Lowering relative humidity level increases epidermal protein deimination and drives human filaggrin breakdown

Laura Cau, Valérie Pendaries, Emeline Lhuillier, Paul R Thompson, Guy Serre, Hidenari Takahara, Marie-Claire Méchin and Michel Simon

S1. Supplementary materials and methods

S1.1. Targeted DNA sequencing

Genomic DNA was isolated from keratinocytes corresponding to the four different donors using the GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, St Louis, MO). DNA quality and quantity were assessed with a Nanodrop ND-1000 spectrophotometer, and a Qubit v3 fluorometer (Thermo Fisher Scientific, Waltham, MA) and the Qbit dsDNA HS Assay kit (Invitrogen Life Technologies, Carlsbad, CA). Mutation screening was performed by next generation sequencing using the Personal Genome Machine (PGM, Ion Torrent, Thermo Fisher Scientific) and the AmpliSeq technology (Ion Torrent). Using the AmpliSeq Designer tool (ampliseq.com; v4.2), an AmpliSeq Custom Panel was designed to cover a panel of genes, comprising exonic regions and exon-intron boundaries. In particular, 97% of *FLG* was covered. Library construction and sequencing were performed at the GeT-Purpan core facility (Genome and Transcriptome, GenoToul, France). Briefly, libraries were produced using 10 ng of genomic DNA for each sample and each pool of AmpliSeq primers, with the Ion AmpliSeq library kit v2.0 (Ion Torrent) and Ion Xpress Barcodes, following the guidelines of the supplier. Final libraries were individually controlled and quantified on High Sensitivity DNA chips of a BioAnalyzer (Agilent Technologies, Waldbronn, Germany).

Libraries were then pooled, templated on Ion Sphere Particles using the One Touch 2 instrument with HiQ kit, and finally sequenced on the Ion Torrent PGM using the sequencing HiQ chemistry, 400 bases run workflow, on a 316 v2 Ion chip. Data produced were processed using the Ion Torrent Suite Software tools (Thermo Fisher Scientific) for quality filtering, trimming, demultiplexing, aligning on the AmpliSeq hg19 reference human genome, and variant calling. A mean of 115804 usable reads was produced per donor DNA and the mean coverage per *FLG* base was 595. Variant caller files were then transferred to the Ion Reporter Software (v5.0, http://ionreporter.lifetechnologies.com/, Thermo Fisher Scientific) for filtering and annotation of detected variants. Mutations with an allele ratio lower than 0.1 were filtered out.

S1.2. Cell viability assay

Cell viability of RHEs produced with keratinocytes from 3 different donors (3 RHEs/condition/donor) was determined using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma-Aldrich) reduction assay as described previously [R1,R2]. RHEs topically treated with 3% SDS were used as a control. Optical density was measured at 550 nm.

S1.3. Analysis of Lucifer Yellow permeability

To test the epidermis outside-in permeability, 500 µL of 1 mM lucifer yellow (Sigma-Aldrich) was added to RHEs (3 RHEs/condition/donor; 3 different donors). After incubation at 37°C and 5% CO_2 for 6 hours, the culture medium dye concentration was measured using a Varioskan Flash spectrophotometer (Thermo Fisher Scientific; $\lambda_{excitation}$ = 425 nm / $\lambda_{emission}$ = 550 nm). An immature RHE (harvested at day 2) was used as positive control.

S1.4. Transepidermal water loss measurement

Transepidermal water loss (TEWL) of RHEs produced with keratinocytes from 3 different donors (3 RHEs/condition/donor) was measured using the tewameter TM300 (Courage & Khazaka, Cologne, Germany). Measurements were performed at stable temperature (20.5±0.1°C) and relative humidity (48.0±0.2%) according to the manufacturer's instructions. Before measurements, plate covers and sealing films were removed, and culture plates containing RHEs were maintained in the room for at least 20 min to allow equilibration with ambient conditions. Just prior to TEWL measurement, culture inserts were placed on a drop of medium into a Petri dish and a rubber seal was placed between the culture insert and the probe to ensure airtightness. For each RHE, 100 measurements were collected (1 measurement/second) and mean ± standard deviation was evaluated.

