chain reaction data on 12 informative genes, i.e. genes that showed differential expression between the five diagnoses normal skin (NS), psoriasis (PS), atopic dermatitis (AD), allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD). Mean $\pm$ SD fold induction was expressed relative to normal skin (set at 1). Statistical analysis was performed on ΔC_t values. ANOVA showed that for each gene there was a significant effect of the factor diagnosis on the gene expression level. Post-hoc analysis (Bonferroni test) was used to analyse significant differences between diagnoses. * and ** indicate significant P-values of < 0.01 and < 0.05 , respectively, compared with normal skin.

high levels of expression in lesional skin of patients with psoriasis (Fig. 2). Sixteen of 17 patients with ACD showed no or hardly any hBD-2 staining. In all patients with AD and ICD hBD-2 staining was minimal to absent. Elafin, however, was strongly expressed in the stratum granulosum of all patients with ICD. Staining for elafin in patients with ACD and AD was variable and less intense. Normal skin was, as expected, always negative for hBD-2 as well as for elafin. In general there was a good correlation between mRNA and protein expression levels of elafin and hBD-2.

In earlier studies we showed that CAII mRNA and protein is upregulated in lesional skin of AD, whereas in psoriasis a downregulation of CAII mRNA is found.¹⁴ In Figure 1 of this study we show that CAII induction is found in all three eczematous conditions as it is also elevated in ACD and ICD. Immunohistochemistry, however, was not suitable to give reliable detection of quantitative differences in CAII staining between AD, ACD, ICD and normal skin (not shown). To investigate whether upregulation of CAII protein could be demonstrated in ACD, a flow cytometric analysis was

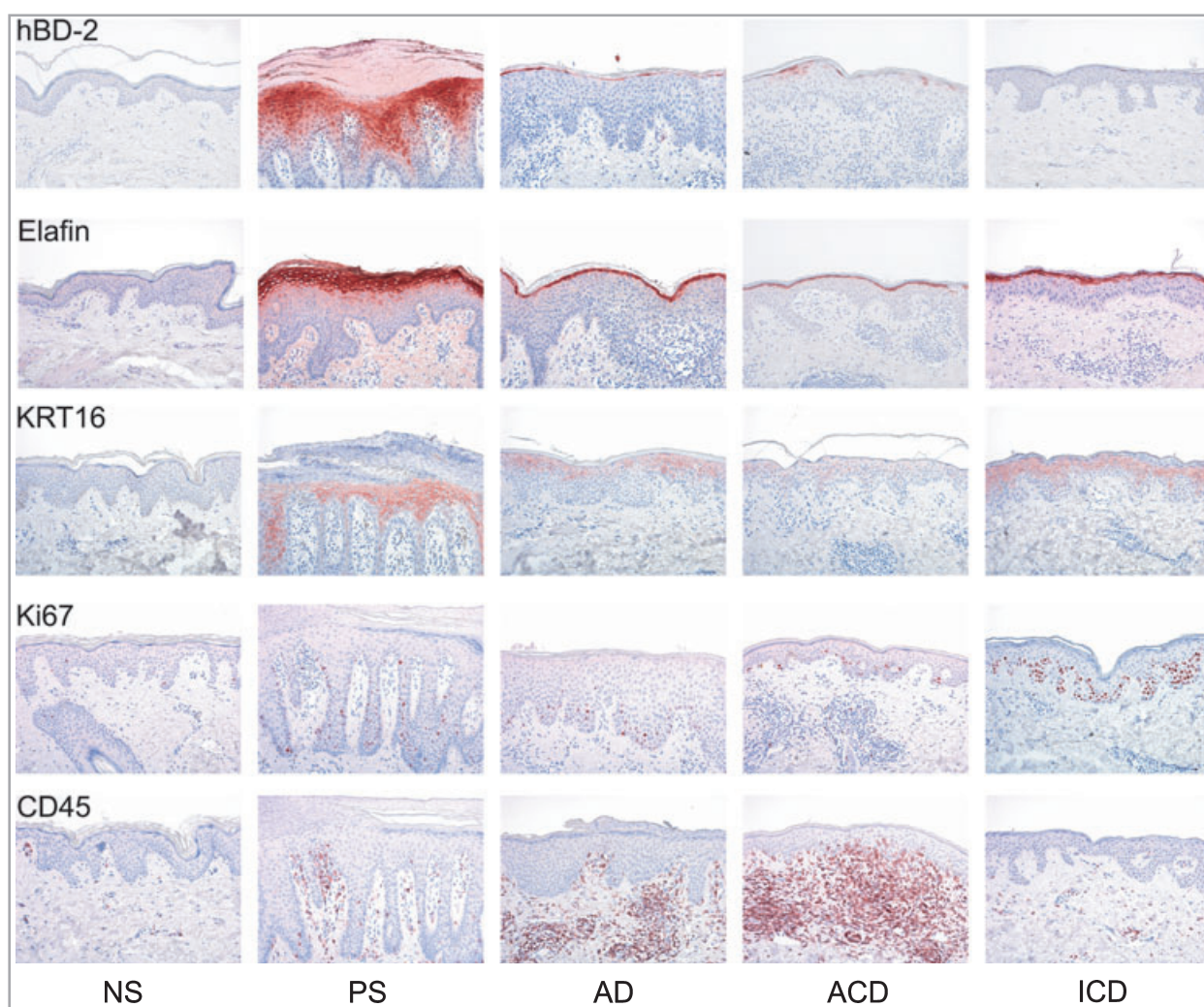


Fig 2. Immunohistochemical staining for five marker proteins associated with epidermal activation (hBD-2, elafin and KRT16), cellular proliferation (Ki67) and infiltration by cells of haematopoietic origin (CD45). Staining patterns were largely similar to the quantitative data obtained by real-time quantitative polymerase chain reaction for hBD-2, elafin, KRT6 and CD45. A representative picture is shown here, without further quantification. NS, normal skin; PS, psoriasis; AD, atopic dermatitis; ACD, allergic contact dermatitis; ICD, irritant contact dermatitis.

performed on epidermal cells obtained from nonlesional and lesional ACD skin. Staining of epidermal cell populations for CAII was found to be heterogeneous. All six patients showed an elevated CAII signal and/or a higher percentage of epidermal cells positive for CAII (Fig. 3). These results indicate that, similar to AD, CAII protein is also upregulated in ACD, albeit less strongly than at the mRNA level.

KRT16 is generally regarded as a keratin that is induced in the context of epidermal hyperproliferation, as found in psoriasis. KRT16 forms a heterodimer with KRT6, which was not found to be informative at the mRNA level to distinguish between the four inflammatory conditions. Recent studies in our laboratory have shown that KRT16 expression and epidermal hyperproliferation are also present in AD. Here we also investigated ACD and ICD for KRT16 and Ki67 staining. Increased numbers of Ki67-positive nuclei correlated with suprabasal KRT16 expression, but both were found to be quite

variable among all inflammatory conditions. KRT16 expression and increases of Ki67-positive nuclei were least prominent in ACD. Collectively, Ki67 and KRT16 staining appeared to be poor discriminators for these inflammatory conditions as was already suggested by qPCR experiments (not shown).

