

Severe dermatitis, multiple allergies, and metabolic wasting syndrome caused by a novel mutation in the N-terminal plak domain of desmoplakin

Maeve A. McAleer, MB, MRCP,^{a,b,c} Elizabeth Pohler, PhD,^d Frances J. D. Smith, PhD,^d Neil J. Wilson, MSc,^d Christian Cole, PhD,^e Stuart MacGowan, PhD,^e Jennifer L. Koetsier, BS,^f Lisa M. Godsel, PhD,^{f,g} Robert M. Harmon, PhD,^f Robert Gruber, MD,^h Debra Crumrine, BS,ⁱ Peter M. Elias, MD,ⁱ Michael McDermott, FRCPath,^c Karina Butler, FRCPI,^j Annemarie Broderick, MRCPI,^k Ofer Sarig, PhD,^{l,m} Eli Sprecher, MD, PhD,^{k,l,m} Kathleen J. Green, PhD,^{f,g} W. H. Irwin McLean, PhD, DSc, FRS, FRSE, FMedSci,^d and Alan D. Irvine, MD^{a,b,c} *Dublin, Ireland, Dundee, United Kingdom, Chicago, Ill, Innsbruck, Austria, San Francisco, Calif, and Tel Aviv, Israel*

Background: Severe dermatitis, multiple allergies, and metabolic wasting (SAM) syndrome is a recently recognized syndrome caused by mutations in the desmoglein 1 gene (*DSG1*). To date, only 3 families have been reported. **Objective:** We studied a new case of SAM syndrome known to have no mutations in *DSG1* to detail the clinical,

histopathologic, immunofluorescent, and ultrastructural phenotype and to identify the underlying molecular mechanisms in this rare genodermatosis.

Methods: Histopathologic, electron microscopy, and immunofluorescent studies were performed. Whole-exome sequencing data were interrogated for mutations in desmosomal and other skin structural genes, followed by Sanger sequencing of candidate genes in the patient and his parents.

Results: No mutations were identified in *DSG1*; however, a novel *de novo* heterozygous missense c.1757A>C mutation in the desmoplakin gene (*DSP*) was identified in the patient, predicting the amino acid substitution p.His586Pro in the desmoplakin polypeptide.

Conclusions: SAM syndrome can be caused by mutations in both *DSG1* and *DSP*. Knowledge of this genetic heterogeneity is important for both analysis of patients and genetic counseling of families. This condition and these observations reinforce the importance of heritable skin barrier defects, in this case desmosomal proteins, in the pathogenesis of atopic disease. (*J Allergy Clin Immunol* 2015;136:1268-76.)

Key words: Atopy, skin barrier, atopic dermatitis, desmosome, desmoplakin, atopic sensitization, eosinophilic esophagitis

From ^aClinical Medicine, Trinity College Dublin; ^bPediatric Dermatology, ^cthe National Children's Research Centre, and ^dthe Infectious Disease Department, Our Lady's Children's Hospital Crumlin, Dublin; ^eDermatology and Genetic Medicine, University of Dundee; ^fthe Division of Computational Biology, College of Life Sciences, University of Dundee; the Departments of ^gPathology and ^hDermatology, Northwestern University Feinberg School of Medicine, Chicago; ⁱthe Department of Dermatology and Venereology, Innsbruck Medical University; ^jthe Dermatology Service, Veterans Affairs Medical Center, San Francisco, and the Department of Dermatology, University of California, San Francisco; ^kthe Department of Gastroenterology, Our Lady's Children's Hospital Crumlin and School of Medicine and Medical Science, University College Dublin; ^lthe Department of Dermatology, Tel Aviv Sourasky Medical Center; and ^mthe Department of Human Molecular Genetics & Biochemistry, Sackler Faculty of Medicine, Tel Aviv University.

