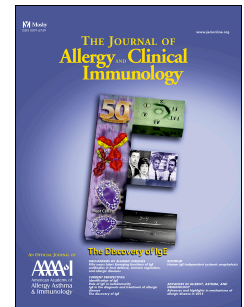


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Epithelial barrier dysfunction in Desmoglein-1 deficiency

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Short summary

Desmoglein-1 deficiency is a missing link between impaired epithelial barrier function and immunological dysregulation. Our characterization of SAMEC syndrome highlighted the pivotal role of the desmoglein-1/ERBIN interaction as an inhibitor of skin inflammation via the NF- κ B signaling pathway.

Keywords: atopy, epithelial barrier, desmosome, desmoglein-1, desmoplakin, arrhythmogenic cardiomyopathy, ERBIN, NF- κ B signaling pathway, eosinophilic esophagitis, SAMEC

Abbreviations

DSG1: desmoglein-1

DSP: desmoplakin

ERBIN: ERBB2-interacting protein

HEK293T cells: human embryonic kidney 293 cells with the SV40 large T-antigen

MEF: mouse embryo fibroblast

ML120B: N-[6-chloro-7-methoxy-9H- β -carbolin-8-yl]-2-methylnicotinamide

SAM: severe dermatitis, multiple allergies and metabolic wasting

SAMEC: severe dermatitis, multiple allergies, metabolic wasting, ectodermal dysplasia and arrhythmogenic cardiomyopathy

TSLP: thymic stromal lymphopoietin

To the Editor,

Mutations in desmoplakin (*DSP*) and desmoglein-1 (*DSG1*) genes have been implicated in the inherited inflammatory skin disease known as severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome (MIM#603165, Tables E1 and E2).^{1,2} The *DSP* and *DSG1* genes encode desmosome components that are critical for the structure of intercellular junctions and the maintenance of epithelial barrier integrity. *DSP* and *DSG1* are also key regulators of signaling pathways involved in differentiation, epidermal homeostasis, and carcinogenesis. *DSG1* promotes keratinocyte differentiation by inhibiting EGFR/ERK signaling via ERBB2-interacting protein (ERBIN), a scaffolding and signaling protein.³ Through the characterization of a new syndrome featuring severe allergic dermatitis and *DSG1* deficiency, we highlighted the pivotal role of the functional desmoglein-1/ERBIN interaction as an inhibitor of skin inflammation via the NF- κ B signaling pathway.

Patient#1 and Patient#2 (13 and 9 years old, respectively) are two unrelated boys, born to healthy parents. They were referred for life-long desquamative erythroderma associated to sparse and wooly hair and dysplastic enamel. Both patients developed painful palmoplantar keratoderma and dystrophic nails (Figures 1A-B). Skin manifestations combined recurrent, painful erythrodermic skin flares triggered by infections and episodes of aseptic pustular psoriasiform dermatitis. Patient#1 (but not Patient#2) displayed failure to thrive, eosinophilic esophagitis, colitis, and a variety of food allergies (total serum IgE level: 2968 kIU/mL [N<114]). Cardiac examination of Patient#1 revealed an asymptomatic, biventricular, dilated cardiomyopathy. At 9 years, Patient#2 received a heart transplant due to a severe left-dominant arrhythmogenic cardiomyopathy.

For both patients, cutaneous histopathology showed an epidermal acantholysis and a dermal inflammatory lymphocytic infiltration (Figures 1C and E1). Ultrastructural examination revealed rare, large numbers of abnormal clusters of desmosomes in the epidermis (Patient#1, Figure 1D). Histopathology of the explanted heart showed the characteristic fibro-fatty myocardial infiltration of arrhythmogenic dysplasia (Figure E1).

Two different heterozygous *de novo* missense mutations were identified by whole exome sequencing in exon 14 of the *DSP* gene: c.A1757C (p.H586P, Patient#1) and c.T1828C (p.S610P, Patient#2). Substitution of H586 or S610 by a proline is expected to induce a kink in the alpha-helix of the DSP's plakin domain that perturbs DSP's three-dimensional structure (Figure E1).

In both patients, skin immunohistochemistry showed: i) low DSP and DSG1 expression in the epidermis (as in Patient#1 primary keratinocytes), ii) irregular and less intense DSP and DSG1 staining at the keratinocyte plasma membrane, iii) abnormal cytoplasmic accumulation of DSP and DSG1 proteins in keratinocytes (Figure 1E). Esophageal immunohistochemistry revealed low level of DSP staining which was irregular and mottled at the cell border and the absence of DSG1 expression (Patient#1, Figure 1F). The expression of DSP protein was also low in heart tissue (Patient#2, Figure E1) (DSG1 is not expressed in heart).

Abnormally high levels of mRNAs encoding pro-inflammatory cytokines (*IL6*, *IL8* and *IL-1 β*), three NF- κ B target genes, and *TSLP* were found in Patient#1's keratinocytes (Figure 2A). Overexpression of IL-6 was confirmed by ELISA (Figure E2). In contrast, mRNA levels of *TNF α* and other pro-TH2 cytokines (*IL13*, *CCL5* and *IL4*, data not shown) were not elevated. Inhibition of the NF- κ B signaling pathway by ML120B, which selectively targets the catalytic subunit of IKK β , restored the normal expression of *IL8* mRNA by Patient#1's keratinocytes

(Figure E2). In view of the primary DSG1 deficiency reported in SAM syndrome and the very low levels of DSG1 protein expression in our patients, we hypothesized that DSG1 could play a role in the inflammatory phenotype. We demonstrated that DSG1 (but not DSP) inhibits NF- κ B reporter activity in a dose-dependent manner following stimulation by IL-1 β or TNF α in HEK293T cells (Figures 2B and E2). Concomitant downregulation of *IL6* and *IL8* expression was observed upon transfection with the DSG1-encoding plasmid (Figure E2). Silencing of *DSG1* by shRNA (with a mean decrease of DSG1 mRNA of 32%) enhanced transcription of *IL6*, *IL8*, *IL-1 β* , *TNF α* and *TSLP* genes in control keratinocytes (regardless of stimulation with IL-1 β , Figures 2C and E2). Retroviral transduction of WT-*DSG1* into Patient#1's keratinocytes rescued the inflammatory cellular phenotype by restoring *IL8* production (Figure 2D).

Given that DSG1 might regulate ERBIN's cellular localization and thus modulate the latter's ability to block ERK signaling, we hypothesized that ERBIN may play a role in DSG1-mediated NF- κ B inhibition.³ We observed little ERBIN/DSG1 co-localization at the cell membrane, and an accumulation of ERBIN in the cytoplasm in a skin biopsy from Patient#1, relative to healthy controls (mean proportion of ERBIN/DSG1 co-localization: 40.7% vs 71.2%, $p < 0.001$; Figure E3). Experiments in control keratinocytes highlighted the recruitment of ERBIN to the cell membrane and an increase in ERBIN/DSG1 co-localization following stimulation by IL-1 β . Interestingly, this recruitment did not occur in Patient#1's keratinocytes (Figure E3). Contrary to Patient#1's keratinocytes, the level of ERBIN protein increased after stimulation of control keratinocytes by IL-1 β (Figure E3). These results demonstrated that the localization of ERBIN at the cell membrane and the membrane co-localization of ERBIN/DSG1 in keratinocytes were regulated by inflammatory stimuli (such as IL-1 β , at least).