Although most of the aforementioned marker genes investigated here are predominantly expressed by keratinocytes, there could be a small contribution of infiltrating cells to the mRNA levels described in Figure 1. To investigate the presence of cells of haematopoietic origin in the epidermis, immunohistochemical staining for CD45 was performed, which showed a massive amount of immune cells in the dermis of patients with ACD, and to a lesser extent in AD and psoriasis. In ICD, only a mild perivascular infiltrate was observed compared with normal skin. Epidermal presence of immune cells, as determined by CD45 expression, was highest in ACD, less so in psoriasis and AD, and nearly absent in ICD skin. These

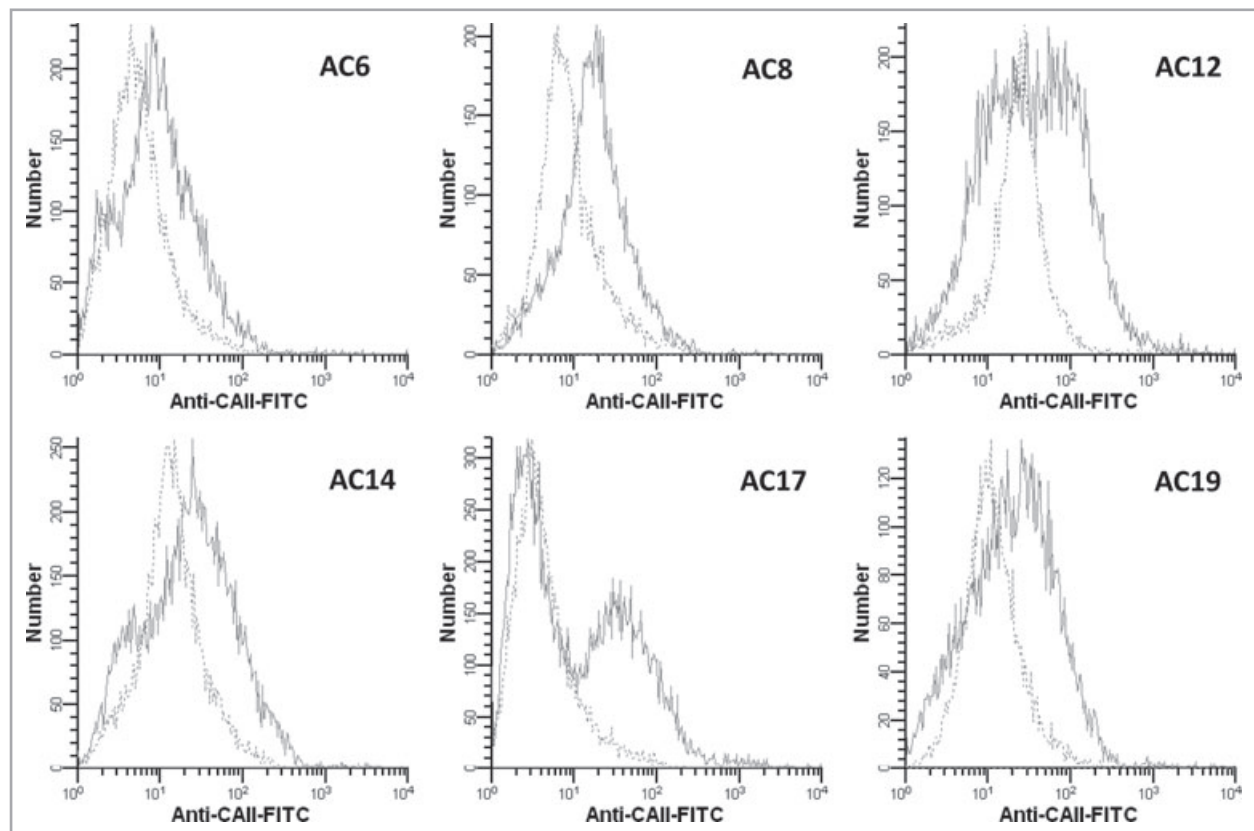


Fig 3. Flow cytometric analysis of CAII protein expression in epidermal cell suspensions derived from six individuals with allergic contact dermatitis (ACD). Cell suspensions were labelled indirectly using a polyclonal anti-CAII antibody and an Alexa Fluor 488-conjugated secondary antibody. CAII expression of six patients (AC6, AC8, AC12, AC14, AC17 and AC19) is shown in frequency histograms. Lesional skin (solid line) and normal skin (dotted line) are overlaid after normalization. Expression of CAII in epidermal cells was heterogeneous and in all cases it was higher in cell suspensions derived from lesional skin compared with normal skin. In three of six patients (AC12, AC14 and AC19) part of the cells in the lesional skin showed a lower CAII expression compared with control skin, suggesting that these cells were newly formed in the basal layer and had lowest CAII protein content. Overall CAII expression in ACD involved skin was on average two times higher than in uninvolved skin from the same patient. FITC, fluorescein isothiocyanate.

immunohistochemical data confirm the observed qPCR data on CD45, which indicated that ACD epidermis showed the highest number of infiltrating cells.

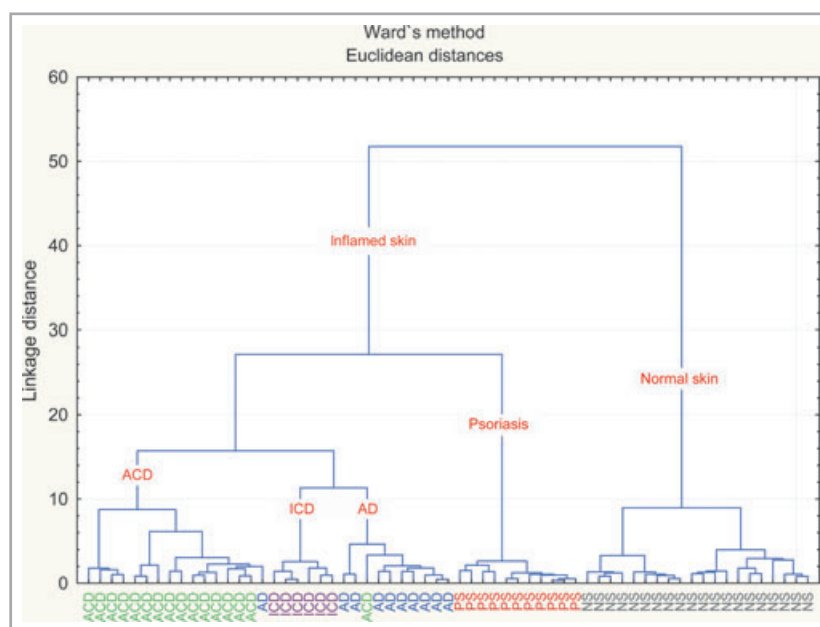
Classification of inflammatory skin conditions based on seven selected genes

In the previous paragraphs we showed that several genes were selectively expressed in one or more specific inflammatory conditions. It remains to be investigated whether these genes are involved in the pathogenesis, as will be discussed hereafter. We next wondered whether a limited set of informative genes could be used to distinguish the four inflammatory dermatoses that we studied based on their expression levels. We used a one-way clustering approach to analyse the similarity between the inflammatory conditions based on the ΔC_t values obtained from our qPCR data of CAII, NELL2, hBD-2, IL-1F9, CXCL8, CXCL10 and CCL17. Figure 4 shows that clustering of the 63 individuals based on expression of these seven genes provides a good separation of sample groups. Normal skin samples

are distinct from all biopsies of inflamed skin. Secondly, psoriasis could be discriminated from the different forms of eczema (AD, ACD and ICD) which are all clustered together. Within the eczematous skin conditions, three distinct clusters emerge for AD, ACD and ICD. Only two samples (one AD and one ACD) were classified in other clusters. The separation of psoriasis and AD is not surprising because the genes were selected on the basis of known differential expression in these conditions. It is, however, striking that ACD and ICD could also be discriminated on the basis of the same gene set.