The Irvine group is funded by the National Children's Research Centre, Dublin, Ireland. M.A.M. is supported by the National Children's Research Centre, Dublin, Ireland. A.D.I. and W.H.I.M. are supported by the Wellcome Trust (090066/B/09/Z and 092530/Z/10/Z). The Centre for Dermatology and Genetic Medicine, University of Dundee, is supported a Wellcome Trust Strategic Award (098439/Z/12/Z to W.H.I.M.). F.J.D.S. and N.J.W. are supported by grants from the Pachyonychia Congenita Project. Work in K.J.G.'s laboratory is supported by the National Institutes of Health (R37 AR043380 and R01 AR041836) and in part by the J.L. Mayberry Endowment. P.M.E. is supported by National Institutes of Health grant R01AR061106. C. Cole is supported by the Wellcome Trust (WT strategic awards WT097945, WT092340, and WT083481).

Disclosure of potential conflict of interest: M. A. McAleer has received research support from and is employed by the National Children's Research Centre. E. Pohler is employed by the University of Dundee. C. Cole has received research support from the Wellcome Trust (092530/Z/10/Z, 098439/Z/12/Z, Strategic awards 097945, 092340, and 083481), has received travel support from the University of Nottingham, and has received funding for bioinformatics user group meetings from Edinburgh Genomics. J. L. Koetsier, L. M. Godsel, R. M. Harmon, and K. J. Green have received research support from the National Institutes of Health. R. Gruber has received travel support from the Rene Touraine Foundation. W. H. I. McLean has received research support from the Wellcome Trust. A. D. Irvine has consultant arrangements with Regeneron and has received research support from the National Children's Research Centre. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 4, 2015; revised May 1, 2015; accepted for publication May 2, 2015.

Available online June 12, 2015.

Corresponding author: Alan D. Irvine, MD, National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin 12, Ireland. E-mail: irvinea@ted.ie. 0091-6749

© 2015 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

<http://dx.doi.org/10.1016/j.jaci.2015.05.002>

Abbreviations used

aCGH:	Array comparative genome hybridization
AD:	Atopic dermatitis
DSG1:	Desmoglein 1 gene
DSP:	Desmoplakin gene
PPK:	Palmoplantar keratoderma
SAM:	Severe dermatitis, multiple allergies, and metabolic wasting
SNP:	Single nucleotide polymorphism
SPINK5:	Serine protease inhibitor Kazal-type 5 gene
WES:	Whole-exome sequencing

have now been reported, all caused by *DSG1* mutations.^{7,8} Here we report the first case of SAM syndrome (MIM #615508) attributed to a missense mutation affecting the plakin domain of the desmoplakin gene (*DSP*).

METHODS

Clinical history

We report the case of a white boy, now aged 6 years, with SAM syndrome. The patient is the first child of Irish, nonconsanguineous healthy parents. He was born at 39 weeks and 5 days' gestation by means of an emergency cesarean section because of fetal bradycardia after rupture of the membranes. He weighed 3.18 kg at birth (25th percentile). The perinatal history was suggestive of a collodion membrane. Erythroderma with "skin scaling and peeling" was reported to be present from the first weeks of life. He was referred to our department at 6 months of age. He had marked erythroderma, ichthyosis, palmoplantar keratoderma (PPK) with nail dystrophy, and diffuse hypotrichosis (Fig 1, A, C, and D). Intermittently, especially with erythrodermic flares, he had a widespread superficial pustulosis (Fig 1, E). He also had severe and intractable itch. He was on the second percentile for weight and the fourth percentile for height. He also had macrocephaly, with his occipitofrontal circumference being greater than the 99th percentile; global developmental delay; and nystagmus.

In the first 3 years of life, the patient had repeated episodes of systemic sepsis necessitating hospital admission for intravenous antibiotics and supportive management. These septic episodes were accompanied by photophobia, with a flare in ichthyosiform erythroderma and pustulosis and an exacerbation in itch. The most frequently isolated pathogens causing sepsis were both the methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*. An extensive metabolic, immunologic, and infectious disease workup did not yield a specific diagnosis. The consensus opinion was that the source of infection was the patient's skin. He failed to thrive and had frequent vomiting, abdominal pain, diarrhea, and food aversion. Several therapeutic approaches to improve his nutrition failed. A percutaneous endoscopic gastrostomy tube was sited when he was 10 months of age to provide essential supplemental feeding.