S1.5. Western-blotting

Either equal amounts of proteins (according to Ponceau staining) or equal volumes (for deiminated filaggrin-enriched extracts) were resolved by SDS-polyacrylamide gel electrophoresis and electrotransferred onto nitrocellulose membranes (GE Healthcare, Danderyd, SE). Membranes were blocked in a Tris-HCl buffer containing 5% skimmed milk and 0.05% Tween-20, then incubated overnight at 4°C with primary antibodies (for details, see Table S1) and finally incubated for 2 hours at room temperature with secondary horseradish peroxidase-conjugated antibodies diluted to 1:10000 (Goat Anti-Rabbit IgG-HRP and Swine-Anti-Goat IgG-HRP; SouthernBiotech, Birmingham, AL; Goat Anti-Mouse IgG-HRP; Bethyl Laboratories, Montgomery, TX). The detection was realized with ECL Prime system (GE Healthcare) and images were acquired with a G:BOX Chemi XT4 CCD camera (Syngene, Cambridge, United Kingdom) and GeneSys software (Genesys, Daly City, CA).

ImageJ software was used to quantify immunoreactive bands. Signals were normalized to actin immunodetection. Quantification was performed for extracts of RHEs produced with keratinocytes from 3 or 4 different donors and results are illustrated for 3 donors.

S1.6. Light microscopy and indirect immunofluorescence

RHEs (1 RHE/condition/donors; 3 different donors) were fixed in 4% formaldehyde buffer (Sigma-Aldrich) for 24 hours at 4°C, and paraffin-embedded. For light microscopy analyses, five-micron sections were stained with hematoxylin-eosin. For indirect immunofluorescence analyses, after deparaffinization, rehydration and incubation in a blocking solution (2% bovine serum albumin and 0.05% Tween-20 in PBS), sections were first incubated with primary antibodies (for details, see Table S1) and then with the appropriate Alexa-fluor conjugated secondary antibodies (Invitrogen Life Technologies,) diluted to 1:1000 and 4'-6-diamidino-2-phenylindole (DAPI) diluted at 1 µg/ml. Slides were mounted in Mowiol 4-88 (Calbiochem, San Diego, CA). Images were captured with a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan), with a Zeiss Apotome microscope (Carl Zeiss, Jena, Germany) or with a confocal SP8 microscope (Leica Microsystems, Nanterre, France). Ki67 positive nucleus counting was performed with ImageJ software on 20x enlargement pictures corresponding to 3 independent areas of each epidermis. The same brightness threshold was set for all pictures and positive nuclei were counted using the "Analyses Particles" tool. For quantification of deiminated proteins detected with the AMC antibody, images corresponding to 3 independent areas of each RHE were acquired using a confocal SP8 microscope with a 63x oil immersion objective. The area and mean gray value of fluorescence was measured on each pictured with ImageJ software using a fixed threshold and amount of deiminated proteins were evaluated as [Area of fluorescence x Mean gray value of fluorescence]. For the visual

representation, the intensity of the fluorescence signal was coded as a color gradient with a 16-color lookup table using ImageJ software, as previously described [R3].

S1.7. In situ transglutaminase activity assay

In situ transglutaminase assay was performed on cryosections of RHEs produced with keratinocytes from 3 different donors (1 RHE/condition/donor) as described previously [R4,R5]. Briefly, five-micron cryosections were blocked with 1% BSA in 0.1 M Tris-HCl pH 8.4 for 30 min and then incubated for 2 hours at room temperature with Alexa-Fluor-555–cadaverine (Invitrogen Life Technologies) in 0.1 M Tris-HCl pH 8.4 and either 5 mM CaCl2 or 5 mM EDTA (negative control). The reaction was stopped by incubating the sections with 25 mM EDTA in PBS for 5 min. Nuclei were stained with DAPI diluted at 1 µg/ml in PBS and slides were mounted in Mowiol 4-88. For each RHE, 3 pictures corresponding to 3 independent areas were acquired with a 63x oil immersion objective using a confocal SP8 microscope (Leica Microsystems) and the area of fluorescence of each image was measured with ImageJ software using a fixed threshold and mean ± standard deviation was calculated.