In a reporter transactivation assay, we found that ERBIN transfection in both non-stimulated

and IL-1 β -stimulated HEK293T cells led to NF- κ B activation in a dose-dependent manner (Figure 2E). Erbin^{-/-} keratinocytes, derived from Erbin^{-/-} mice, showed abnormally low levels of the pro-inflammatory cytokines *IL6* and *IL-1 β* mRNAs (Figure 2F). In addition, abnormally low levels of *IL-1 β* mRNA were found in the Erbin^{-/-} MEFs, regardless of stimulation with lipopolysaccharide (Figure 2G). Biochemical analysis of NF- κ B activation showed that in Erbin^{-/-} MEFs, I κ B α degradation was delayed in response to IL-1 β (Figure 2H).

Here, we reported on two unrelated patients in whom severe dermatitis and loss of epithelial barrier integrity were related to two different, heterozygous mutations in *DSP*. Both patients presented with a combination of SAM syndrome, Ectodermal dysplasia (combination of hair, nail, and tooth anomalies) and arrhythmogenic Cardiomyopathy (hereby named SAMEC syndrome). Due to the major risk of severe cardiac involvement in patients carrying *DSP* mutation, a thorough cardiac assessment should always be carried out in patients presenting with the SAM syndrome phenotype, unless *DSP* mutations have been ruled out.

Our results demonstrate that DSG1 inhibits skin inflammation by inhibiting the NF- κ B signaling pathway at least. Various observations support that DSG1 deficiency is linked to inflammation. Firstly, DSG1 deficiency has been reported in cases of atopic dermatitis and Netherton syndrome^{4,5}, sharing with SAMEC chronic inflammatory dermatitis and allergic manifestations. Secondly, two siblings presented with Netherton syndrome and normal DSG1 epidermal staining, whereas no skin inflammation was observed.⁶ Thirdly, DSG1 deficiency is probably responsible for the mucosal barrier impairment and inflammation observed in eosinophilic esophagitis.⁷

We suggest that DSG1 normally inhibits NF- κ B signaling pathway at least in part by retaining ERBIN at the cell membrane. The involvement of ERBIN in other inflammatory diseases has been shown. In the context of inflammatory bowel disease, ERBIN seems to specifically

inhibit the NF- κ B signaling pathway *via* NOD2.⁸ In addition, five related patients presenting with similar allergic inflammatory manifestations as SAMEC syndrome (eczema, eosinophilic esophagitis and an elevated serum IgE level) were shown to carry a heterozygous mutation in the gene coding for ERBIN.⁹ Thus, ERBIN mis-localization in SAMEC syndrome might explain our patients' allergic manifestations.

Through the characterization of SAMEC syndrome, we showed that DSG1 acts as a novel, hitherto unexpected inhibitor of epithelial inflammation by inhibiting the NF- κ B signaling pathway, probably *via* the functional desmoglein-1/ERBIN interaction. The lack of an epithelial barrier protein, here DSG1, appears to be a crucial link between loss of epithelial barrier integrity and immunological dysregulation.

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Figure legends

Figure 1. Clinical and histopathological features of patients #1 and #2

- (A) Clinical phenotype of Patient#1: desquamative erythroderma, thickening of the skin (a-e); sparse hair (a); diffuse PPK (b, c), and thickening of the nail plate (d, e).
- (B) Clinical phenotype of Patient#2: desquamative erythroderma (a), sparse hair (a, b), onycholysis (c), and plantar keratoderma (d).

(C) Skin histology (Patient#1) showing epidermal acanthosis, hyperorthokeratosis, extensive acantholysis (inset), and inflammation in the superficial dermis (lymphocytes).

(D) Ultrastructural features of skin biopsy from Patient#1 (a, b, d) and healthy control (c). (a) Many desmosomes clustered in the upper epidermis (arrows). Keratin filaments strongly aggregated to the desmosome (inset). (b) Complete absence of desmosomes and widened spaces between keratinocytes (cell membrane features filipodium-like processes; inset), in the lower epidermis. (d) Patient#1's desmosome lacks the inner plaque compared to control (arrow, c).

(E) Immunofluorescence on skin sections from a healthy control (a-c), Patient#1 (d-f) and Patient#2 (g-i) showed a drastic reduction and mislocalization in the staining of DSP and DSG1 in both patients.

(F) Immunofluorescence on esophageal sections from a healthy control (a-c) and Patient#1 (d-f), showing low levels of DSP staining and absence of DSG1 staining in Patient #1.

Figure 2. Role of DSG1 and ERBIN in NF- κ B-mediated epithelial inflammation

(A) Relative mRNA expression levels of pro-inflammatory cytokines in primary keratinocytes.

(B) NF- κ B luciferase reporter assay in HEK293T cells transfected with DSG1 vector or with an empty vector, and stimulated with IL-1 β or TNF α (AU: arbitrary units). Inset: Western blot of DSG1 expression in HEK293T cells transfected with DSG1 vector.

(C) Relative mRNA expression of *IL8* (a), *IL6* (b), *IL-1 β* (c), *TNF α* (d) and *TSLP* (e) in control (ctrl) keratinocytes infected with a lentivirus expressing shRNA against *DSG1* and stimulated or not (NS) with IL-1 β .

(D) Relative mRNA expression of in Patient#1's keratinocytes infected with a retrovirus expressing FLAG-*DSG1*. Inset: Western blot of DSG1 and FLAG expression in Patient#1's

keratinocytes after transduction.

(E) NF- κ B luciferase reporter assay in HEK293T cells transfected with ERBIN or empty vector, and stimulated with IL-1 β . Inset: Western blot of ERBIN expression in HEK293T cells transfected with ERBIN vector.

(F) Relative mRNA expression levels of *IL-1 β* and *IL6* in non-stimulated Erbin^{+/+} and Erbin^{-/-} keratinocytes.

(G) Relative mRNA expression levels of *IL-1 β* in Erbin^{+/+} and Erbin^{-/-} MEFs before/after stimulation with LPS.

(H) Immunoblot assays showing the degradation of I κ B α proteins in Erbin^{+/+} and Erbin^{-/-} MEFs induced by IL-1 β stimulation.

Disease	Total of patients	Past familial history	Dermatological symptoms	Features of ectodermal dysplasia				Allergic details	Cardiac involvement	DSG1 mutation
				Hair	Teeth	Nails	Sweating			
SAM syndrome	2 sisters	Diffuse plantar keratoderma (mother)	PPK, skin fragility, congenital ichthyosiform erythroderma	Sparse	NS	NS	NS	Severe food allergies, Elevated total IgE levels, Eosinophilic esophagitis (one patient)	No (1) / Muscular ventricular-septal defects (1)	c.49-1G>A (homozygous) ²
	2 sisters	Focal palmar keratoderma (father)	Congenital erythroderma	Sparse	NS	NS	NS	Multiple food allergies, Elevated total IgE levels	No	c.1861delG (homozygous) ²
	1	Focal plantar keratoderma (mother)	PPK, atopic dermatitis since the first month of life with severe generalized flares of dermatitis	Curly	No	No	NS	Multiple allergies, Elevated total IgE levels	No	c.2659C>T, p.R887*, exon 15 (homozygous) ³
	2 half brothers	Mild PPK (mother)	PPK, skin erosions, psoriasiform and eczematous erythroderma in the first few months of life	No	No	No	No (1) / NS (1)	Multiple allergies, Elevated total IgE levels	No	c.2614delA (p.Ile872Serfs*10), exon 15 (homozygous) ⁴
	2 brothers and sisters	PPK (both parents)	PPK, skin erosions, congenital erythroderma	Curly and hard	No	No (1) / "affected nails" wo precision (1)	NS	Isolated elevated total IgE levels for the boy	No	c.1892-1delG / p.Gly631Glnfs*8, exon 14 (homozygous) ⁵
	1	PPK (father)	PPK, transient erythematous patches	Curly	No	No	No	Slight elevated total IgE, no clinical manifestation of allergy	No	c.811_812delAC, (p.Q271Vfs*20), exon 7 + c.2100+4A>G, IVS14+4A>G, intron 14 ⁶