Neurologic assessment confirmed mild global developmental delay. Computed tomographic brain scanning demonstrated mild dilatation of the lateral and third ventricles, with diffuse prominence of sulci over both cerebral hemispheres. The brain appeared otherwise normal. Ophthalmology assessment demonstrated keratitis. Binocular visual evoked responses and electroretinographic results were within normal limits. Despite extensive assessment, no cause of nystagmus was established. The results of cardiology workup, including echocardiography, were unremarkable.

Several therapeutic approaches to treat the patient's skin and systemic infection were tried with variable success. Potent topical corticosteroids and corticosteroid and antimicrobial combination therapy yielded minimal improvement. Topical tacrolimus ointment, with careful monitoring of tacrolimus blood levels, resulted in moderate improvement. The patient's skin was observed to improve when he received antibiotics for systemic sepsis. Prophylactic anti-staphylococcal antimicrobials had no effect. A trial of anakinra proved ineffective. Systemic acitretin (0.5 mg/kg/d) had good effect,

with a significant improvement in PPK and ichthyosis. Intravenous immunoglobulin infusions were also effective in reducing the frequency of septic episodes. The combined beneficial effects of acitretin, intravenous immunoglobulin, and percutaneous endoscopic gastrostomy feeding have allowed improvement in the patient's dermatitis, growth and weight gain, and development (Fig 1, B). His hair started to grow when he was 6 years old and is a woolly hair phenotype. Also, he has had ongoing poor periodontal health, with marked hypodontia.

The patient was atopic, with multiple food allergies. Blood investigations were consistent with atopy, with persistent eosinophilia ($0.4\text{--}0.5 \times 10^9/\text{L}$) and increased IgE levels (611 kU/L). Analysis of specific IgEs confirmed sensitization to egg, peanut, and wheat, despite having no oral exposure to these foods.

Histopathologic findings

Skin biopsies performed when the patient was 3 and 16 months of age showed similar findings (Fig 2, A). There was hyperkeratosis and parakeratosis overlying an acanthotic epidermis. The granular layer was absent, and there was no evidence of acantholysis. A superficial dermal inflammatory infiltrate composed of lymphocytes, admixed neutrophils, and histiocytes was present (Fig 2, B). A skin biopsy of the pustular eruption performed when the patient was 26 months of age showed a florid pustular dermatosis superimposed on the previously reported histologic findings (Fig 2, B). The pustule was intraepidermal and restricted to the stratum corneum. Hair microscopy was noncontributory, and notably, there was no trichorrhexis invaginata. Esophageal biopsies were done when the patient was 10 and 16 months old. They showed separation, detachment, and acantholytic sloughing of the superficial squamous epithelial cells in the absence of any significant inflammatory process or spongiosis (Fig 2, C).

Molecular genetics analysis

Whole-exome sequencing (WES) was performed in the proband with the Agilent SureSelect v4 capture kit (Agilent Technologies, Santa Clara, Calif) and sequenced on an Illumina HiSeq 2000 (Illumina, San Diego, Calif; GenePool, Edinburgh, United Kingdom). The resulting 88.7M 100-bp PE sequencing reads were aligned to the human genome (Ensembl release 68) with Bowtie2 (version 2.0.2; 98.8% aligned).⁹ Read duplicates were removed with Picard Tools (version 1.79; <http://picard.sourceforge.net>), and variants were called by using the UnifiedGenotyper in GATK-lite (version 2.2-8) according to the GATK best practices.¹⁰ The 69,732 called variants were annotated with the Variant Effect Predictor (version 72),¹¹ and any variants with the following consequences were filtered out: downstream_gene_variant, upstream_gene_variant, synonymous_variant, intergenic_variant, and intron_variant. Filtered data were put in a MySQL database, allowing querying through a Django interface. The desmoplakin variant p.His586Pro was confirmed by means of Sanger sequencing with the primers and conditions previously reported for exon 14 of the *DSP* gene.¹² Array comparative genome hybridization (aCGH) was carried out with the Cytoscan 750K high-density oligo array (Affymetrix UK, High Wycombe, United Kingdom), according to the manufacturer's recommended conditions (analysis performed under contract by the South East Scotland Cytogenetics Laboratory, Western General Hospital, Edinburgh, United Kingdom).