S1.8. In vitro calpain activity assay

Calpain activity of RHE extracts (3 RHEs/condition/donor; 3 different donors) was evaluated using a fluorometric calpain activity assay kit (Abcam, Cambridge, United Kingdom) according to the manufacturer's instructions. Briefly, RHEs were homogenized on ice in the extraction buffer provided, which specifically extracts cytosolic proteins. After centrifugation (15000g, 10 min, 4°C), RHE extracts were incubated in the reaction buffer with a calpain substrate (Ac-LLY-AFC) for 1 hour at 37°C in darkness. For each sample, controls were performed in the presence of 2.5 mM of the calpain inhibitor N-Acetyl-Leu-Leu-Nle-CHO (Sigma-Aldrich) to assess the specificity of calpain-independent release of fluorescence.

Upon cleavage of the substrate by calpain, free AFC emits a yellow-green fluorescence that was quantified using a Varioskan Flash spectrophotometer (Thermo Fisher Scientific; $\lambda_{excitation}$ = 400 nm/ $\lambda_{emission}$ = 505 nm). The fluorescence intensity of controls was subtracted from the total fluorescence intensity of the sample to determine calpain activity specific fluorescence. Results are expressed as relative fluorescence units.

S1.9. Reverse transcription

RHEs (1 RHE/condition/donor; 4 different donors) were placed in RNAlater RNA stabilization solution (Qiagen, Hilden, Germany) for at least one day at 4°C and total RNA was extracted with RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. Quality of total RNA was validated for RNA Integrity Number > 8.5 using an Agilent 2100 Bioanalyzer with the Agilent RNA 6000 Nano Kit (Agilent Technologies) and RNA quantity was evaluated using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). RT was performed from 400 ng of total RNA by using PrimeScript II 1st strand cDNA synthesis kit (Takara Bio Inc., Kusatsu, Japan) with combination of oligo(dT) and random hexamer primers according to the manufacturer's instructions.

S1.10. Quantitative polymerase chain reaction

For quantitative polymerase chain reaction (qPCR), each amplification was performed twice with the 7300 Real Time PCR System (Applied BioSystems, Foster City, CA) using the Sybr qPCR SuperMix W/ROX (Invitrogen Life Technologies). The qPCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. The steady-state mRNA levels of each gene were determined using *YWHAZ* expression for normalization, according to the $2(^{-\Delta\Delta Ct})$ calculation method [R6]. Sequences of primers used in this study are presented in Table S2. For some genes,

primer pairs were newly designed with Primer3 software (http://primer3.ut.ee/). All primer pair specificities were verified by *in silico* nBLAST analysis, and by loading on 2.5% agarose gel and Sanger-sequencing amplicons. Moreover, qPCR primer efficiencies were checked on RHE cDNA serial dilutions (1/10, 1/20, 1/40, 1/80, 1/160 and 1/320).

S1.11. Analysis of the expression of histidase at the mRNA level

The mRNAs encoding histidase variants 2 (*HAL-V2*) and 3 (*HAL-V3*) were analyzed by RT-qPCR using specific primers (Table S2), whereas the variant 1 (*HAL-V1*) was analyzed by a classical PCR approach. PCR was performed by using the EconoTaq PLUS GREEN 2X PCR Master Mix (Lucigen, Middleton, WI). The PCR conditions were as follows: 95°C for 4 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 sec, and elongation at 72°C for 1 min, followed by a final elongation at 72°C for 5 min.

