SUPPLEMENTARY INFORMATION

Desmoglein 1 deficiency results in severe dermatitis, multiple allergies and metabolic wasting

Liat Samuelov[1,20], Ofer Sarig[1,20], Robert M Harmon[2,20], Debora Rapaport[3], Akemi Ishida-Yamamoto[4], Ofer Isakov[5], Jennifer L Koetsier[2], Andrea Gat[6], Ilan Goldberg[1], Reuven Bergman[7,8], Ronen Spiegel[8,9], Ori Eytan[1,10], Shamir Geller[1], Sarit Peleg[8,11,12], Noam Shomron[5], Christabelle S M Goh[13], Neil J Wilson[13], Frances J D Smith[13], Elizabeth Pohler[13], Michael A Simpson[14], W H Irwin McLean[13], Alan D Irvine[15-17], Mia Horowitz[3], John A McGrath[18], Kathleen J Green[2,19] & Eli Sprecher[1,10]

[1]Department of Dermatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. [2]Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA. [3]Department of Cell Research and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel. [4]Department of Dermatology, Asahikawa Medical University, Asahikawa, Japan. [5]Department of Cell and Developmental Biology, Faculty of Medicine, Tel-Aviv University, Ramat-Aviv, Israel. [6]Department of Pathology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel. [7]Department of Dermatology, Rambam Health Care Campus, Haifa, Israel. [8]Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa, Israel. [9]Institute of Human Genetics, Haemek Medical Center, Afula, Israel. [10]Department of Human Molecular Genetics & Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel. [11]Pediatric Department B, Haemek Medical Center, Afula, Israel. [12]Pediatric Gastroenterology Unit, Haemek Medical Center, Afula, Israel. [13]Centre for Dermatology and Genetic Medicine, University of Dundee, Dundee, UK. [14]Division of Genetics and Molecular Medicine, King's College London (Guy's Campus), London, UK. [15]Paediatric Dermatology, Our Lady's Children's Hospital Crumlin, Dublin, Ireland. [16]National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin, Ireland. [17]Clinical Medicine, Trinity College Dublin, Dublin, Ireland. [18]St John's Institute of Dermatology, King's College London (Guy's Campus), London, UK. [19]Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA. [20]These authors contributed equally to this work. Correspondence should be addressed to E.S. (elisp@tlvmc.gov.il) or K.J.G. (kgreen@northwestern.edu).

Supplementary Note

Patient recruitment

Patients and their families were referred for evaluation to our clinic. All affected and healthy family members or their legal guardian provided written and informed consent according to a protocol approved by our institutional review board and by the Israel National Committee for Human Genetic Studies in adherence to the Helsinki guidelines.

Supplementary Table 1

Exome sequencing details – family A

Family A	**II-1**	**II-2**
Total sequence (reads)	83,607,454	88,089,786
QC Passed Aligned reads (duplicates removed)	66,646,163	80,004,380
QC passed aligned to target reads (+/-150 bp)	57,396,818	65,757,675
Mean read depth	99.84	105.51
% of exome >1x	96.41	98.19
% of exome >5x	89.09	96.60
% of exome >10x	81.82	94.94
% of exome >20x	72.00	90.36

Supplementary Table 2

Exome sequencing details – family B

Family B	IV-10	IV-8	III-2	IV-7
Total sequence (raw reads)	83,425,092	83,749,934	83,797,198	83,968,158
Total sequence (clean reads)	80,000,000	80,000,000	80,000,000	80,000,000
QC Passed Aligned data to genome (duplicates removed)	6,688.65 Mb	6,639.69 Mb	6,586.59 Mb	6,616.77 Mb
QC passed aligned data to target or near target (+/-200bp)	3,891.96 Mb	5,782.83 Mb	5,809.56 Mb	5,796.25 Mb
Mean read depth on target	40.88	61.18	61.97	61.65
% of target >1x	99.00	99.10	99.20	98.90
% of target >4x	97.50	98.10	98.10	97.80
% of target >10x	94.60	96.40	96.40	96.20
% of target >20x	82.20	92.20	92.10	92.00