Ultrastructure

Skin biopsy specimens were prefixed in half-strength Karnovsky fixative, followed by postfixation in reduced 1% OsO₄ containing 1.5% potassium ferrocyanide. After embedding in an Epon epoxy resin, ultrathin sections (600 Å) were mounted on Formvar-coated grids, counterstained with uranyl acetate and lead nitrate, and examined in a Jeol JEM 100 CX electron microscope (60 kV).

Immunohistochemistry of skin biopsy specimens

Four-micrometer paraffin-embedded sections were baked overnight at 60°C and deparaffinized with xylene, followed by a gradient of ethanol

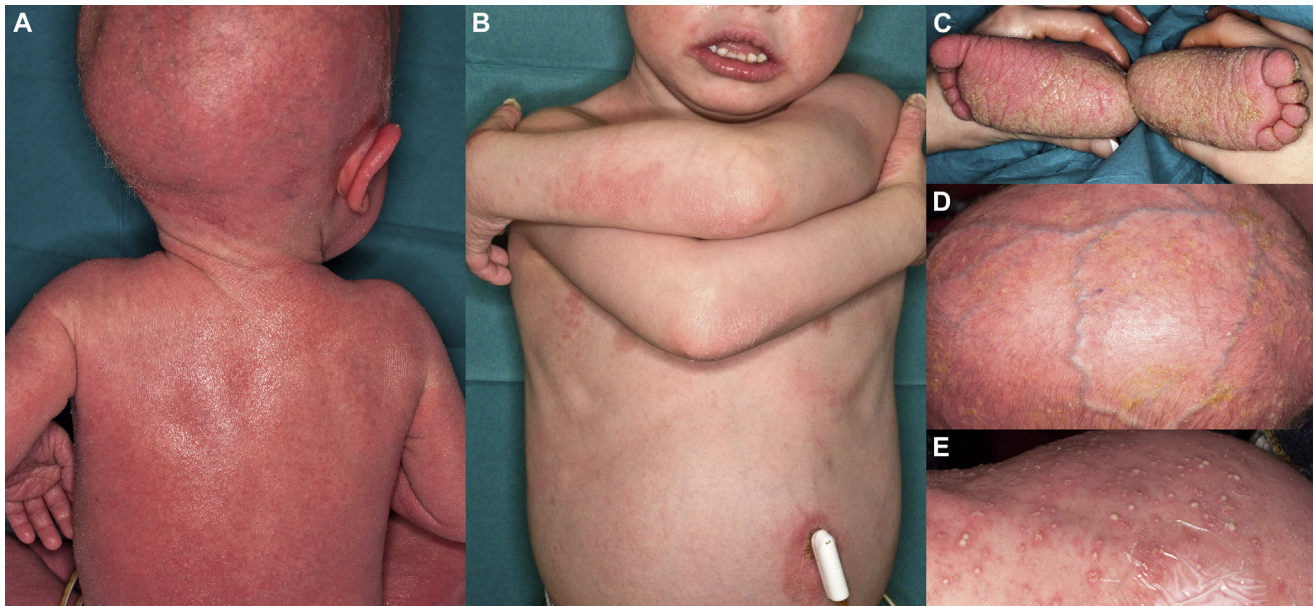


FIG 1. Clinical phenotype. **A**, Generalized erythroderma and ichthosis when the patient was 6 months of age. Hypotrichosis and macrocephaly are also evident. **B**, Improvement in erythrodermic ichthosis after treatment with acitretin. **C**, Plantar keratoderma. **D**, Erythema, scaling, and diffuse hypotrichosis of the scalp. **E**, Pustulosis on the abdomen during an episode of systemic sepsis.