S2. Supplementary tables

Table S1. Primary antibodies

Antigen	Antibody	Source (Reference)	Dilution	
			WB	IIF
Actin	clone C4	Merck Millipore, Billerica, MA	1:10000	-
Bleomycin hydrolase	polyclonal HPA039548	Sigma-Aldrich, St Louis, MO	1:1000	-
Calpain 1	polyclonal HPA005992	Sigma-Aldrich	1:1000	-
Caspase 14	clone D-10	Santa Cruz Biotech., Santa Cruz, CA	1:100	-
Corneodesmosin	clone G36-19	[R7]	1:1000	-
Elastase 2	polyclonal K-14	Santa Cruz Biotech.	1:200	-
Filaggrin	clone AHF3	[R8]	1:1000	1:1000
Histidase	clone 4F2	Abnova, Taipei, Taiwan	1:1000	-
Involucrin	clone SY5	Sigma-Aldrich	1:1000	-
Kallikrein 5	polyclonal AF1108	R&D Systems, Minneapolis, MN	1:500	-
Keratin 10	polyclonal PRB-159P	Covance, Princeton, NJ	1:40000	-
Keratin 14	polyclonal HPA023040	Sigma-Aldrich	1:40000	-
Ki-67	clone SP6	Abcam, Cambridge, United Kingdom	-	1:100
Loricrin	polyclonal AF62	Covance	1:10000	-
Matriptase	clone D-7	Santa Cruz Biotech.	1:100	-
Modified deiminated proteins	Anti-Modified Citulline (AMC)*	[R9]	1:2500	1:1000
Peptidyl-arginine deiminase 1	polyclonal anti-peptide anti-PAD1	[R10,R11]	1:200	-
Peptidyl-arginine deiminase 3	polyclonal anti-peptide anti-PAD3	Abcam, Cambridge	1:200	-
SASPase	polyclonal C-13	Santa Cruz Biotech.	1:200	-
Transglutaminase 1	polyclonal A018	Zedira, Darmstadt, Germany	1:200	-
Transglutaminase 3	polyclonal A015	Zedira	1:200	-
Transglutaminase 5	polyclonal A008	Zedira	1:500	-

* When AMC antibodies were used, citrullyl residues were chemically modified before the saturation step by incubation at 37°C in 0.0125% $FeCl_3$, 2.3M H_2SO_4, 1.5M H_3PO_4, 0.25% diacetyl monoxime and 0.125% antipyrine for 1 hour for western blot and 3 hours for indirect immunofluorescence, as previously described [R3,R9,R12].

Table S2. Sequences of PCR primers

Gene	Forward primer	Reverse primer	Reference
ASPRV1	GTTTCTCTTCTTGGCGTGGG	ATGGTGCCAATGGTTGAGGT	*this work*
BLMH	GTGGTGGACAGGAAGCATGT	TCCTTTGCAGCTACGTCAGG	[R2]
CAPN1	CAAACACCCCTCCCCCAGGATGT	CGCACCCGCAGCTGCTCATA	[R2]
CASP14	TGCACGTTTATTCCACGGTA	TGCTTTGGATTTCAGGGTTC	[R2]
CDSN	ACTGCTGCTGGCTGGTCT	AGAGCTTCTGGCACTGGAAA	*this work*
FLG	GCAAGGTCAAGTCCAGGAGAA	CCCTCGGTTTCCACTGTCTC	[R12]
IVL	GCCAGGTCCAAGACATTCAAC	GGGTGGTTATTTATGTTTGGGTGG	[R12]
KLK5	AGTCAGAAAAGGTGCGAGGA	TGAACTTGCAGAGGTTCGTG	*this work*
KRT10	TGATGTGAATGTGGAAATGAATGC	GTAGTCAGTTCCTTGCTCTTTTCA	[R12]
KRT14	CTCATCCTCCCGCTTCTCCT	AAAGCCACTACCAAAGCTGCT	[R2]
LOR	CGAAGGAGTTGGAGGTGTTT	ACTGGGGTTGGGAGGTAGTT	[R13]
PADI1	AGAGTGACATCGTGGACATTC	GCTCGTGGTAGGACAAGTAGTC	[R12]
PADI2	GGTGGGATGAGCAGCAAGCGAATC	GAACAGAGCGGGCAGGTCAATGATG	[R12]
PADI3	GCAGAGTGTGACATCATTGACATCC	GACCGCACCTTCTCCTCCAG	[R12]
PADI4	CCACACGGGGCAAACTGTC	CAGCAGGGAGATGGTGAGGG	[R12]
PADI6	CGTGGAGAAGTGCATTCACCTGAAC	GCCTCGCAAAGGACCTCTTGGG	[R12]
ST14	ATCGCCTACTACTGGTCTGA	GTTTTGGAGTCCGTGGGGAAA	*this work*
TGM1	CCCCCGCAATGAGATCTACA	ATCCTCATGGTCCACGTACACA	*this work*
TGM3	GGAAGGACTCTGCCACAATGTC	TGTCTGACTTCAGGTACTTCTCATACTG	*this work*
TGM5	CGGAGCAGGTTGAGGACTGT	GAGGACTCCAAGGAAGACTTTCTG	*this work*
HAL-V1	AAAGAGAAAACAGTTGTTTACGGTAT TAC	GCGATCTTTTATCCAGGGCCTTAC	*this work*
HAL-V2	AGAGAAAACAGGGAGCTTCAGG	CAAGATGAGAGAGTGGGGCA	*this work*
HAL-V3	GTTGACTCCCTCTCCACCAG	TACTTCCCAAACCTTACAACAGA	*this work*
YWHAZ	ACTTTTGGTACATTGTGGCTTCAA	CCGCCAGGACAAACCAGTAT	[R12]