Polytomous logistic regression analysis, in combination with a forward selection procedure, using the expression levels of the seven selected genes as continuous predictors and the diagnosis group (five levels: normal skin, psoriasis, AD, ACD, ICD) as the nominal outcome variable, resulted in 'perfect' prediction with error rate $0/63 = 0\%$. Only the genes CCL17, hBD-2 and NELL2 were selected by the forward selection procedure, that is to say that the 'perfect' prediction rule used only these three genes. To account for possible overfitting, leave-out-one cross-validation was performed, including

Fig 4. Real-time quantitative polymerase chain reaction data from skin biopsy samples were analysed using a one-way clustering approach to assess the similarity between the inflammatory conditions based on the ΔC_t values from CAII, NELL2, hBD-2, IL-1F9, CXCL8, CXCL10 and CCL17. A good separation of diagnosis groups is obtained. Normal skin (NS) samples are distinct from all biopsies of inflamed skin. Secondly, psoriasis (PS) could be discriminated from the different forms of eczema [atopic dermatitis (AD), allergic contact dermatitis (ACD) and irritant contact dermatitis (ICD)] which are clustered under a separate node. Within the eczematous skin conditions, three distinct clusters emerge for AD, ACD and ICD. Only two samples (one AD and one ACD) were classified in other clusters.



the same forward selection procedure. This resulted in an internally validated error rate of $8/63 = 12.7\%$. In 55 of the 63 models (87.3%) of the leave-out-one analyses the same genes CCL17, hBD-2 and NELL2 were selected as predictors.

Discussion

Recent genetic and cell biological investigations from various laboratories including our own have indicated that epidermal keratinocytes are not passive bystanders in cutaneous inflammation but play an active role in diseases such as psoriasis and various forms of eczema. For this reason we have studied gene expression in isolated epidermal sheets rather than full-thickness skin biopsies. Our data specify the contribution of the epidermis and as such they convey a different message than other studies that use full-thickness biopsies, with varying contributions of inflammatory cells and resident dermal cells. In this study we analysed if gene expression signatures as found in AD are also present in lesional epidermis of other eczematous skin conditions such as ACD and ICD. Normal epidermis and psoriatic epidermis were analysed for comparison. While CAII was found in all investigated forms of eczema, NELL2 showed a strong induction only in AD epidermis. CXCL10 and CCL17, in contrast, were highly upregulated predominantly in epidermis from patients with ACD. IL-1F9, elafin and hBD-2 were strongly induced in psoriasis, but not in any type of eczema. The chemokine CXCL8 was highly expressed in psoriasis, AD and ACD, but not in ICD. Cluster analysis on a limited set of genes allowed a molecular discrimination between these four inflammatory conditions.

To date, no specific keratinocyte-derived biomarkers have been identified to distinguish AD from other forms of eczema at the molecular level. Several genes including CAII and NELL2 were, however, found to be overexpressed in AD lesions

compared with psoriatic epidermis.^{9,10,14} Although our data do not allow conclusions on the involvement of these genes in the disease-specific pathogenetic processes, we observed a remarkable, strong expression of CAII in all eczematous conditions (Fig. 1). Previous *in vitro* studies showed that CAII is induced by AD-associated Th2 cytokines but not by Th1 cytokines that are predominantly associated with psoriasis, and to a lesser extent with chronic AD.¹⁴ Although the cytokine environment of AD, ACD and ICD is generally thought to be different, there are several publications that report the involvement of overlapping cytokine/chemokine profiles, including both Th1 and Th2 cytokines, in these conditions.^{25–29} We speculate that the presence of Th2 cytokines in all three eczematous conditions could drive the CAII expression. Another characteristic that is present in all forms of eczema is a decreased barrier function that results in increased transepidermal water loss. As carbonic anhydrases are involved in water transport,³⁰ CAII upregulation could be a response to restore fluid balance following impaired barrier function. Obviously this assumption requires further investigation. Analysis of CAII at the protein level showed a discrepancy between mRNA and protein data. Similar to earlier findings in AD, we here observed a strong increase in ACD at the mRNA level but only a minor increase at the protein level, as determined by flow cytometric analysis. From this analysis it can be concluded that CAII induction is useful as a disease marker only at the mRNA level, in view of the low and heterogeneous expression of the protein. In contrast, NELL2 was predominantly upregulated in AD, to a much lesser extent in ACD, and not at all in ICD. NELL2 is a thrombospondin-like molecule that shows a significant homology with NEL, which is strongly expressed in neuronal tissues of chicken.³¹ NELL2 contains six epidermal growth factor-like repeats and is strongly expressed in adult and fetal brain, weakly in the kidney.³² It promotes the survival of hippocampal and cortical neurones¹⁵ and increases the

differentiation of spinal cord progenitors, while at the same time exerting paracrine proliferative activity.³³ In a previous study we showed that cultured keratinocytes do express NELL2,¹¹ and in the present study we show that NELL2 is expressed in keratinocytes of AD skin *in vivo*. Bearing in mind its function in brain, NELL2 expression could be associated with the increased amount of nerve fibres in the epidermis that are found in lesional skin of AD.³⁴

Earlier studies have shown CXCL10 upregulation in several diseases that are associated with a Th1 response, like ACD³⁵ and psoriasis,³⁶ while in AD only a weak staining was shown by immunohistochemistry.³⁷ We, however, found a high expression of CXCL10 mRNA in ACD, but only a comparatively minor upregulation in psoriasis. Another chemokine we found to be highly upregulated in ACD and only mildly in AD is CCL17. This chemokine can bind to the CC chemokine receptor (CCR) 8³⁸ and CCR4,³⁹ which is expressed by Th2 type lymphocytes, and is a chemoattractant for these cells.⁴⁰ Injection of CCL17 in the skin of mice results in a Th2-dominated inflammation.⁴¹ CCL17 expression is found in lesional skin of patients with AD, and high serum levels of CCL17 correspond to disease severity.^{42,43} The high CCL17 expression we found in ACD suggests that besides a Th1 type of inflammation also Th2 type lymphocytes are involved. This is in agreement with several studies that have reported an overlap of cytokine/chemokine profiles in eczematous diseases (see above).

ICD is caused mainly by epidermal reactivity to skin-penetrating agents, resulting in inflammation largely mediated by innate immunity.²⁰ The types of cytokines released after exposure to an irritant are not the same for all irritants. TNF- α , for example, is induced after exposure to tributyltin⁴⁴ and SDS⁴⁵ in mice, but not after benzalkonium chloride exposure.⁴⁶ Despite a vigorous inflammatory response, most of the cytokines and chemokines we investigated were only weakly upregulated in experimental ICD compared with other eczematous conditions. Another difference we observed was the absence of a strong inflammatory infiltrate in ICD, both in the dermis (Fig. 2) and in the epidermis (Figs 1 and 2). In previous studies IL-1 β and CXCL10 were upregulated only after allergen painting and not after irritant exposure in BALB/c mice⁴⁵ and also in human studies CXCL10 and CCL17 were upregulated in ACD but not in ICD.⁴⁷ This is in contrast with data on elevated levels of CXCL10 in (epi)dermal fluid samples after exposure of forearm skin to 10% SDS.²⁷ This could be due to the time point measured: samples were collected 16 h after exposure to SDS, while the biopsies in our study were taken after 48 h. Alternatively, the CXCL10 could be derived entirely from the dermal compartment and not from the keratinocytes. The acute ICD reaction that we observed here has features of both psoriasis (elafin induction, hyperproliferation and KRT16 expression) and AD (absence of hBD-2, induction of CAII).