Supplementary Table 3

Oligonucleotide sequences

Exon / Gene	Sequence (5'-3')	Product size (bp)	Tm
Genomic *DSG1* amplification			
1	F-CTCCCTTGGTCTTGGATG R-CAATGGGCACGATTATGAAG	128	52
2	F-GACTCACAAGCCTATGGTTTC R-GATGTTTAATTGGGAAAATTGG	299	57
3	F-ATACCTTTCTTTATTGCTGTC R-GTTATGCTTTGGAGTTG	185	50
4	F-CACATGCAACAACTCTC R-GGATGCTCGTGAATTAAGA	384	50
5	F-CATAATGCTCAAAGTAAGAAC R-GGAAGCCACTACATTAC	235	51
6	F-GAAGACAGTGAAGTCCACATC R-GTTCCTTTGAGACCAAAAACCCAC	333	61
7	F-TGATATTGCCTGTAATATG R-ATGTAAGCAGAATTGTT	239	46
8	F-AACATTACAGTATAAGC R-GCCAAGTTTGTGAAATG	356	49
9	F-GATTTTCTTTCACCTGGAACG R-GTTCAATATGTAAGGAAAATTAG	366	49
10	F-GCTCCATATTGCTAAGACT R-GTCTATAATGCCCAGTG	434	49
11	F-CTTCCATTTTGAACGTTATTAC R-GGGACACATATACATAGG	341	49
12	F-GCACCCAGTGCTAACTC R-GCGGCCATCTTGGTTCA	253	49
13	F-AAGCTAATGGAGAAAATGAAGAGG R-TTCTAAGTACTCTTTGATGGTTGAGG	527	49
14	F-CACATATTACAAGGCAAGTTG R-AAGTGCTCAGGTCAGAGCT	472	50
15_1	F-TCCTCTTTGGAAATGCTCTGG R-GTCCACTAACAACGGGCTCTG	341	50
15_2	F-TTGATCCTTCTTGGCCACCAC R-CTCGCAAGTCAGGCATCTCTA	351	66
15_3	F-ACGATAACCGACCAGCATCAA R-GTGGTGCCACTAATTCCAGTT	343	65
15_4	F-TAGCCAATGCCCACAATGTCA R-TCTAAACCACAATGACTATGA	365	66

Quantitative Real-Time PCR (qPCR)			
DSG1	F- CCCTCCAGTGTTTTCAATGGC R- AATTGTTCGGTTCATCTGCG	110	60
CCL5	F- TGCCCACATCAAGGAGTATTTC R- CCATCCTAGCTCATCTCCAAAG	150	60
IL-4	F- CAAGCAGCTGATCCGATTCC R- TCCAACGTACTCTGGTTGGC	102	60
IL-5	F- CAAGTGCATTGGTGAAAGAGAC R- AGTGTGCCTATTCCCTGAAAG	145	60
TSLP	F- CCTAACCTTCAATCCCACCG R- TTCTCCTCTTCTTCATTGCCTG	143	60
TNFα	F- ACTTTGGAGTGATCGGCC R- GCTTGAGGGTTTGCTACAAC	139	60

Primers to screen for the *DSG1* c.49-1G>A mutation:

Forward: 5’-ATTTAATTAAAAAATAACTAGTGTGATTATCTTACCTTTTAC-3’

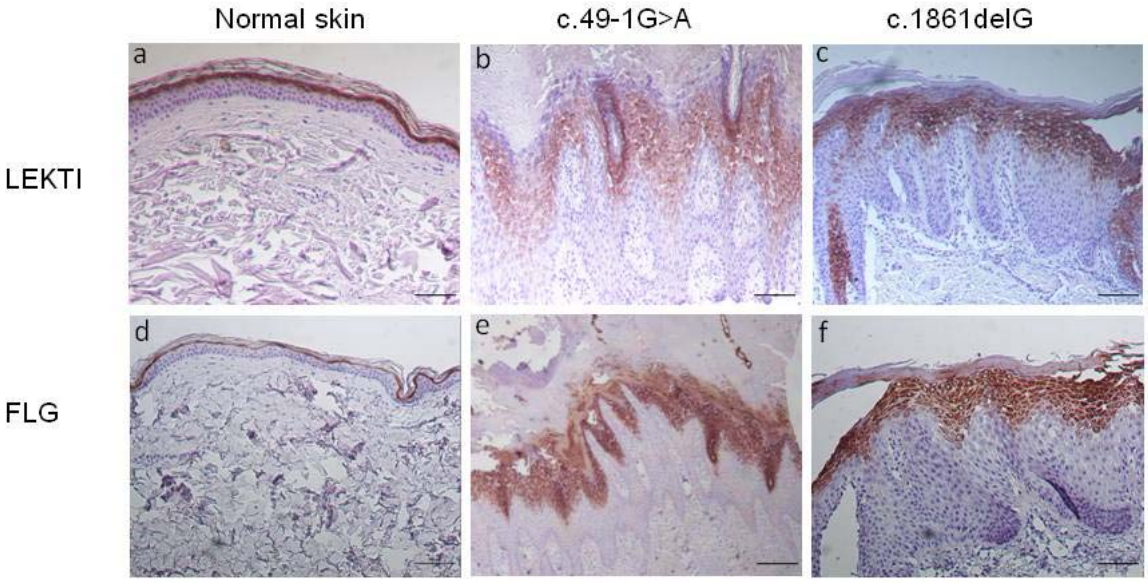
Reverse: 5’-GAAATAGAGGATGTTTAATTGGG-3’.