Table E1. Main clinical characteristics of reported patients with SAM syndrome due to recessive mutation in *DSG1* gene. These 10 patients do not seem to have an ectodermal dysplasia even they presented with ectodermal dysplasia features (NS: not specified, PPK: palmoplantar keratoderma, wo: without)

Disease	Total of patients	Past familial history	Dermatological symptoms	Features of ectodermal dysplasia				Allergic details	Cardiac involvement	DSP mutation
				Hair	Teeth	Nails	Sweating			
SAMEC syndrome	1 (M)	NS	Diffuse PPK, erythroderma from the first week of life with superficial pustulosis	Yes, hypotrichosis	Yes, hypodontia	Yes, Nail dystrophy	NS	Multiple food allergies Elevated total IgE level	Normal at 6 yo (clinical exam and ultrasound)	c.1757A>C (p.His586Pro-, exon 14 (heterozygous)) ⁷
	1 (M)	NS	PPK, erythrokeratoderma	Yes, sparse hair at birth, scalp hair was absent after	Yes, enamel defect, no hypodontia on primary teeth (but died at 3 yo)	Yes, Nail dystrophy	NS	No clinical manifestation of allergy Normal level of total IgE	Yes, marked left atrial and ventricular dilation and right ventricular dilation with an ejection fraction of 20%, and he died of heart failure at 3 years of age	Q616P, exon 14 (heterozygous) ⁸
	1 (M)		PPK, erythrokeratoderma	Yes, sparse eyebrows, eyelashes and scalp hair	Yes, hypodontia, enamel defects	Yes, Nail dystrophy	NS	No clinical manifestation of allergy Normal level of total IgE	Yes, pronounced left ventricular dilation and right atrial dilation	H618P, exon 14 (heterozygous) ⁸
	1 (F)		PPK, erythrokeratoderma	Yes, sparse eyebrows, eyelashes and scalp hair	Yes, hypodontia	Yes, Nail dystrophy	NS	No clinical manifestation of allergy Normal level of total IgE	Yes, a moderately dilated left ventricle with low normal systolic function	L622P, exon 14 (heterozygous) ⁸

Table E2. Main clinical characteristics of reported patients with SAM syndrome due to dominant mutation in *DSP* gene. These 4 patients have an ectodermal dysplasia (F: female, M: male, NS: Not specified, PPK: palmoplantar keratoderma).

Figure 1

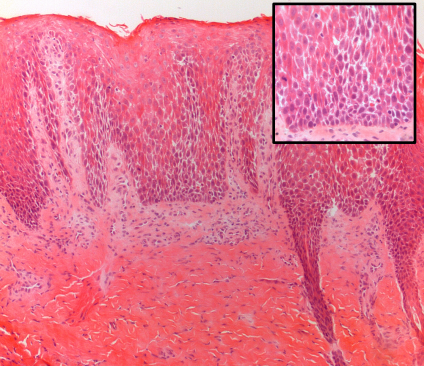
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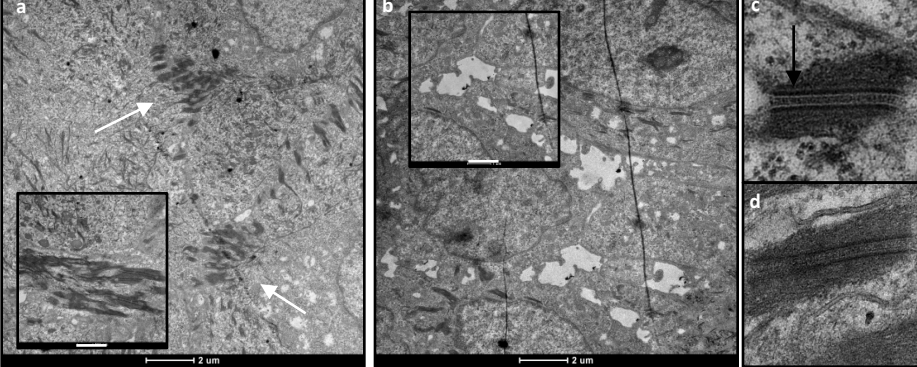
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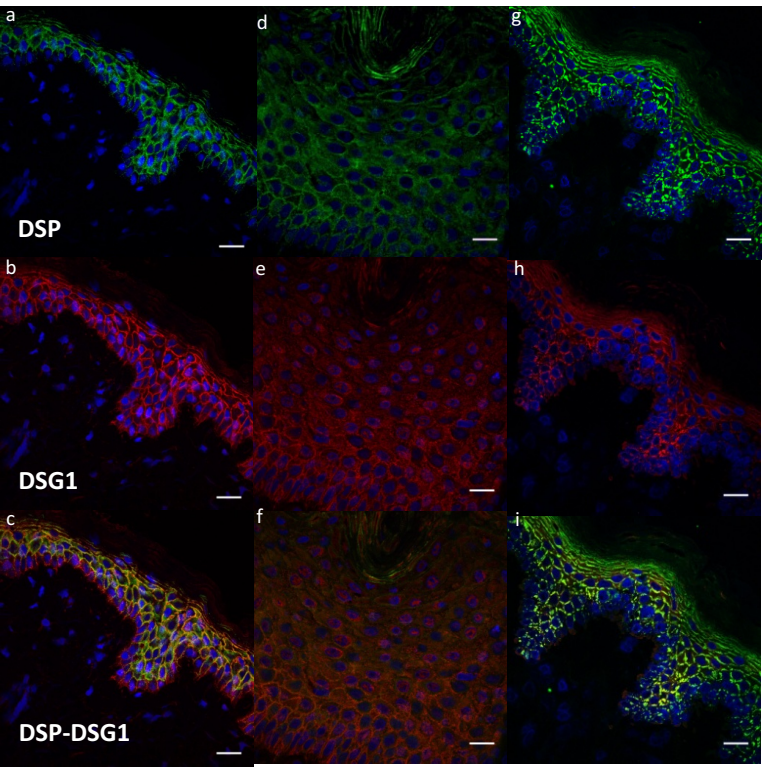
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E