Cluster analysis clearly shows that psoriasis is distinct from all forms of eczema, and the high expression levels of genes such as hBD-2, IL-1F9 and VNN3 were unique to psoriasis. Recent studies have indicated that the transcriptome of psoriasis resembles that of keratinocytes stimulated by IL-1¹² or a

combination of IL-1, TNF- α and IFN- γ .¹¹ Indeed, all psoriasis-associated genes reported here are strongly induced by these cytokines.^{11,12,16} We would speculate that the similarities between the eczematous conditions are caused by their similar, albeit not identical, cytokine environments, which could include IL-4 and/or IL-13 produced by Th2 lymphocytes. The question of whether the induced genes also contribute to the disease process remains to be addressed.

A number of caveats should be considered for our study. For practical reasons the number of samples is limited and internal validation of the predictive power indicated a 12.7% error rate. We have not considered the effect of disease severity on expression of genes and disease classification, as we included only strongly inflamed skin lesions. Another limitation is the fact that AD and psoriasis biopsies were taken from existing lesions in patients, whereas in ACD and ICD induced lesions were biopsied at 48 h after elicitation by allergen or irritant. Recent studies have shown that cytokine profiles of acute and chronic irritant reactions are largely similar, but some cytokines like CCL5 are different.²⁷

Finally, the question arises whether a classification based on molecular diagnostic criteria has any practical significance for a group of inflammatory conditions. Although these conditions can usually be discriminated quite well by clinical criteria, it is not uncommon to encounter diagnostic difficulties in discriminating ICD from ACD, e.g. in hand eczema.⁴⁸ Likewise, nonpustular palmoplantar psoriasis is often difficult to delineate from hyperkeratotic eczema of the palms or soles.⁴⁹ It would be interesting to analyse expressed genes in these conditions, and to see if clear-cut molecular patterns emerge. Alternatively, molecular diagnostics could also result in mixed, inconclusive results thereby confirming the assertion that these conditions represent intermediate phenotypes both clinically and molecularly.

Acknowledgments

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Supporting information

Additional supporting information may be found in the online version of this article:

Data S1. Gene selection.

Table S1. List of all genes that were found to be differentially expressed in a microarray analysis performed on isolated epidermal cells taken from lesional epidermis of six patients with psoriasis and six patients with atopic dermatitis.

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Supplementary text

Gene selection

A number of microarray studies on gene expression in psoriasis, atopic dermatitis and normal skin have been performed.¹⁻⁵ These analyses have identified a large number of genes that were differentially expressed between lesional psoriatic epidermis and normal skin. Two of these studies^{3,4} have focused on a comparison of lesional psoriasis and atopic dermatitis skin in order to identify disease-specific expression patterns. These data have been deposited at the NCBI (Gene Expression Omnibus). A limited number of these genes have been verified by qPCR or immunohistochemistry. Our own study³ focused on host defense proteins, and we could indeed confirm most of the array data by qPCR. In this study we identified 180 genes that were differentially expressed between atopic dermatitis and psoriasis. These genes showed a significant difference after correcting for false discovery. Supplemental table 1 provides a list of all these genes. From this list we tested a number of genes with qPCR to verify the differential expression, including CA2, IL1F9, PI3 and VNN3. As the observed expression could be confirmed, they were included in the analyses of the current manuscript. Other genes that we examined were selected on the basis of available literature such as NELL2, CCL17 and VNN1.^{4,6} The remaining genes were selected for reasons that they have been associated with psoriasis or atopic dermatitis in other studies (e.g. KRT6 and CXCL10). IL1B and IL18 were selected because of the reported gene expression signature of psoriasis resembling that of IL-1 stimulated keratinocytes. Finally PTPRC (CD45) was not selected on the basis of known differential expression, but as a convenient marker to assess the presence of hematopoietic cells in our isolated epidermal sheets. We could indeed demonstrate, using qPCR and histology, that CD45+ cells are largely absent from normal skin, but clearly present in Psoriasis, ACD and AD.