washes. Sections were permeabilized with 0.5% Triton X-100. Antigen retrieval was done by heating sections to 95°C for 15 minutes in 0.01 mol/L citrate buffer (pH 6.0). Slides were blocked in 1% BSA and 2% normal goat serum and incubated for 1 hour at 37°C. Primary antibodies were diluted in 1% BSA and 2% normal goat serum and incubated overnight at +4°C. Mouse mAbs used were as follows: 4B2 (desmoglein 1; diluted 1:100)¹³ and 115F (desmoplakin, a gift from David Garrod; diluted 1:100). Polyclonal antibodies used were as follows: keratin 10 (a gift from Julie Segre; diluted 1:2000) and 1407 (plakoglobin, diluted 1:2000). Secondary staining was done with Alexa Fluor-conjugated antibodies to mouse, rabbit, or chicken (Life Technologies, Grand Island, NY) diluted 1:300 in 1% BSA and 2% normal goat serum for 30 minutes at 37°C. All washes were done with PBS. Coverslips were mounted with polyvinyl alcohol for microscopic analysis by using a DMR Leica microscope (×40 NA 1.0 Plan-Fluotar Plan-Apochromat objective) and a charge-coupled device camera (Orca 100 model CA7 42-95; Hamamatsu Photonics, Hamamatsu City, Japan). 4',6-Diamidino-2-phenylindole was added during the secondary antibody incubation at a final concentration of 2 μg/mL.

RESULTS

Molecular genetics

Initial mutation screening of the proband's genomic DNA isolated from peripheral blood was unremarkable. This included Sanger sequencing of the entire coding sequences of *SPINK5* and ADAM metalloproteinase domain 17 (*ADAM17*), as well as 8 genes known to cause autosomal recessive congenital ichthyosis (*NIPAL4*, *CYP4F22*, *ALOXE3*, *ALOXE12B*, *TGM1*, *PNPLA1*, *ABCA12*, *CERS3*, *FATP4*, and *ABHD5*) and pustular psoriasis (*CARD14* and *IL36RN*). Results of DNA repair studies were entirely normal. The patient had a normal male karyotype, and aCGH showed no major deletions or duplications.

The proband's DNA was then subjected to WES. Given the known association of *DSGI* mutations in prior cases of SAM syndrome, we screened WES variant data for mutations in

DSGI and related desmosomal genes. Repetitive analysis was initially focused on the *DSGI* gene, which was previously reported to be causative in patients with SAM syndrome. No potentially pathogenic variants were identified in the *DSGI* gene, and therefore genes encoding other desmosomal proteins were examined. Only nonpathogenic single nucleotide polymorphisms (SNPs) that have been found in healthy populations by the 1000 Genomes Project were identified in the desmosomal protein genes listed in Table I, with the exception of the novel point mutation c.1757A>C in *DSP* (encoding desmoplakin) predicting the amino acid substitution mutation p.His586Pro in the desmoplakin polypeptide.

The mutation was confirmed by means of Sanger sequencing (Fig 3, A and B) and excluded from 100 unrelated, ethnically matched healthy control subjects (200 alleles) by using Sanger sequencing. The mutation was not detected in the proband's parents, indicating that it represents a *de novo* change. This mutation lies within the plakin domain of desmoplakin, which is shared by other members of the plakin protein family (Fig 3, C). The crystal structure of residues 175 to 630 of desmoplakin was described by Choi and Weis.¹⁴ Histidine 586 is located in an α-helix within spectrin repeat 6, a structure that is conserved in bullous pemphigoid antigen 1 and plectin.¹⁴ Histidine is a hydrophilic charged amino acid, whereas proline is a hydrophobic residue, and therefore this is a nonconservative amino acid substitution. More importantly, proline introduces a strong turn structure within polypeptide chains, and therefore this variant is strongly predicted to completely disrupt the conserved α-helix within desmoplakin's spectrin repeat 6.^{15,16}