Primers to screen for the *DSG1* c.1861delG mutation:

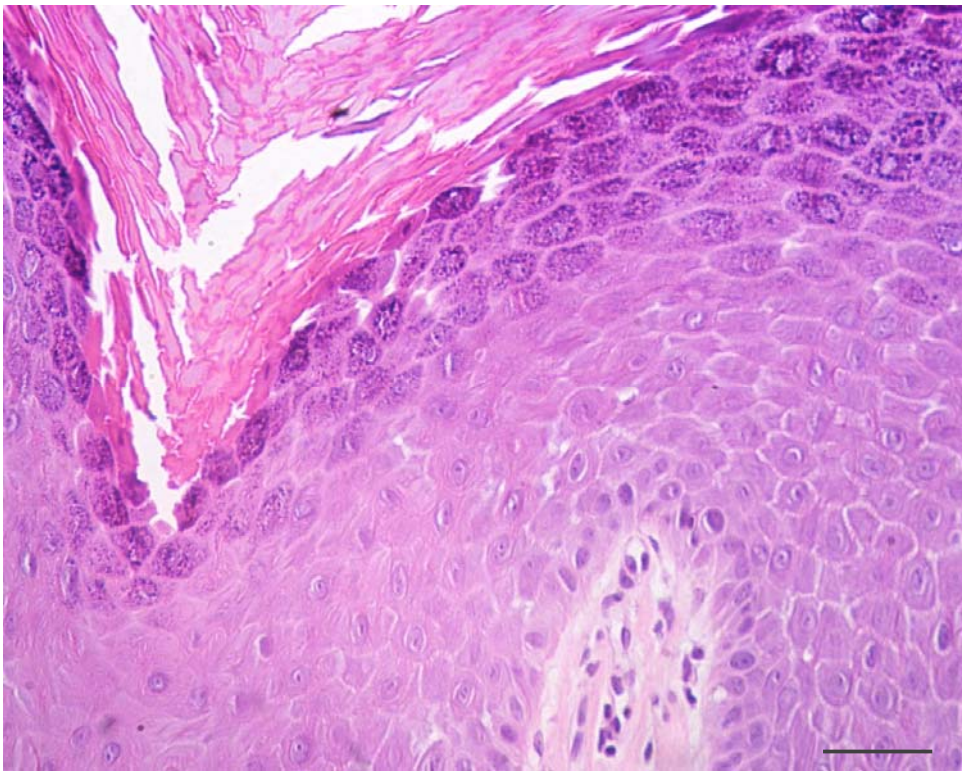
Forward: 5’-AAGCTAATGGAGAAAATGAAGAGG-3’

Reverse: 5’-CTTTTTTCTTACCTGAGTTGTCAATGCATTCAATTATATTCG-3’