F

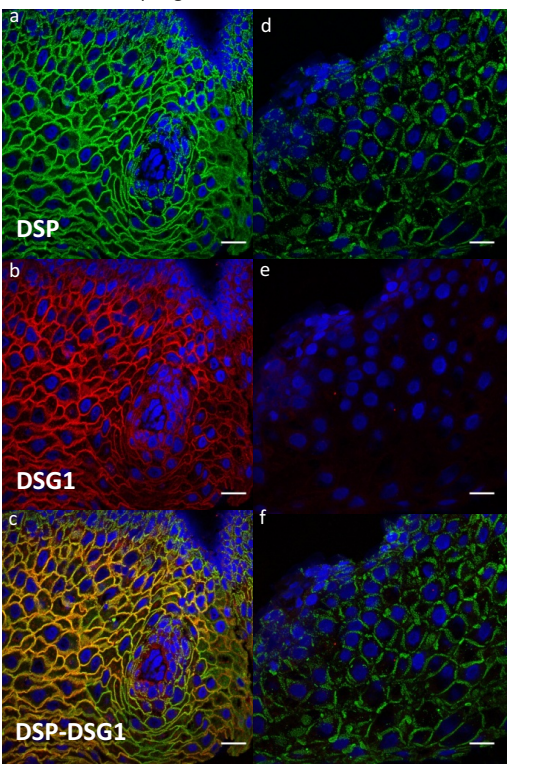
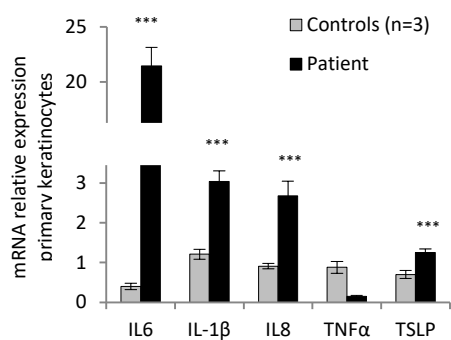
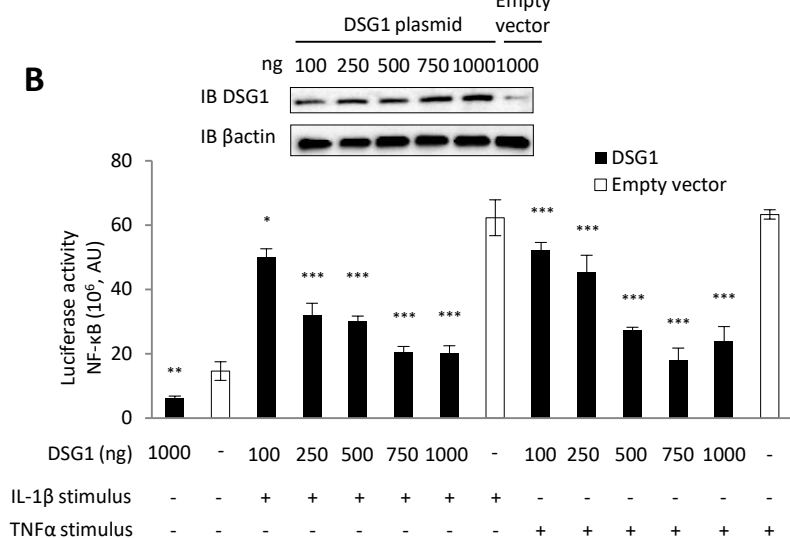
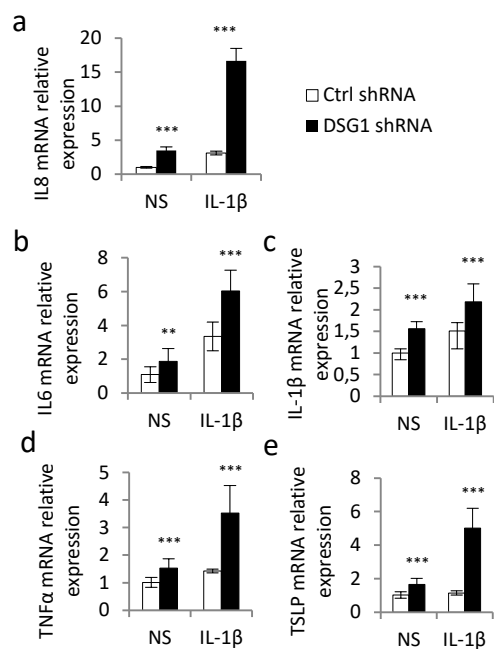
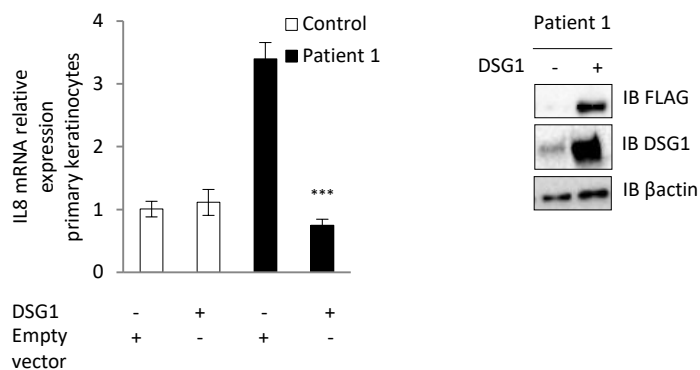
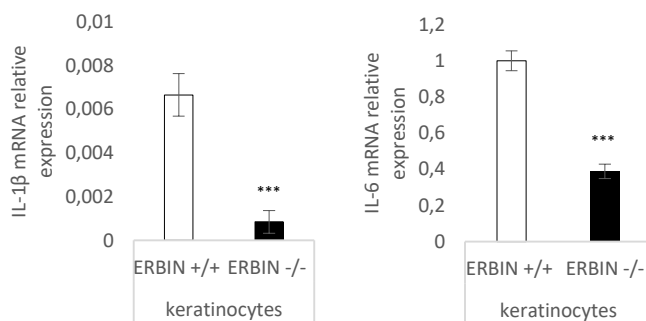
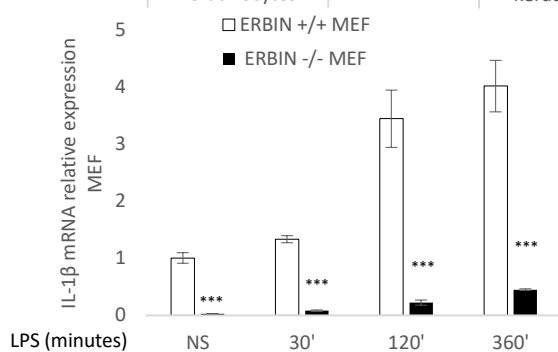
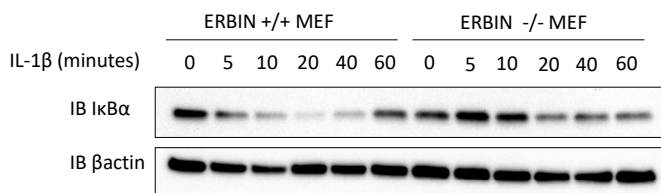