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GenID	Mean	Stdev	N	UniGene_ID	Description	Gene_ontology
NM_018399	4.2	0.6	6	Hs.183656	Homo sapiens vanin 3 (VNN3), transcript variant 1, mRNA.	cell motility [0006928]
NM_004942	3.0	0.8	4	Hs.105924	Homo sapiens DEFB4, mRNA	response to pest/pathogen/parasite
NM_005621	2.8	0.9	6	Hs.19413	Homo sapiens S100 calcium binding protein A12 (calgranulin C) (S100A12), mRNA.	signal transduction [0007165]
NM_007231	2.8	0.9	6	Hs.162211	Homo sapiens solute carrier family 6 (neurotransmitter transporter), member 14 (SLC6A14), mRNA.	neurotransmitter transport [0006836]
NM_002462	2.6	1.0	6	Hs.76391	Homo sapiens myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) (MX1), mRNA.	signal transduction [0007165]
NM_014438	2.5	0.9	6	Hs.278909	Homo sapiens interleukin 1 family, member 8 (eta) (IL1F8), mRNA.	response to pest/pathogen/parasite [0009613]
NM_006417	2.4	1.0	6	Hs.82316	Homo sapiens interferon-induced protein 44 (IFI44), mRNA.	response to viruses [0009615]
NM_012337	2.4	0.8	6	Hs.158450	Homo sapiens nasopharyngeal epithelium specific protein 1 (NESG1), mRNA	developmental processes [0007275]
NM_017654	2.3	1.0	6	Hs.65641	Homo sapiens hypothetical protein FLJ20073 (FLJ20073), mRNA	biological_process unknown [0000004]
AF289028	2.2	0.8	6	Hs.14155	Homo sapiens transmembrane protein B7-H2 ICOS ligand mRNA, complete cds.	positive regulation of activated T-cell proliferation [0042104]
AF068236	2.2	0.5	5	Hs.193788	Homo sapiens inducible nitric oxide synthase (NOS) mRNA, complete cds.	electron transport [0006118]
AY009090	2.1	0.9	6	Hs.161786	Homo sapiens putative transmembrane protein dectin-1 mRNA, complete cds.	cell surface receptor linked signal transduction [0007166]
NM_004559	2.1	0.8	6	Hs.74497	Homo sapiens nuclease sensitive element binding protein 1 (NSEP1), mRNA.	regulation of transcription, DNA-dependent [0006355]
NM_019618	2.1	0.8	6	Hs.211238	Homo sapiens interleukin 1 family, member 9 (IL1F9), mRNA.	response to pest/pathogen/parasite [0009613]
NM_001901	2.1	0.7	6	Hs.75511	Homo sapiens connective tissue growth factor (CTGF), mRNA.	regulation of cell growth [0001558]
X56692	2.0	0.7	6	Hs.76452	H.sapiens mRNA for C-reactive protein.	acute-phase response [0006953]
NM_016590	2.0	0.8	6	Hs.96744	Homo sapiens prostate androgen-regulated transcript 1 (PART1), mRNA.	cytoskeleton organization and biogenesis [0007010]
NM_000673	1.9	0.6	6	Hs.389	Homo sapiens alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide (ADH7), mRNA.	ethanol oxidation [0006069]
NM_005156	1.9	0.7	5	Hs.374634	Homo sapiens ROD1 regulator of differentiation 1 (S. pombe) (ROD1), mRNA.	embryogenesis and morphogenesis [0007345]
NM_000045	1.9	0.8	6	Hs.332405	Homo sapiens arginase, liver (ARG1), mRNA.	arginine catabolism [0006527]
AL117423	1.9	0.8	6	Hs.61628	Homo sapiens mRNA; cDNA DKFp564C246 (from clone DKFp564C246); complete cds.	defense response [0006952]
AL137489	1.9	0.5	4	Hs.7517	Homo sapiens mRNA; cDNA DKFp434O1230 (from clone DKFp434O1230); partial cds.	biological_process unknown [0000004]
NM_002963	1.8	0.6	6	Hs.112408	S100A7 S100 calcium binding protein A7 (psoriasis 1)	S100A7 S100 calcium binding protein A7 (psoriasis 1)
NM_017973	1.8	0.5	4	#N/B	#N/B	#N/B
NM_004673	1.8	0.3	6	Hs.241519	Homo sapiens angiotensin-like 1 (ANGPTL1), mRNA.	development [0007275]
NM_003465	1.8	0.5	6	Hs.91093	Homo sapiens chitinase 1 (chitotriosidase) (CHIT1), mRNA.	response to pest/pathogen/parasite [0009613]
NM_006440	1.7	0.6	6	Hs.12971	Homo sapiens thioredoxin reductase 2 (TXNRD2) transcript variant 1, nuclear gene, mRNA.	response to pest/pathogen/parasite [0009613]
AB007978	1.7	0.5	6	Hs.158278	Homo sapiens mRNA, chromosome 1 specific transcript KIAA0509.	ribosomal protein-nucleus import [0006610]
NM_014683	1.7	0.5	6	Hs.151406	Homo sapiens unc-51-like kinase 2 (C. elegans) (ULK2), mRNA.	signal transduction [0007165]
NM_002377	1.7	0.5	4	Hs.99900	Homo sapiens MAS1 oncogene (MAS1), mRNA.	cell proliferation [0008283]
NM_005503	1.6	0.5	6	Hs.26468	Homo sapiens amyloid beta (A4) precursor protein-binding, family A, member 2 (X11-like) (APBA2), mRNA.	neurogenesis [0007399]
NM_018456	1.6	0.3	6	Hs.26892	Homo sapiens uncharacterized bone marrow protein BM040 (BM040), mRNA	biological_process unknown [0000004]
AK022035	1.5	0.6	6	Hs.151504	Homo sapiens cDNA FLJ11973 fis, clone HEMBB1001221	biological_process unknown [0000004]
AK022213	1.5	0.2	4	Hs.287493	Homo sapiens cDNA FLJ12151 fis, clone MAMMA1000431.	development [0007275]
NM_019599	1.5	0.4	6	Hs.168278	Homo sapiens taste receptor, type 2, member 1 (TAS2R1), mRNA.	chemosensory behavior [0007635]
AL157455	1.5	0.4	6	Hs.22543	#N/B	#N/B
NM_018974	1.5	0.6	6	Hs.267749	Homo sapiens unc93 (C.elegans) homolog A (UNC93A), mRNA.	protein secretion [0009306]
AB033044	1.5	0.6	6	Hs.114012	Homo sapiens mRNA for KIAA1218 protein, partial cds.	nuclear organization and biogenesis [0006997]
NM_016606	1.5	0.6	6	Hs.28088	Homo sapiens SGC32445 protein (LOC51308), mRNA.	hypersensitive response [0009626]
NM_018473	1.5	0.5	6	Hs.9676	Homo sapiens uncharacterized hypothalamus protein HT012 (HT012), mRNA.	translational initiation [0006413]
AJ293294	1.5	0.6	6	Hs.24678	Homo sapiens mRNA for sphingosine-1-phosphatase (ORF1).	signal transduction [0007165]
AL050035	1.5	0.4	6	Hs.133130	Homo sapiens mRNA; cDNA DKFp566H0124 (from clone DKFp566H0124).	metabolism [0008152]
AL110259	1.5	0.5	6	Hs.383803	Homo sapiens mRNA; cDNA DKFp586B1221 (from clone DKFp586B1221).	biological_process unknown [0000004]
AJ227863	1.5	0.2	6	Hs.310286	Homo sapiens partial mRNA; ID YG39-2B.	biological_process unknown [0000004]
NM_003137	1.4	0.5	6	Hs.75761	Homo sapiens SFRS protein kinase 1 (SRPK1), mRNA.	RNA splicing [0008380]
AF105067	1.4	0.5	6	Hs.154078	Homo sapiens lipopolysaccharide-binding protein (LBP) mRNA, complete cds.	response to pathogenic bacteria [0009618]
AF169158	1.4	0.6	6	Hs.117694	Homo sapiens CaBP3 (CABP3) mRNA, complete cds.	signal transduction [0007165]