As an incidental finding, both the proband and his father were heterozygous for a c.6208G>A missense SNP in *DSP*, predicting the protein change p.Asp2070Asn (Table I). This SNP (rs41302885) was absent in maternal DNA. SNP rs41302885 has been found at a low frequency in healthy populations, with

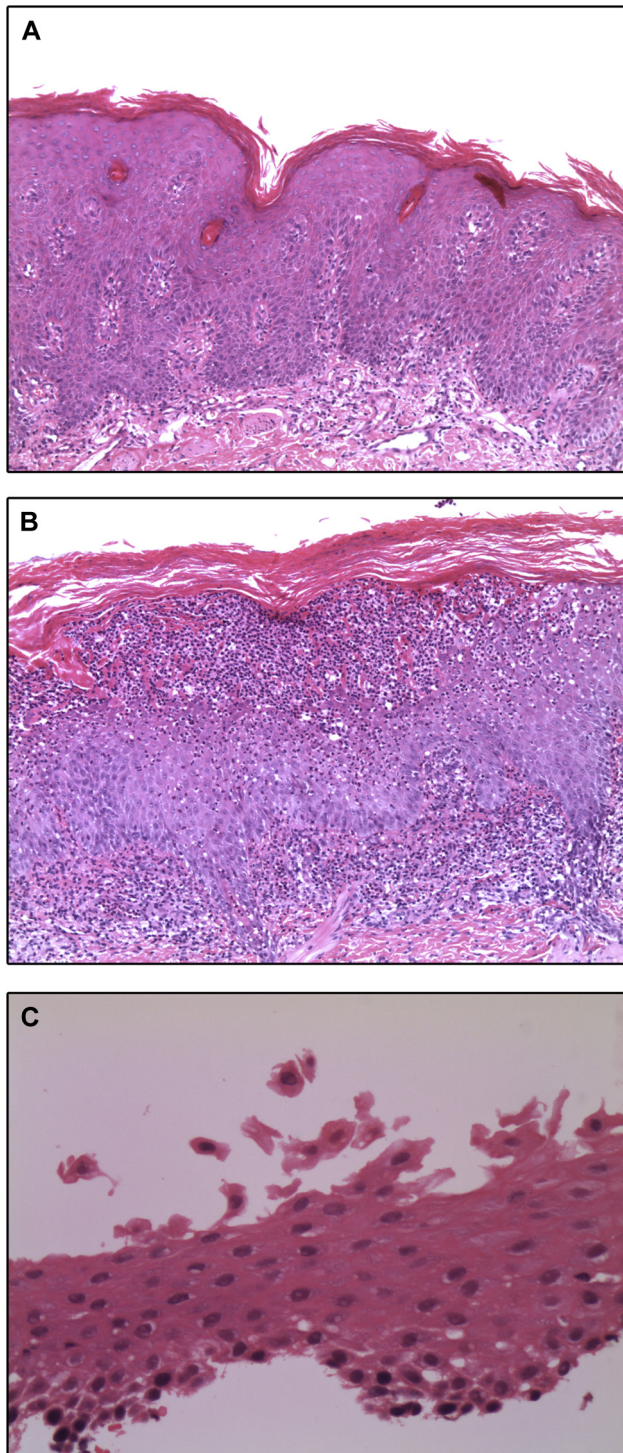


FIG 2. Histopathology. **A**, Skin showing irregular psoriasiform hyperplasia, hyperkeratosis and parakeratosis, and a mild superficial dermal inflammatory infiltrate. **B**, Skin showing a florid subcorneal pustular dermatosis superimposed on background changes similar to those in Fig 2, A, with irregular hyperplasia and marked hyperkeratosis and parakeratosis. **C**, Esophageal squamous mucosa showing separation and detachment of superficial squamous epithelial cells in the absence of any mucosal inflammatory process.