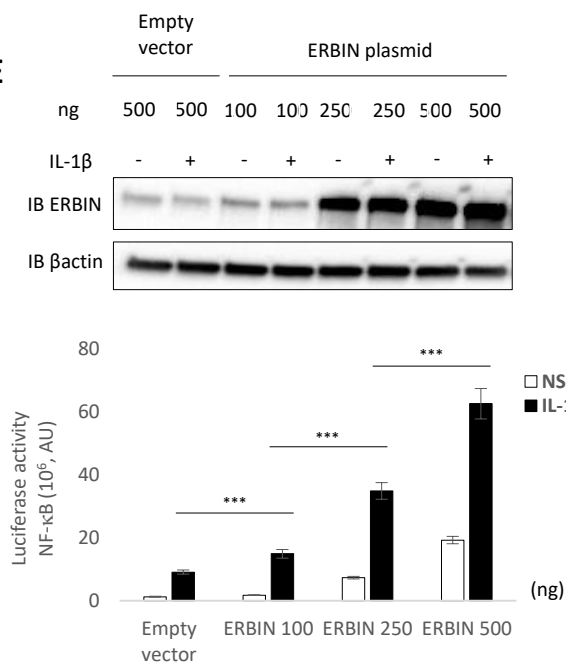
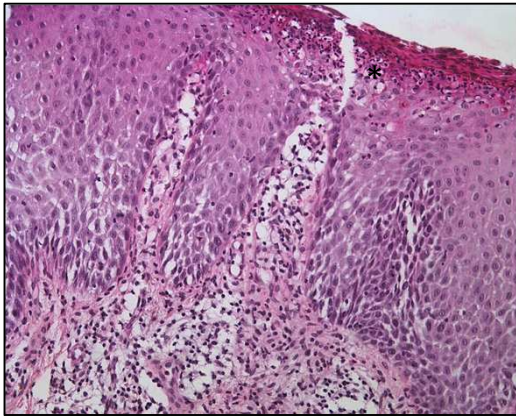
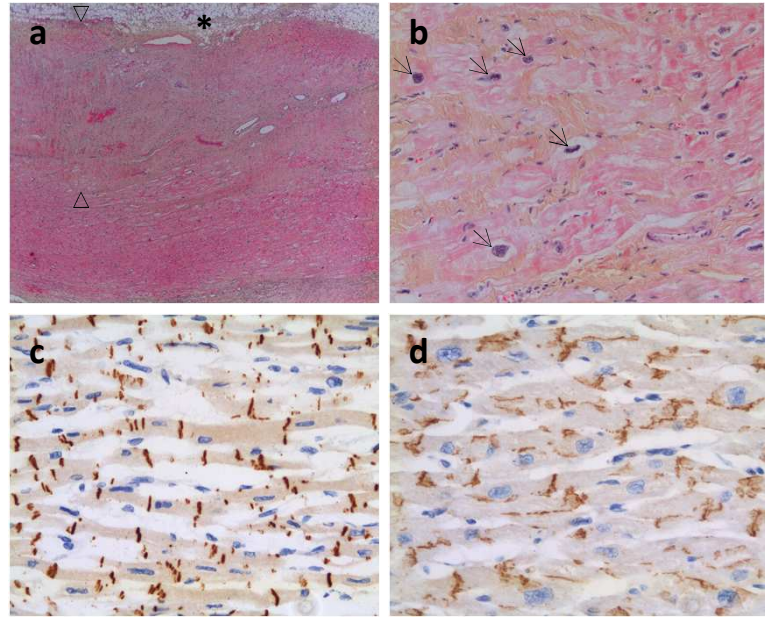
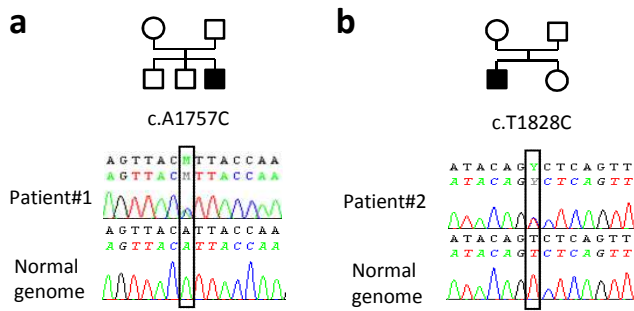
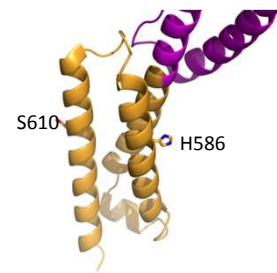
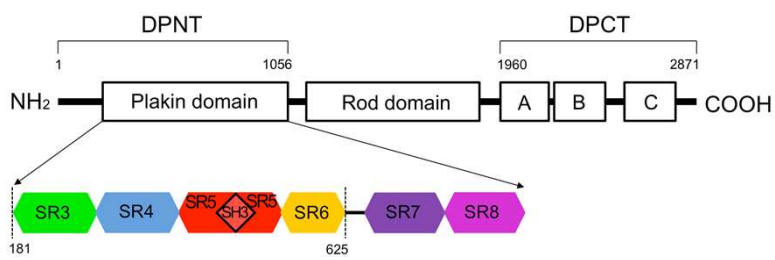
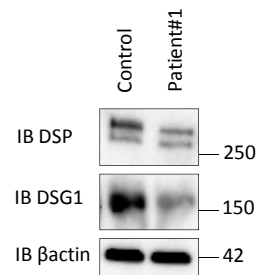
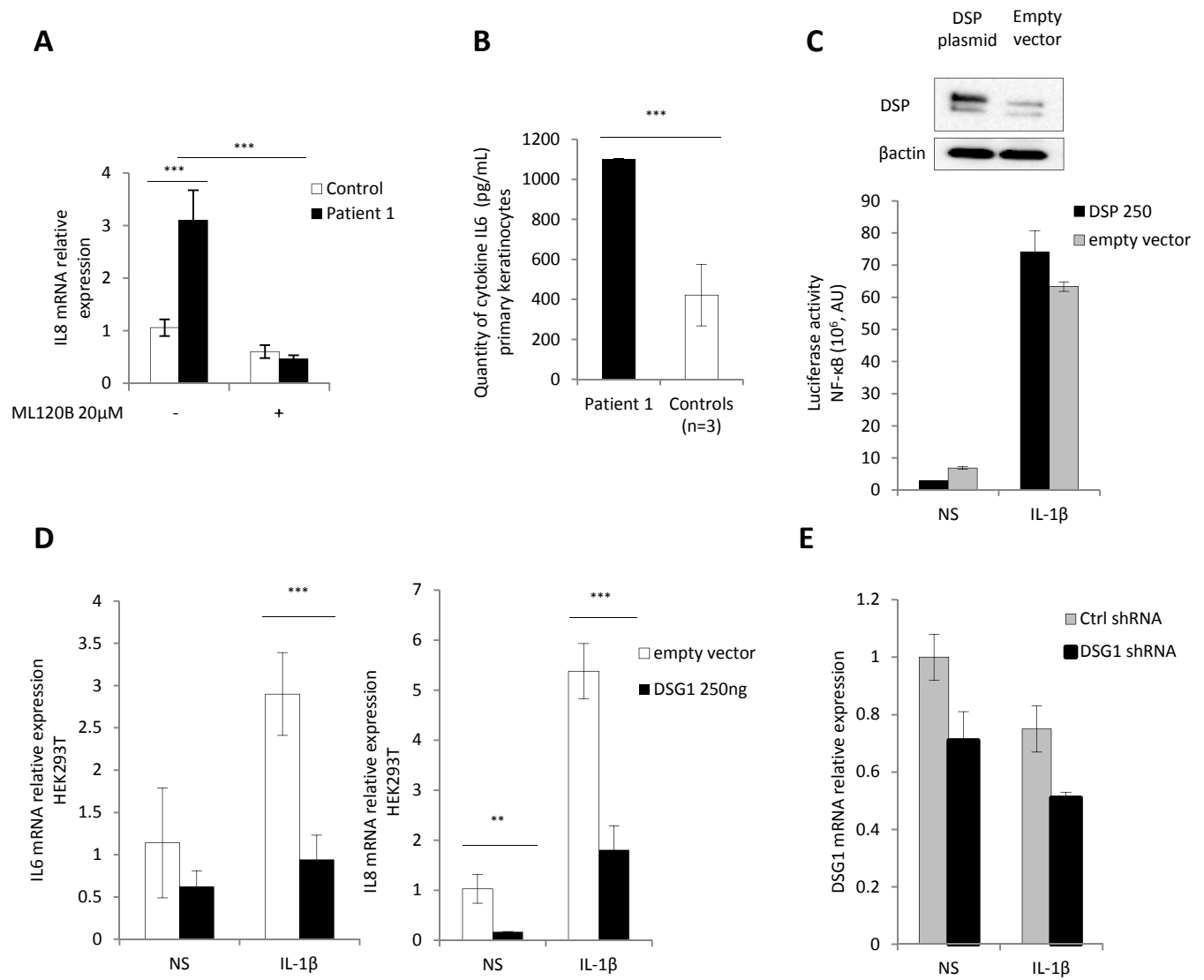