GenID	Mean	Stdev	N	UniGene_ID	Description	Gene_ontology
AK024534	1.4	0.4	6	Hs.406845	Homo sapiens cDNA: FLJ20881 fis, clone ADKA03177.	regulation of transcription, DNA-dependent [0006355]
X74613	1.4	0.3	6	-	H.sapiens HERV-K mRNA (252 bp).	biological_process unknown [0000004]
AK022464	1.4	0.6	6	Hs.143303	Homo sapiens cDNA FLJ12402 fis, clone MAMMA1002807.	biological_process unknown [0000004]
L48516	1.4	0.4	6	Hs.335322	Homo sapiens paraoxonase 3 (PON3) mRNA, 3' end of cds.	DNA transposition [0006313]
NM_000116	1.4	0.3	6	Hs.389108	Homo sapiens tafazzin (cardiomyopathy, dilated 3A (X-linked); Barth syndrome) (TAZ), mRNA.	metabolism [0008152]
NM_004580	1.4	0.6	6	Hs.50477	Homo sapiens RAB27A, member RAS oncogene family (RAB27A), mRNA.	intracellular protein transport [0006886]
NM_007199	1.4	0.3	6	Hs.268552	Homo sapiens interleukin-1 receptor-associated kinase 3 (IRAK3), mRNA.	protein amino acid phosphorylation [0006468]
AF085981	1.3	0.2	4	0	Homo sapiens full length insert cDNA clone YT94C01	biological_process unknown [0000004]
NM_005420	1.3	0.5	6	Hs.54576	Homo sapiens sulfotransferase, estrogen-preferring (STE), mRNA.	steroid metabolism [0008202]
AK000792	1.3	0.5	5	Hs.306405	Homo sapiens cDNA FLJ20785 fis, clone COL02504.	biological_process unknown [0000004]
NM_016564	1.3	0.5	4	Hs.22140	Homo sapiens BM88 antigen The protein encoded by this gene is a neuron-specific protein.	transmembrane receptor protein tyrosine phosphatase signaling pathway
NM_000718	1.3	0.5	6	Hs.69949	Homo sapiens calcium channel, voltage-dependent, L type, alpha 1B subunit (CACNA1B), mRNA.	synaptic transmission [0007268]
NM_001225	1.3	0.5	4	Hs.74122	Homo sapiens caspase 4, apoptosis-related cysteine protease (CASP4), transcript variant alpha, mRNA.	induction of apoptosis [0006917]
NM_005322	1.3	0.4	4	Hs.131956	Homo sapiens H1 histone family, member 5 (H1F5), mRNA.	nucleosome assembly [0006334]
AL137507	1.3	0.4	6	Hs.255348	Homo sapiens mRNA; cDNA DKFZp761P211 (from clone DKFZp761P211)	biological_process unknown [0000004]
NM_005564	1.3	0.3	6	Hs.204238	Homo sapiens lipocalin 2 (oncogene 24p3) (LCN2), mRNA.	prostaglandin metabolism [0006693]
NM_014228	1.2	0.4	5	Hs.241597	Homo sapiens solute carrier family 6 (neurotransmitter transporter, L-proline), member 7 (SLC6A7), mRNA.	proline transport [0015824]
NM_016303	1.2	0.3	6	Hs.15984	Homo sapiens pp21 homolog (LOC51186), mRNA.	negative regulation of transcription from Pol II promoter [0000122]
AF263541	1.2	0.2	6	Hs.17154	Homo sapiens protein kinase DYRK4 (DYRK4) mRNA, partial cds.	cell cycle [0007049]
NM_005218	1.2	0.5	6	Hs.32949	Homo sapiens defensin, beta 1 (DEFB1), mRNA.	xenobiotic metabolism [0006805]
AK024799	1.2	0.3	5	Hs.17433	Homo sapiens cDNA: FLJ21146 fis, clone CAS09305.	signal transduction [0007165]
NM_017835	1.2	0.5	6	Hs.5811	Homo sapiens chromosome 21 open reading frame 59 (C21orf59), mRNA.	metabolism [0008152]
L77571	1.2	0.2	6	Hs.106311	Homo sapiens DGS-A mRNA, 3' end	biological_process unknown [0000004]
NM_002055	1.2	0.4	5	Hs.406397	Homo sapiens glial fibrillary acidic protein (GFAP), mRNA.	cytoskeleton organization and biogenesis [0007010]
AK026656	1.1	0.4	6	Hs.381566	Homo sapiens cDNA: FLJ23003 fis, clone LNG00331.	cholesterol catabolism [0006707]
NM_005694	1.1	0.5	6	Hs.16297	Homo sapiens COX17 homolog, cytochrome c oxidase assembly protein (yeast) (COX17)	copper ion transport [0006825]
AK025203	1.1	0.4	6	Hs.141269	Homo sapiens cDNA: FLJ21550 fis, clone COL06258.	biological_process unknown [0000004]
AF061556	1.1	0.4	4	Hs.147174	Homo sapiens ubiquitin-protein ligase E3-alpha (UBR1) mRNA, partial cds.	protein polyubiquitination [0000209]
AK023335	1.1	0.3	6	Hs.126119	Homo sapiens cDNA FLJ13273 fis, clone OVARC1001010.	cell cycle [0007049]
NM_001163	1.1	0.3	6	Hs.4880	Homo sapiens amyloid beta (A4) precursor protein-binding, family A, member 1 (X11) (APBA1), mRNA.	neurogenesis [0007399]
AL049434	1.1	0.2	6	Hs.302062	Homo sapiens mRNA; cDNA DKFZp586M151 (from clone DKFZp586M151).	DNA replication dependent nucleosome assembly [0006335]
AL110185	1.1	0.5	6	Hs.171625	Homo sapiens mRNA; cDNA DKFZp566D211 (from clone DKFZp566D211).	protein biosynthesis [0006412]
AB002446	1.1	0.2	6	Hs.407689	Homo sapiens mRNA from chromosome 5q21-22, clone:sF2.	biological_process unknown [0000004]
NM_004083	1.1	0.3	6	Hs.400353	Homo sapiens DNA-damage-inducible transcript 3 (DDIT3), mRNA.	cell cycle arrest [0007050]
NM_003143	1.1	0.4	6	Hs.923	Homo sapiens single-stranded DNA binding protein (SSBP1), mRNA.	cell growth and/or maintenance [0008151]
AL133573	1.1	0.4	6	Hs.272312	Homo sapiens mRNA; cDNA DKFZp434J2235 (from clone DKFZp434J2235).	ribosomal protein-nucleus import [0006610]
NM_007238	1.1	0.3	6	Hs.241205	Homo sapiens 24 kDa intrinsic membrane protein (PMP24), mRNA	peroxisome organization and biogenesis [0007031]
AL117415	1.1	0.3	6	Hs.173716	Homo sapiens mRNA; cDNA DKFZp434K0521 (from clone DKFZp434K0521)	biological_process unknown [0000004]
M14200	1.1	0.3	6	Hs.78888	Human diazepam binding inhibitor (DBI) mRNA, complete cds.	lipid metabolism [0006629]
NM_006731	1.1	0.2	6	Hs.55777	Homo sapiens Fukuyama type congenital muscular dystrophy (fukutin) (FCMD), mRNA.	muscle development [0007517]
AL110240	1.0	0.4	6	Hs.32990	Homo sapiens mRNA; cDNA DKFZp566F084 (from clone DKFZp566F084); partial cds	biological_process unknown [0000004]
NM_000160	1.0	0.4	6	Hs.208	Homo sapiens glucagon receptor (GCGR), mRNA.	G-protein signaling, coupled to cAMP nucleotide second messenger [0007188]
AF217525	1.0	0.3	6	Hs.49002	Homo sapiens clone cDSC1 Down syndrome cell adhesion molecule (DSCAM) mRNA, complete cds.	neurogenesis [0007399]
NM_017876	1.0	0.3	6	Hs.69554	Homo sapiens hypothetical protein FLJ20552 (FLJ20552), mRNA.	cell cycle [0007049]
NM_004617	1.0	0.4	5	Hs.11881	Homo sapiens transmembrane 4 superfamily member 4 (TM4SF4), mRNA.	negative regulation of cell proliferation [0008285]
D31763	1.0	0.3	5	Hs.70617	Human mRNA for KIAA0065 gene, partial cds.	regulation of transcription, DNA-dependent [0006355]
NM_004397	1.0	0.2	6	Hs.316	Homo sapiens DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 6 (RNA helicase, 54kDa) (DDX6), mRNA.	cell growth and/or maintenance [0008151]