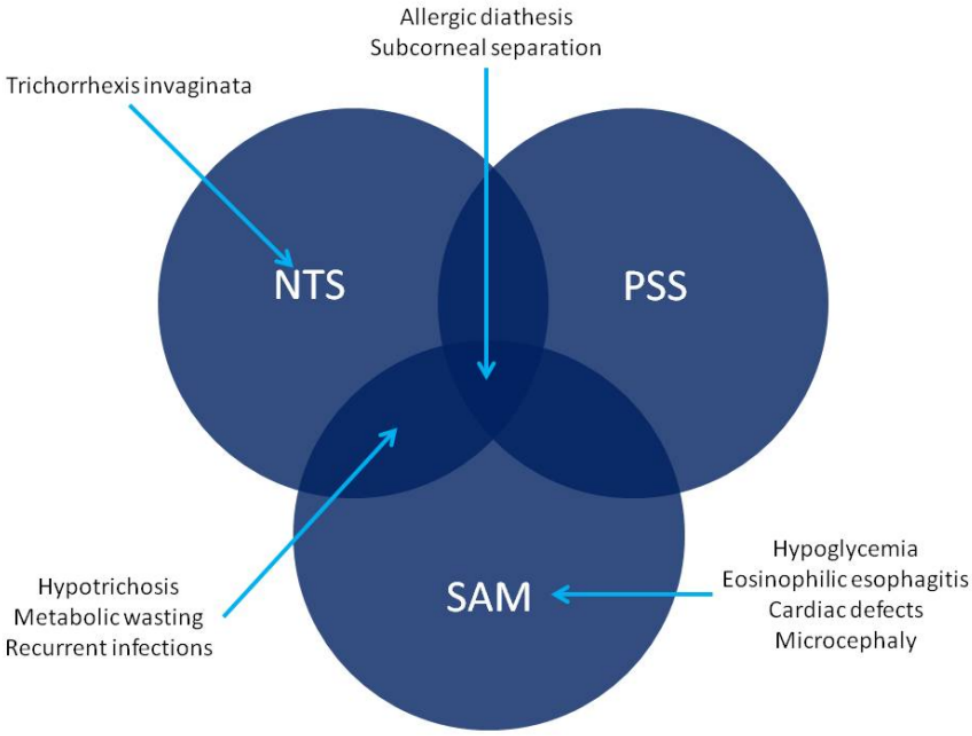
Supplementary Figures



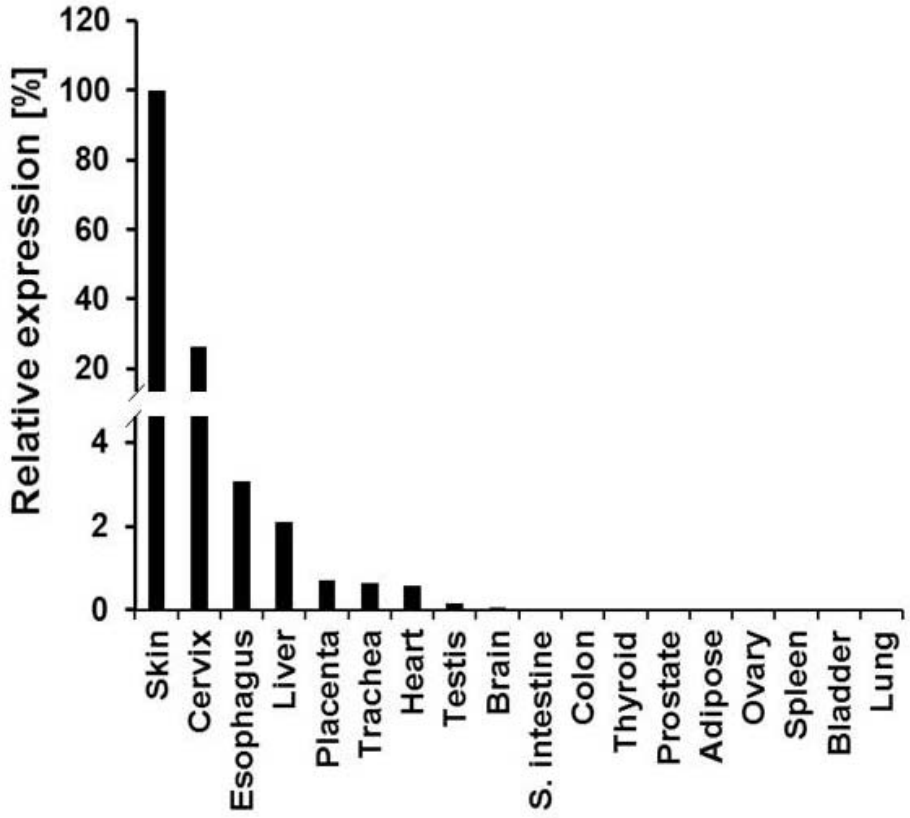
Supplementary Figure 1. Immunohistochemical staining for LEKTI and filaggrin in normal and desmoglein 1 deficient skin biopsies. Biopsies obtained from a healthy individual (a,d), from patient II-1 from family A (b,e) and from patient IV-10 from family B (c,f) were stained for LEKTI (a, ,b, c) and filaggrin (d, e, f,) (scale bar=100µm).



Supplementary Figure 2. Histopathological findings in an heterozygous carrier of c.1861delG in DSG1. A skin biopsy obtained from the palm of a heterozygous carrier of mutation c.1861delG in DSG1 demonstrates widened intercellular spaces mainly in the upper epidermal layers (hematoxylin and eosin; scale bar = 50 μm).



Supplementary Figure 3. Clinical features of the three inherited disorders associated with skin barrier disruption and allergic manifestations. NTS – Netherton syndrome; PSS – peeling skin syndrome type B; SAM - severe skin dermatitis, multiple allergies and metabolic wasting syndrome.



Supplementary Figure 4. DSG1 mRNA expression in normal human tissues. Results are expressed as percentage of expression relative to gene expression in skin ± standard error.