Figure 2**A****B****C****D****F****G****H**

A**B****C****D****E****F**



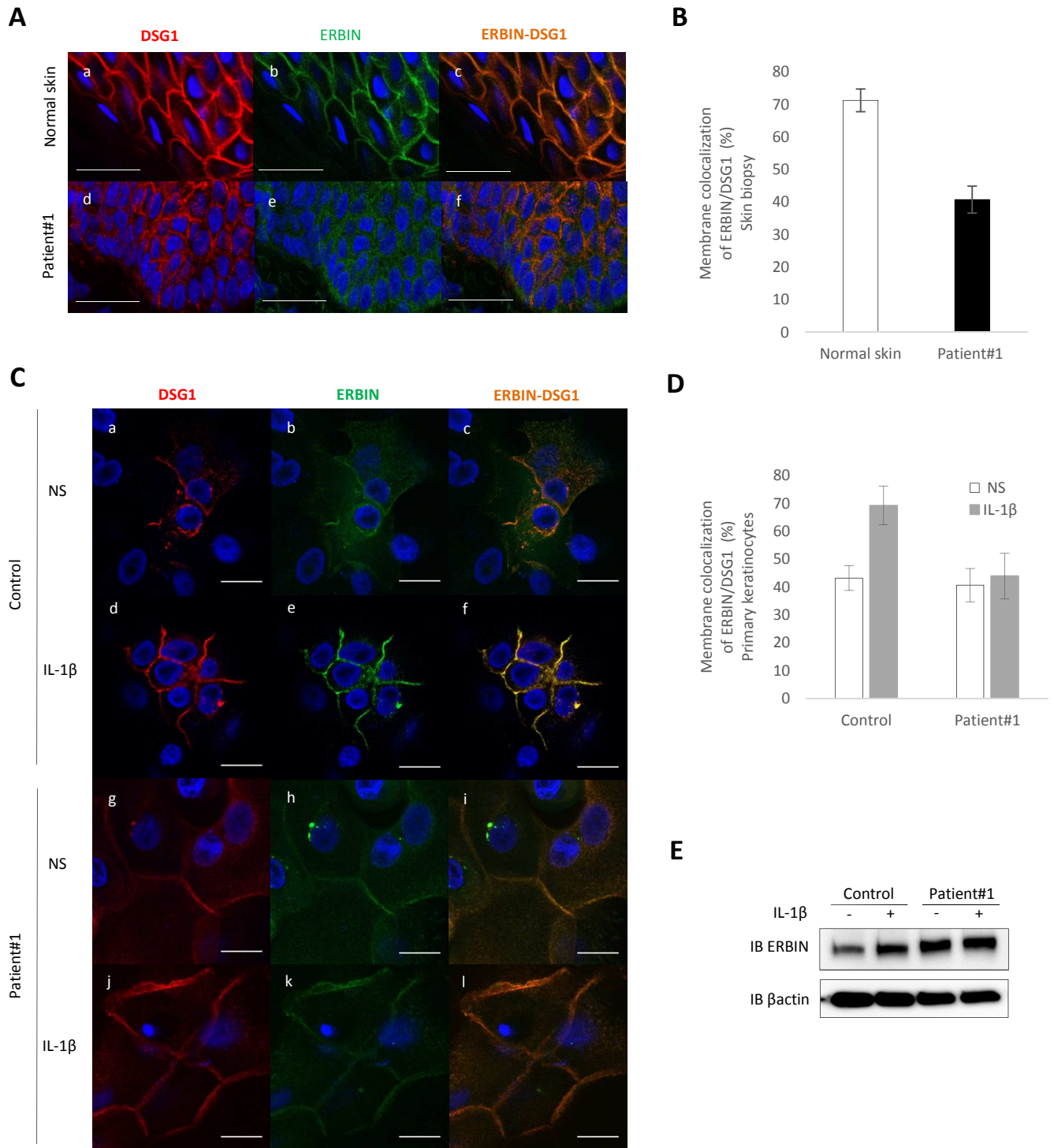


Fig E1. Histopathological and molecular features of patients #1 and #2. A, Histopathology of a skin biopsy (Patient#2) revealed acanthosis, acantholysis (lower part of the epidermis), inflammatory infiltrate (lymphocytes and histiocytes) in the dermis, and a pustula with neutrophils (asterisk). B, Histopathology of heart explants (left ventricle, Patient#2) showing fatty infiltration (epicardium, asterisk) and major fibrous infiltration (subepicardium, arrows, a). Presence of dystrophic nuclei in the cardiomyocytes (fibrotic areas, arrows, b). Immunohistochemical comparison of DSP expression in heart sections: strong and regular pattern of reactivity in the intercalated discs (control, c), compared to the lower and abnormally distributed pattern (Patient#2, d). C, Pedigrees and sequence electropherograms of Patients #1 (a) and #2 (b). D, Locations of the H586 and S610 residues within DSP's SR6 domain (orange), at the surface of straight alpha helices. SR5 domain is in magenta. E, Desmoplakin protein consists of 3 major regions whose amino acid boundaries are indicated. Close-up view of the plakin domain within DSP N-terminal domain (DPNT), with the colored regions representing the 4 spectrin repeats found in the crystal structure of DSP (residues 181–625). Figure extracted from Choi H-J et al, 2011.1 F, Immunoblots of DSP and DSG1 in Patient #1's and control keratinocytes.

Fig E2. DSG1 inhibits NF- κ B-mediated epithelial inflammation. A, Relative mRNA expression of IL8 analyzed by quantitative RT-PCR in primary keratinocytes from Patient#1 and a healthy control, following inhibition of IKK β by ML120B. B, IL6 production by keratinocytes from Patient#1 and controls (n=3). C, NF- κ B luciferase reporter assay in HEK293T cells transfected with 250 ng of DSP. Inset: Western blot analysis of DSP expression in HEK293T cells transfected with 250 ng of DSP. D, IL6 and IL8 mRNA relative expression in HEK293T cells transfected with DSG1 (250 ng) and stimulated with IL-1 β . E, DSG1 mRNA relative expression in control keratinocytes infected with lentivirus expressing shDSG1 and stimulated or not with IL-1 β .