GenID	Mean	Stdev	N	UniGene_ID	Description	Gene_ontology
NM_004477	1.0	0.2	6	Hs.203772	Homo sapiens FSHD region gene 1 (FRG1), mRNA.	pathogenesis [0009405]
U55185	1.0	0.3	6	Hs.385190	Human oral cancer candidate gene mRNA, clone T9, 3' end.	protein biosynthesis [0006412]
NM_004688	1.0	0.3	6	Hs.54483	Homo sapiens N-myc (and STAT) interactor (NMI), mRNA.	inflammatory response [0006954]
NM_005764	1.0	0.4	6	Hs.271473	Homo sapiens epithelial protein up-regulated in carcinoma, membrane associated protein 17 (DD96), mRNA.	transmembrane receptor protein tyrosine phosphatase signaling pathway [0007185]
NM_018474	1.0	0.4	6	Hs.173515	Homo sapiens chromosome 20 open reading frame 19 (C20orf19), mRNA.	regulation of transcription, DNA-dependent [0006355]
NM_015715	-1.0	0.4	6	Hs.149623	Homo sapiens phospholipase A2, group III (PLA2G3), mRNA.	lipid catabolism [0016042]
AF217986	-1.0	0.4	6	Hs.7159	Homo sapiens clone PP1628 unknown mRNA	biological_process unknown [0000004]
AL359401	-1.0	0.3	6	Hs.7436	Isoform 1 of a novel human mRNA from chromosome 22.	metabolism [0008152]
NM_015946	-1.0	0.2	6	Hs.5798	Homo sapiens pelota homolog (Drosophila) (PELO), mRNA.	translational termination [0006415]
NM_003032	-1.0	0.3	6	Hs.2554	Homo sapiens sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase) (SIAT1), mRNA.	protein modification [0006464]
NM_003651	-1.0	0.3	6	Hs.198726	Homo sapiens cold shock domain protein A (CSDA), mRNA.	negative regulation of transcription from Pol II promoter [0000122]
NM_002189	-1.0	0.4	4	Hs.12503	Homo sapiens interleukin 15 receptor, alpha (IL15RA), mRNA.	cell proliferation [0008283]
AK022649	-1.0	0.3	6	Hs.301338	Homo sapiens cDNA FLJ12587 fis, clone NT2RM4001217, mRNA.	development [0007275]
AK000554	-1.0	0.3	6	Hs.406646	Homo sapiens cDNA FLJ20547 fis, clone KAT11527.	cell growth and/or maintenance [0008151]
AL079314	-1.1	0.2	6	Hs.16537	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 469780.	cell cycle [0007049]
NM_012116	-1.1	0.2	6	Hs.156637	Homo sapiens Cas-Br-M (murine) ecotropic retroviral transforming sequence c (CBLC), mRNA.	cell surface receptor linked signal transduction [0007166]
NM_005804	-1.1	0.3	6	Hs.311609	DDX39 DEAD box proteins, characterized by the conserved motif Asp-Glu-Ala-Asp (DEAD), are putative RNA helicases.	ATP-dependent helicase activity
NM_014344	-1.1	0.3	6	Hs.39384	Homo sapiens four jointed box 1 (Drosophila) (FJX1), mRNA.	cell communication [0007154]
NM_004308	-1.1	0.3	6	Hs.138860	Homo sapiens Rho GTPase activating protein 1 (ARHGAP1), mRNA.	cytoskeleton organization and biogenesis [0007010]
NM_006848	-1.1	0.4	6	Hs.66713	Homo sapiens hepatitis delta antigen-interacting protein A (DIPA), mRNA.	virulence [0009406]
NM_001941	-1.1	0.4	6	Hs.41690	Homo sapiens desmocollin 3 (DSC3),transcript variant Dsc3a, mRNA.	homophilic cell adhesion [0007156]
NM_004430	-1.1	0.3	4	Hs.74088	Homo sapiens early growth response 3 (EGR3), mRNA.	regulation of transcription, DNA-dependent [0006355]
NM_012414	-1.1	0.3	4	Hs.197289	RAB3-GAP150 rab3 GTPase-activating protein, non-catalytic subunit	GTPase activator activity
AK023496	-1.1	0.3	6	Hs.268843	Homo sapiens cDNA FLJ13434 fis, clone PLACE1002578.	protein amino acid phosphorylation [0006468]
NM_001300	-1.1	0.5	6	Hs.285313	Homo sapiens core promoter element binding protein (COPEB), mRNA.	regulation of transcription, DNA-dependent [0006355]
AK021858	-1.1	0.4	6	Hs.298020	Homo sapiens cDNA FLJ11796 fis, clone HEMBA1006158.	biological_process unknown [0000004]
AK002173	-1.1	0.3	6	Hs.5518	Homo sapiens cDNA FLJ11311 fis, clone PLACE1010102.	biological_process unknown [0000004]
M95929	-1.2	0.5	6	Hs.443452	PRRX1 The protein functions as a transcription co-activator,	regulation of transcription, DNA-dependent
AK000745	-1.2	0.4	6	Hs.243901	Homo sapiens cDNA FLJ20738 fis, clone HEP08257.	development [0007275]
NM_002414	-1.2	0.4	6	Hs.433387	Homo sapiens antigen identified by monoclonal antibodies 12E7, F21 and O13 (MIC2), mRNA.	ATP/ADP exchange [0006854]
NM_002101	-1.2	0.2	4	Hs.81994	Homo sapiens glycophorin C (Gerbich blood group) (GYPC) transcript variant 1, mRNA.	virulence [0009406]
NM_003472	-1.2	0.5	6	Hs.110713	Homo sapiens DEK oncogene (DNA binding) (DEK), mRNA.	regulation of transcription from Pol II promoter [0006357]
NM_018226	-1.2	0.4	6	Hs.5345	Homo sapiens arginyl aminopeptidase (aminopeptidase B)-like 1 (RNPEPL1), mRNA.	leukotriene metabolism [0006691]
NM_003897	-1.2	0.4	5	Hs.76095	Homo sapiens immediate early response 3 (IER3), transcript variant short, mRNA.	cell growth and/or maintenance [0008151]
X91195	-1.2	0.3	6	Hs.406326	H.sapiens SOM172 mRNA.	protein amino acid phosphorylation [0006468]
AB040883	-1.2	0.5	6	Hs.83243	Homo sapiens mRNA for KIAA1450 protein, partial cds.	signal transduction [0007165]
NM_015874	-1.2	0.5	6	Hs.347340	Homo sapiens H-2K binding factor-2 (LOC51580), mRNA.	DNA transposition [0006313]
AK026985	-1.2	0.5	6	Hs.28794	Homo sapiens cDNA: FLJ23332 fis, clone HEP12754	biological_process unknown [0000004]
NM_002823	-1.2	0.3	6	Hs.250655	Homo sapiens prothymosin, alpha (gene sequence 28) (PTMA), mRNA.	immune response [0006955]
AL110280	-1.2	0.3	4	Hs.301152	Homo sapiens mRNA; cDNA DKFZp434F053 (from clone DKFZp434F053).	proteolysis and peptidolysis [0006508]
AL050214	-1.2	0.4	5	Hs.55044	Homo sapiens mRNA; cDNA DKFZp586H2123 (from clone DKFZp586H2123); partial cds.	proteolysis and peptidolysis [0006508]
NM_006697	-1.2	0.5	6	Hs.166066	Homo sapiens cisplatin resistance associated (CRA), mRNA.	protein amino acid dephosphorylation [0006470]
NM_006664	-1.2	0.4	6	Hs.225948	Homo sapiens chemokine (C-C motif) ligand 27 (CCL27), mRNA.	chemotaxis [0006935]
AF039944	-1.2	0.5	6	Hs.318567	This gene is a member of the N-myc downregulated gene family	biological_process unknown [0000004]
AK024270	-1.3	0.4	4	Hs.4094	Homo sapiens cDNA FLJ14208 fis, clone NT2RP3003264	biological_process unknown [0000004]
NM_005842	-1.3	0.6	6	Hs.18676	Homo sapiens sprouty (Drosophila) homolog 2 (SPRY2) mRNA	histogenesis and organogenesis [0007397]
AF036364	-1.3	0.3	6	Hs.110708	Homo sapiens epsilon-sarcoglycan (ESG) mRNA, complete cds.	muscle development [0007517]