S3. Supplementary references

[R1] A. Frankart, A. Coquette, K.-R. Schroeder, Y. Poumay, Studies of cell signaling in a reconstructed human epidermis exposed to sensitizers: IL-8 synthesis and release depend on EGFR activation, Arch. Dermatol. Res. 304 (2012) 289–303.

[R2] V. Pendaries, J. Malaisse, L. Pellerin, M. Le Lamer, R. Nachat, S. Kezic, A.-M. Schmitt, C. Paul, Y. Poumay, G. Serre, M. Simon, Knockdown of Filaggrin in a Three-Dimensional Reconstructed Human Epidermis Impairs Keratinocyte Differentiation, J. Invest. Dermatol. 134 (2014) 2938–2946.

[R3] M.-C. Méchin, L. Cau, M.-F. Galliano, S. Daunes-Marion, S. Poigny, J.-L. Vidaluc, S. Bessou-Touya, H. Takahara, G. Serre, H. Duplan, M. Simon, Acefylline activates filaggrin deimination by peptidylarginine deiminases in the upper epidermis, J. Dermatol. Sci. 81 (2016) 101–106.

[R4] M. Raghunath, H.C. Hennies, F. Velten, V. Wiebe, P.M. Steinert, A. Reis, H. Traupe, A novel in situ method for the detection of deficient transglutaminase activity in the skin, Arch. Dermatol. Res. 290 (1998) 621–627.

[R5] E.A. Leclerc, A. Huchenq, S. Kezic, G. Serre, N. Jonca, Mice deficient for the epidermal dermokine β and γ isoforms display transient cornification defects, J. Cell Sci. 127 (2014) 2862–2872.

[R6] T. Senshu, K. Akiyama, S. Kan, H. Asaga, A. Ishigami, M. Manabe, Detection of Deiminated Proteins in Rat Skin: Probing with a Monospecific Antibody After Modification of Citrulline Residues, J. Invest. Dermatol. 105 (1995) 163–169.

[R7] G. Serre, V. Mils, M. Haftek, C. Vincent, F. Croute, A. Réano, J.P. Ouhayoun, S. Bettinger, J.-P. Soleilhavoup, Identification of late differentiation antigens of human

cornified epithelia, expressed in re-organized desmosomes and bound to cross-linked envelope, J. Invest. Dermatol. 97 (1991) 1061–1072.

[R8] M. Simon, M. Sebbag, M. Haftek, C. Vincent, E. Girbal-Neuhauser, J. Rakotoarivony, G. Sommé, D. Schmitt, G. Serre, Monoclonal Antibodies to Human Epidermal Filaggrin, Some Not Recognizing Profilaggrin, J. Invest. Dermatol. 105 (1995) 432-437.

[R9] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nat. Protoc. 3 (2008) 1101–1108.

[R10] M. Guerrin, A. Ishigami, M.-C. Méchin, R. Nachat, S. Valmary, M. Sebbag, M. Simon, T. Senshu, G. Serre, cDNA cloning, gene organization and expression analysis of human peptidylarginine deiminase type I, Biochem. J. 370(Pt 1) (2003) 167–174.

[R11] R. Nachat, M.-C. Méchin, H. Takahara, S. Chavanas, M. Charveron, G. Serre, M. Simon, Peptidylarginine Deiminase Isoforms 1–3 Are Expressed in the Epidermis and Involved in the Deimination of K1 and Filaggrin, J. Invest. Dermatol. 124 (2005) 384–393.

[R12] M.-C. Méchin, F. Coudane, V. Adoue, J. Arnaud, H. Duplan, M. Charveron, A.-M. Schmitt, H. Takahara, G. Serre, M. Simon, Deimination is regulated at multiple levels including auto-deimination of peptidylarginine deiminases, Cell. Mol. Life Sci. 67 (2010) 1491–1503.