AU: arbitrary units, NS: non stimulated

Fig E3. Expression and subcellular localization of ERBIN in the keratinocytes of Patient #1. A, Immunofluorescence analysis of skin sections from a healthy control (a, b, c) and from Patient#1 (d, e, f), showing a reduction in DSG1 staining (in red) and ERBIN staining (in green) at the plasma membrane of Patient #1's keratinocytes. The two proteins accumulated in the cytoplasm of Patient#1's keratinocytes. Scale bar: 20 μ m. Data are representative of three independent experiments. B, Quantification of ERBIN/DSG1 co-localization at the plasma membranes in skin keratinocytes from a healthy control and from Patient#1. C, Immunofluorescence analysis of primary keratinocytes from a healthy control (a-f) and from Patient#1 (g-l). Note the increase in DSG1 staining (in red) and ERBIN staining (in green) at the plasma membrane of control keratinocytes (d-f), following stimulation by IL-1 β . This increase was absent in Patient#1's keratinocytes (j-l). Scale bar: 20 μ m. Data are representative of two independent experiments (controls, n=3). D, Quantification of ERBIN/DSG1 co-localization at the plasma membrane of primary keratinocytes. E, Immunoblotting of ERBIN in keratinocytes from Patient#1 and from a healthy control. Primary keratinocytes were either stimulated with IL-1 β or not stimulated (NS).

Data are quoted as the mean $\pm$ standard deviation. ***p<0.001.

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Methods

Molecular genetics. DNA was extracted from peripheral blood lymphocytes using the Nucleon BACC3 DNA extraction kit (GE Healthcare, Piscataway, NJ, USA), according to the manufacturer's instructions. Samples of genomic DNA (1 µg) from Patient#1 and his parents underwent whole exome sequencing. The exons were captured with an in-solution enrichment technique (SureSelect Human All Exon Kits Version 3, Agilent, Massy, France) based on a biotinylated oligonucleotide probe library (Human All Exon v3 50 Mb, Agilent). Each genomic DNA fragment was sequenced using the paired-end strategy and an average read length of 75 bases (Illumina HiSeq sequencer, Illumina, San Diego, CA, USA). Image analysis and base calling were performed with the Illumina Real-Time Analysis pipeline (version 1.9, Illumina), using its default parameters. Sequences were aligned with the Human Genome Project reference sequence (hg19 assembly), and SNPs were called on the basis of allele calls and read depth using the Consensus Assessment of Sequence and Variation pipeline (version 1.8, Illumina). Genetic variations were annotated using an in-house pipeline (IntegraGen, Evry, France), and the results for each sample were made available online for analysis with ERIIS (<http://eris.integrangen.com/>). The DSP variant was confirmed by Sanger sequencing with specific primers for exon 14 of the DSP gene. For Patient#2 and his relatives, DSP's 24 exons were amplified by PCR with specific primers. The PCR products were sequenced using Sanger's method and a BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit (version 3.1, Applied Biosystems, Foster City, CA, USA) and then analyzed with SeqScape® software (version 3.0, Applied Biosystems). A whole exome sequencing was then performed for Patient#2.

Primary keratinocytes culture. Primary human keratinocytes were obtained from 4-mm punch biopsies from Patient#1 and three healthy controls. Keratinocytes were cultured on a feeder layer of lethally irradiated mouse 3T3-J2 fibroblasts, as described previously.^{1,2} May be due to intrinsic factors linked to desmosomal disease, several tentative keratinocyte cultures for Patient#2 failed. Similarly, keratinocytes from Patient#1 biopsy were obtained after several culture assays.

Cell lines. HEK293T cells were purchased from ATCC (Teddington, Middlesex, UK).

Isolation and culture of mouse embryo fibroblasts and epidermal keratinocytes from *erbin* null mice

- Primary mouse embryo fibroblasts (MEFs) were isolated from 14.5-day-old *Erbin*^{+/+} and *Erbin*^{-/-} FVB mouse embryos. After dissection, fetuses were transferred in sterile PBS, livers and heads were removed and discarded, the remaining part of each fetus was teased into fine pieces. The tissues were broken up into a suspension by pipetting vigorously and after a short sedimentation the remaining cell suspension was grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics.
- Primary mouse epidermal keratinocytes were isolated from 19.5-day-old *Erbin*^{+/+} and *Erbin*^{-/-} Balb/C mouse embryos. After dissection, fetuses were transferred in sterile PBS, head, paws and tails were removed and discarded, the back skins were removed and treated with Dispase (Gibco/Invitrogen). The epidermis was separated from the dermis with forceps and was incubated with a trypsin/versene mixture. The epidermis was cut in small pieces and after pipetting vigorously up and down the basal layer keratinocytes were separated out from the epidermis. The cell suspension was filtered, washed with medium and grown in CnT-57 medium (CELLnTEC) containing low concentration of Bovine Pituitary Extract (BPE).

RNA extraction, RT-PCR and real-time PCR. Total RNA was isolated from HEK293T cells, mouse embryo fibroblasts (MEFs) and cultured keratinocytes from Patient #1 and different controls using the RNeasy Plus Minikit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. RNA samples were reverse-transcribed into cDNA using the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems). Real-time PCR was carried out using the Fast SYBR Green PCR Master Mix (PE Applied Biosystems, Foster City, CA, USA) on an ABI prism 7000 (PE Applied Biosystems). RT-qPCR primers were designed using the sequences available in Ensembl, spanned an intron-exon boundary. The amounts of the various mRNAs were normalized against the amount of *ACTB* RNA in each sample (measuring using RT-qPCR). The results were analyzed with DataAssist® (version 3.01, Applied Biosystems), which uses the comparative Ct (ddCt) method. All experiments were performed in triplicate. All data are expressed as the mean ± standard deviation. Values were calculated using an unpaired *t* test. *: *p*<0.05; **: *p*<0.01; ***: *p*<0.001.

Immunoblotting analysis. HEK293T cells were transiently transfected with increasing doses (100-1000 ng) of DSG1 plasmid (plasmid #55029, Addgene) or with 250 ng of DSP plasmid (plasmid #32227, Addgene) and/or ERBIN plasmid (pEF1-Erbin-Myc-HisA construct) together with 0.2 µg of Igκ-Luc (see above). The cells were then lysed in RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl

pH 8) with protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany). Western blotting was performed using mouse anti-DSP I/II antibody (diluted 1:1000, sc-390975, Santa Cruz Biotechnology, Heidelberg, Germany), rabbit anti-DSG1 antibody (diluted 1:1000, sc-20114, Santa Cruz Biotechnology, Dallas, TX, USA) and rabbit anti-ERBIN antibody (diluted 1:10000, a gift from Lionel Fontao, Laboratory of Dermatology, University Hospitals and Medical School of Geneva, Geneva, Switzerland). Bound antibodies were visualized with horseradish-peroxidase-conjugated antibodies against rabbit or mouse IgG (Santa Cruz Biotechnology) and an Enhanced Chemiluminescence kit (SuperSignal West Dura Extended Duration Substrate, Thermo Scientific, Rockford, IL, USA). Western blotting to assess the degradation of I κ B α proteins in MEFs was performed with a mouse anti-I κ B α antibody (diluted 1:1000, L35A5, Cell Signaling Technology, Danvers MA, USA). MEFs were stimulated with 20 ng/ml mouse IL-1 β (R&D Systems, Minneapolis MN, USA).