GenID	Mean	Stdev	N	UniGene_ID	Description	Gene_ontology
NM_006180	-1.3	0.5	6	Hs.47860	Homo sapiens neurotrophic tyrosine kinase, receptor, type 2 (NTRK2), mRNA.	neurogenesis [0007399]
NM_007229	-1.3	0.4	5	Hs.18842	Homo sapiens protein kinase C and casein kinase substrate in neurons 2 (PACSIN2), mRNA.	signal transduction [0007165]
NM_003016	-1.4	0.4	6	Hs.73965	Homo sapiens splicing factor, arginine/serine-rich 2 (SFRS2), mRNA.	RNA processing [0006396]
NM_018165	-1.4	0.4	5	Hs.44143	Homo sapiens polybromo 1 (PB1), mRNA.	DNA packaging [0006323]
NM_001311	-1.4	0.4	6	Hs.423190	Homo sapiens cysteine-rich protein 1 (intestinal) (CRIP1), mRNA.	cell proliferation [0008283]
NM_012282	-1.4	0.6	6	Hs.146372	Homo sapiens potassium voltage-gated channel, Isk-related family, member 1-like (KCNE1L), mRNA.	regulation of heart [0008016]
NM_020400	-1.4	0.4	6	Hs.155538	Homo sapiens G protein-coupled receptor 92 (GPR92), mRNA.	chemotaxis [0006935]
NM_003174	-1.5	0.6	6	Hs.154567	Homo sapiens supervillin (SVIL), transcript variant 1, mRNA.	cellular morphogenesis [0000902]
NM_019897	-1.5	0.5	6	Hs.283806	Homo sapiens olfactory receptor, family 2, subfamily S, member 2 (OR2S2), mRNA.	G-protein coupled receptor protein signaling pathway [0007186]
NM_007326	-1.5	0.3	6	Hs.274464	Homo sapiens diaphorase (NADH) (cytochrome b-5 reductase) (DIA1), nuclear gene encoding mitochondrial protein,	circulation [0008015]
AF205437	-1.5	0.6	6	Hs.7837	Homo sapiens G-protein-coupled receptor induced protein GIG2 (GIG2) mRNA, complete cds.	cell proliferation [0008283]
NM_003875	-1.6	0.3	5	Hs.5398	Homo sapiens guanine monphosphate synthetase (GMPS), mRNA.	GMP biosynthesis [0006177]
NM_000304	-1.6	0.3	6	Hs.103724	Homo sapiens peripheral myelin protein 22 (PMP22), mRNA.	synaptic transmission [0007268]
NM_017694	-1.6	0.5	4	Hs.390013	Homo sapiens hypothetical protein FLJ20160 (FLJ20160), mRNA.	transport [0006810]
NM_001614	-1.6	0.3	6	Hs.14376	Homo sapiens actin, gamma 1 (ACTG1), mRNA.	cytoskeleton organization and biogenesis [0007010]
NM_006022	-1.6	0.4	6	Hs.114360	Homo sapiens transforming growth factor beta-stimulated protein TSC-22 (TSC22), mRNA.	regulation of transcription, DNA-dependent [0006355]
AK022798	-1.6	0.6	6	Hs.147434	T3JAM TRAF3-interacting Jun N-terminal kinase (JNK)-activating modulator	muscle contraction [0006936]
NM_018043	-1.6	0.5	6	Hs.26176	Homo sapiens hypothetical protein FLJ10261 (FLJ10261), mRNA.	mitochondrial processing [0006627]
NM_016205	-1.6	0.5	6	Hs.43080	Homo sapiens platelet derived growth factor C (PDGFC), mRNA.	cell growth and/or maintenance [0008151]
NM_003627	-1.7	0.7	6	Hs.18910	Homo sapiens prostate cancer overexpressed gene 1 (POV1), mRNA.	oncogenesis [0007048]
AL133585	-1.7	0.7	5	Hs.279806	Homo sapiens mRNA; cDNA DKFZp434E109 (from clone DKFZp434E109)	biological_process unknown [0000004]
AK023288	-1.7	0.6	5	Hs.29397	Homo sapiens cDNA FLJ13226 fis, clone OVARC1000068	biological_process unknown [0000004]
AB037725	-1.7	0.7	6	Hs.18995	Homo sapiens mRNA for KIAA1304 protein, partial cds.	Rho protein signal transduction [0007266]
NM_003311	-1.8	0.6	6	Hs.154036	Homo sapiens tumor suppressing subtransferable candidate 3 (TSSC3), mRNA.	embryogenesis and morphogenesis [0007345]
NM_002192	-1.8	0.7	6	Hs.727	Homo sapiens inhibin, beta A (activin A, activin AB alpha polypeptide) (INHBA), mRNA.	cell growth and/or maintenance [0008151]
AK000776	-1.8	0.5	4	Hs.128753	Homo sapiens cDNA FLJ20769 fis, clone COL06674	biological_process unknown [0000004]
AF218008	-1.8	0.5	6	Hs.347803	Homo sapiens clone PP3501 unknown mRNA.	biological_process unknown [0000004]
NM_000043	-1.9	0.7	5	Hs.82359	Homo sapiens tumor necrosis factor receptor superfamily, member 6 (TNFRSF6), transcript variant 1, mRNA.	anti-apoptosis [0006916]
NM_001657	-2.0	0.8	6	Hs.270833	Homo sapiens amphiregulin (schwannoma-derived growth factor) (AREG), mRNA.	cell-cell signaling [0007267]
NM_001692	-2.0	0.7	4	Hs.64173	Homo sapiens ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 1	excretion [0007588]
NM_001748	-2.1	0.5	5	Hs.76288	Homo sapiens calpain 2, (mII) large subunit (CAPN2), mRNA.	proteolysis and peptidolysis [0006508]
AK024805	-2.1	0.6	4	Hs.375709	Homo sapiens cDNA: FLJ21152 fis, clone CAS09594.	electron transport [0006118]
NM_001109	-2.3	0.9	6	Hs.86947	Homo sapiens a disintegrin and metalloproteinase domain 8 (ADAM8), mRNA.	proteolysis and peptidolysis [0006508]
NM_012132	-2.4	0.9	5	Hs.162209	Homo sapiens claudin 8 (CLDN8), mRNA.	biological_process unknown [0000004]
AK001903	-2.5	0.9	6	Hs.28792	Homo sapiens cDNA FLJ11041 fis, clone PLACE1004405	biological_process unknown [0000004]
NM_001663	-2.6	0.6	4	Hs.89474	Homo sapiens ADP-ribosylation factor 6 (ARF6), mRNA.	intracellular protein transport [0006886]
NM_000067	-2.9	0.3	6	Hs.155097	Homo sapiens carbonic anhydrase II (CA2), mRNA.	protein targeting [0006605]

Legend to supplemental table 1

Supplemental table 1 lists all genes that were found to be differentially expressed in a microarray analysis performed on isolated epidermal cells taken from lesional epidermis of 6 psoriasis and 6 atopic dermatitis patients. The experimental details of the microarray analysis has been published before^{1,2}, and the data on gene expression levels have been deposited, compliant with MIAME criteria, at <http://www.ncbi.nlm.nih.gov/geo/> and are accessible through GEO Series accession number [GSE6601](#). For convenience of the reader we here present all 180 genes that passed the criterion for false discovery rate. Several genes from this list were used in the current study to define a limited gene set that could differentiate between inflammatory skin diseases.

GenID: the reference sequence from which the oligonucleotide sequence on the chip was derived. Mean and Standard deviation: $^2\log$ of the normalized expression ratio of psoriasis/atopic dermatitis. N: the number of chips that gave a reliable signal and were used for analysis. Unigene_ID: the Unigene entry identification code for each gene (see <http://www.ncbi.nlm.nih.gov/sites/entrez?db=unigene>). Description: the given gene name and HUGO gene name. Gene_Ontology: the putative function of the gene in a biological process.

The list is sorted from the highest mean (4.2 for the VNN3 gene) to the lowest mean (-2.9 for CA2). As the mean is the $2\log$ of the ratio psoriasis/atopic dermatitis, all genes with a mean >0 are overexpressed in psoriasis, whereas all genes with a mean <0 are overexpressed in atopic dermatitis. The selection criterion was set at a mean > 1 and < -1 , combined with false discovery rate correction. This gave a list of 180 genes whose expression was significantly different between psoriasis and atopic dermatitis.

References

1. 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**: e2301.
2. de Jongh GJ, Zeeuwen PL, Kucharekova M *et al*. High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis. *J Invest Dermatol* 2005; **125**: 1163-73.