[R13] M. Visser, D. Zubakov, KN. Ballantyne, M. Kayser, mRNA-based skin identification for forensic applications, Int. J. Legal Med. 125 (2011) 253–263.

S4. Supplementary figure legends

Fig. S1. Unchanged cell viability, outside-in permeability and proliferation in RHEs when exposed to the dry condition. RHEs produced with keratinocytes from 3 different donors (B1-B3) and exposed to humid or dry conditions were collected at day 10. **(a)** Cell viability was measured with an MTT assay. Positive control corresponds to RHEs topically treated with 3% SDS. **(b)** Outside-in permeability was evaluated using the Lucifer Yellow assay. A Lucifer yellow solution was applied to RHEs and, after 6 hours of incubation, the dye concentration in the culture medium was measured. Positive control corresponds to immature RHEs (harvested at day 2). **(c)** Left part: representative immunodetection of Ki67 on sections of fixed RHEs (3 different donors). Scale bar = 50 µm. Right part: quantification of Ki67 positive nuclei per mm of epidermis length. *n. s. = no significant difference*.

Fig. S2. Exposure to the dry condition alters the RHE ultrastructure. RHEs produced with keratinocytes from 3 different donors (B1-B3) and exposed to humid or dry conditions were collected at day 10. **(a)** Quantification of cornified, granular and total viable keratinocyte layers. *n. s. = no significant difference*. **(b)** Transmission electron micrographs of the granular layers. Scale bar = 2 µm. *SC*: *stratum corneum*; G1: first granular layer; G2: second granular layer; G3: third granular layer. Note the increased number of granular layers and the increased size of keratohyalin granules in RHEs cultivated in the dry condition *versus* the humid one. **(c)** Measurement of the keratohyalin granules (KHG) density (area of KHG / area of cytoplasm) and size.

Fig. S3. Gene expression analyzed by RT-qPCR. Expression of genes encoding **(a)** for keratin K14 and various differentiation markers, **(b)** for filaggrin (FLG) and **(c)** for proteases implicated in filaggrin breakdown was analyzed by RT-qPCR in RHEs exposed to humid (H) or dry (D) conditions (4 keratinocyte donors). *n. s. = no significant difference*

Fig.S4. **Imuunodetection of profilaggrin and filaggrin in dry RHEs.** RHEs were produced with keratinocytes from 3 different donors (B1-B3) in humid or dry conditions. Fixed-tissue sections were analyzed by indirect immunofluorescence with AHF3 to detect profilaggrin and filaggrin. Differential contrast images images are superimposed. Nuclei are stained in blue using DAPI, scale bar = 20 µm, the *stratum corneum* (SC) and *stratum granulosum* (SG) are indicated.

Fig. S5. Expression of proteases proposed to be involved in filaggrin metabolism regulation. **(a)** Expression of genes encoding kallikrein 5 (*KLK5*), matriptase (*ST14)* and skin-aspartic protease (SASPase; *ASPRV1*) was analyzed by RT-qPCR in RHEs exposed to humid or dry conditions (4 different donors). *n. s. = no significant difference*. Note: we did not succeed in detecting *elastase 2* transcripts. **(b)** Expression of actin (as a loading control), KLK5, SASPase, matriptase and elastase 2 was analyzed by western blotting of total protein extracts prepared from RHEs produced with keratinocytes from 3 different donors (B1-B3) and exposed to humid (H) or dry (D) conditions.

Fig. S6. Expression of histidase in RHEs. Three variants of histidase (GenBank™ accession numbers: NM_002108.3, NM_001258333.1 and NM_001258334.1) were detected in RHEs by both RT-qPCR and western blotting. **(a)** Expression of the variant 1 encoding mRNAs *(HAL-V1)* analyzed by RT-PCR in RHEs exposed to humid (H) or dry (D) conditions (4

keratinocyte donors). **(b)** Expression of variants 2 and 3 encoding mRNAs *(HAL-V2* and *HAL-V3)* analyzed by RT-qPCR in RHEs exposed to humid or dry conditions (4 different donors). *n. s. = no significant difference*. **(c)** Detection of the three variants of histidase by western blotting of TE-NP40 extracts prepared from RHEs produced with keratinocytes from 3 different donors (B1-B3) and exposed to humid (H) or dry (D) conditions.