Inhibition of IKK-2 by ML120B. Keratinocytes from a healthy control and from Patient#1 were seeded into 12-well plates (100 000 cells/well). 24 hours later, keratinocytes were preincubated with ML120B 20 μ M at 37°C for 1h and then stimulated with 10ng/ml IL-1 β . 24 hours after the stimulation, the cells were pelleted for RNA extraction. ML120B was sent as a gift by Emmanuel Laplantine (Institut Pasteur, Paris, France).

Luciferase NF- κ B reporter assays. HEK293T cells were seeded into 24-well plates and transfected in triplicate using jetPRIMTM reagent (Polyplus Transfection Inc., New York, NY, USA) with increasing doses (100-1000 ng) of DSG1 plasmid [mCherry-Desmoglein1-C-18, a gift from Michael Davidson (Addgene plasmid #55029)] or with 250 ng of DSP plasmid [1136-Desmoplakin-GFP, a gift from Kathleen Green (Addgene plasmid #32227)] and/or increasing doses (100-500 ng) of ERBIN plasmid (pEF1-Erbin-Myc-HisA construct, a gift from Masaki Inagaki, Division of Biochemistry, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya, Aichi 464 8687, Japan)¹⁴ together with 0.2 μ g of a plasmid carrying the firefly luciferase gene under the control of the NF- κ B promoter (Ig κ -Luc, a gift from Gilles Courtois, Biosciences and Biotechnology Institute of Grenoble, Grenoble, France). Sixteen hours after transfection, cells were stimulated with 10 ng/ml IL-1 β or 20 ng/ml TNF α . Eight hours after stimulation, luciferase activity was determined using a dual luciferase assay kit (Promega, Madison, WI, USA).

Retroviral vector production. pRetro-DSG1 was sent as a gift by Kathleen Green (Northwestern University, Chicago, IL, USA). For virus preparation, pRetro-DSG1 or blank vector were co-transfected using jetPRIMETM reagent (Polyplus Transfection Inc.) and packaging vectors pGag/Pol and pVSVG into HEK293T cells. Infectious retroviruses were harvested at 24, 48 and 72hours post-transfection and filtered through 0.8- μ m-pore cellulose acetate filters. Recombinant retroviruses were concentrated by ultracentrifugation (2 hours at 20,000 $\times$ g) and resuspended in Hank's Balanced Salt Solution. The virus aliquots were frozen and stored.

Lentiviral and retroviral transductions. Primary keratinocytes from a healthy control were seeded into 12-well plates. Twelve hours later, keratinocytes were infected with lentivirus containing (or not) DSG1 shRNA (sc-35224-V Santa Cruz Biotechnology). Twenty-four hours after infection, keratinocytes were stimulated or not with 10 ng/ml human IL-1 β (R&D Systems). Twenty-four hours after stimulation, the cells were pelleted for RNA extraction. Expression of DSG1 was assessed by qRT-PCR. Primary keratinocytes from Patient#1 and a healthy control were seeded into 12-well plates (80,000 cells/well). Twelve hours later, keratinocytes (20% confluent) were infected with retrovirus containing (or not) the DSG1 construct. Twenty-four hours after infection, keratinocytes were stimulated with 10 ng/ml of IL-1 β (R&D Systems). Lastly, 24 hours after stimulation, the cells were pelleted for RNA extraction. DSG1 expression was assessed using RT-qPCR.

Reagents. This antibody was used: anti-FLAG (diluted 1:10 000, Sigma Aldrich, St. Louis, MO, USA).

Light microscopy. Skin and heart biopsy specimens were fixed in 10% formalin, embedded in paraffin and processed using standard procedures. Three- μ m-thick sections were stained with ~~H&E~~ hematoxylin-eosin reagent and examined under the LEICA DFC280 light microscopy (Leica, Buffalo Grove, IL, USA) at different magnifications (x25 for Figure E1-Ba, x100 for Figure 1C, x200 for the inset of Figure 1C, Figures E1-A and E1-Bb). Images were acquired with Leica Application Suite Software.

Electron microscopy. The skin biopsy sample was immersed in 2.5% glutaraldehyde fixative in 0.1M cacodylate buffer at pH 7.4 for 3 to 5 hours at 4°C, washed thoroughly in cacodylate buffer overnight at 4°C and then postfixed in 1% osmium tetroxide for 1 hour at room temperature. The skin biopsy slices were then dehydrated in graded ethanol and impregnated with epoxy resin. After selection of suitable areas, the semithin sections were stained with 1% toluidine blue and examined under the light microscope. Ultrathin sections were prepared and stained with uranyl acetate and lead citrate for electron microscopy (Tecnai T12, FEI, Hillsboro, O,

USA). Scale bar: 2 μm for Figures 1D-a and b; scale bar: 500nm for the inset of Figure 1D-a and 1 μm for the inset of Figure 1D-b.

Immunohistochemical analysis of skin and esophagus biopsy specimens. Immunohistochemical reactions were performed on 4- μm -thick frozen tissue sections using rabbit anti-DSG1 antibody (diluted 1:50, sc-20114, Santa Cruz Biotechnology), mouse anti-DSP I/II antibody (diluted 1:50, sc-390975, Santa Cruz Biotechnology) or sheep anti-ERBIN antibody (diluted 1:20, AF-7866, R&D Systems). The secondary antibodies were anti-rabbit Alexa Fluor 546, anti-mouse Alexa Fluor 488 and anti-sheep Alexa Fluor 488 (Life Technologies, Grand Island, NY), diluted 1:500 in 1% normal goat serum and incubated for 1 hour at 37°C. Sections were washed with PBS 1X. Coverslips were mounted with *In Situ* Mounting Medium with DAPI (Duolink, Olink Biosciences, Uppsala, Sweden). Images were acquired and processed with an LSM700 microscope (Zeiss, Jena, Germany) and Zen Software (Zeiss, Jena, Germany). Data were quantified using Image J software (<https://imagej.nih.gov/ij/>). Quantification of the ERBIN/DSG1 co-localization have been made by a surface based co-localization measurement on Fiji (using immunofluorescence and confocal microscopy). Data corresponded to the average of the quantification of 5 images per skin, n=5. Data are quoted as the mean $\pm$ standard deviation. ***p<0.001. All scale bars: 20 μm

Immunohistochemistry of heart biopsy specimens. Immunohistochemistry was performed on formalin-fixed paraffin-embedded sections. After the deparaffination steps, reactions were carried out according to the automatized system BondTM (A.Menarini Diagnosis system, Firenze, Italy). Staining was performed with the Menarini refine detection kit. Anti-desmoplakin 1+ 2 antibody (clone 65146 Progen, Heidelberg, Deutschland) was used.

Study approval. Clinical investigations have been conducted according to Declaration of Helsinki principles and were approved by local ethical committee. A written informed consent was provided for picture of the three patients appearing in the manuscript. All patients, parents and controls provided informed consent for genetic studies.

Statistical analysis. Results were expressed as the mean $\pm$ standard deviation. Statistical significance was determined using unpaired, two-sample t-tests (equal variance). All data were normally distributed, and the variance was similar in groups that were compared in statistical tests. The threshold for statistical significance was set to p<0.05.

References (methods)

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