minor allele frequencies of 0.0026 (1000 Genomes Project; <http://www.ncbi.nlm.nih.gov/SNP/>) and 0.0033 (Exome Variant Server; <http://evs.gs.washington.edu>). Therefore this variant, like those

TABLE I. Desmosome protein variants

Gene	Protein	Missense SNP	Zygoty	dbSNP no.	Minor allele frequency (1000 Genomes Project)
<i>DSP</i>	Desmoplakin	p.Asp2070N	Het	rs41302885	0.0026
		p.His586Pro	Het	None	None
<i>DSG1</i>	Desmoglein 1	p.Met11Val	Hom	rs1426310	0.3626
<i>DSG2</i>	Desmoglein 2	p.Ile293Val	Het	rs2230234	0.0323
		p.Arg773Lys	Het	rs2278792	0.2400
<i>DSG3</i>	Desmoglein 3	p.Thr912Ala	Hom	rs1380866	0.0008
<i>DSG4</i>	Desmoglein 4	p.Ile644Leu	Hom	rs4799570	0.0341
<i>DSC1</i>	Desmocollin 1	p.Met659Thr	Het	rs28620831	0.0220
		p.Cys848Phe	Het	rs985861	0.0933
<i>DSC2</i>	Desmocollin 2	p.Ile776Val	Het	rs1893963	0.1965
<i>DSC3</i>	Desmocollin 3	None			
<i>JUP</i>	Plakoglobin	p.Arg142His	Het	rs41283425	0.0240
		p.Met697Leu	Hom	rs1126821	0.4167
<i>PKP1</i>	Plakophilin 1	None			
<i>PKP2</i>	Plakophilin 2	None			
<i>PKP3</i>	Plakophilin 3	p.Gly95Arg	Het	rs11246148	0.3608
<i>PKP4</i>	Plakophilin 4	None			
<i>CSDN</i>	Corneodesmosin	None			
<i>EVPL</i>	Envoplakin	p.Gln433Arg	Het	rs2071192	0.3241
<i>EVPLL</i>	Envoplakin-like	p.Ser4Asn	Het	rs570145	0.4766
<i>PPL</i>	Periplakin	p.Arg819Ser	Hom	rs2734742	0.0617
		p.Ala1007Val	Het	rs2075639	0.1194

Het, Heterozygous; *Hom*, homozygous.
Variables in boldface are disease causing.

seen in other desmosomal genes (Table I), is predicted to be a nonpathogenic polymorphism.

High-density aCGH was performed to exclude the presence of a second compound heterozygous genomic mutation that might be missed by both WES and targeted PCR-based sequencing, such as deletion or duplication of 1 or more exons. The Affymetrix 750K array used had full probe coverage for all exons of the *DSP* and *DSG1* genes. No copy number changes were observed for either gene by using high-density aCGH. Consistent with this result, the coverage of WES sequence reads across the *DSP* gene was not significantly different from that of matched control samples (data not shown). Thus all molecular genetics data are consistent with the *de novo* missense mutation p.His586Pro in *DSP* being the causative genetic lesion.

Ultrastructural analysis

In normal human epidermis keratin filaments make looping attachments with the inner plaques of desmosomes.¹⁷ Electron microscopy of the patient's skin (Fig 4) showed a striking disassociation between keratin filament bundles (asterisks in the control tissue image) and desmosomes in the spinous layer of the epidermis, where desmosomes are most clearly visualized (Fig 4, A and B). The desmosomal inner plaques were much less electron dense and appeared poorly formed compared with desmosomes observed in normal control skin (Fig 4, C and D). The stratum corneum also displayed striking structural abnormalities (Fig 5), including marked attenuation of cornified envelopes (Fig 5, J, double arrows), a virtual absence of

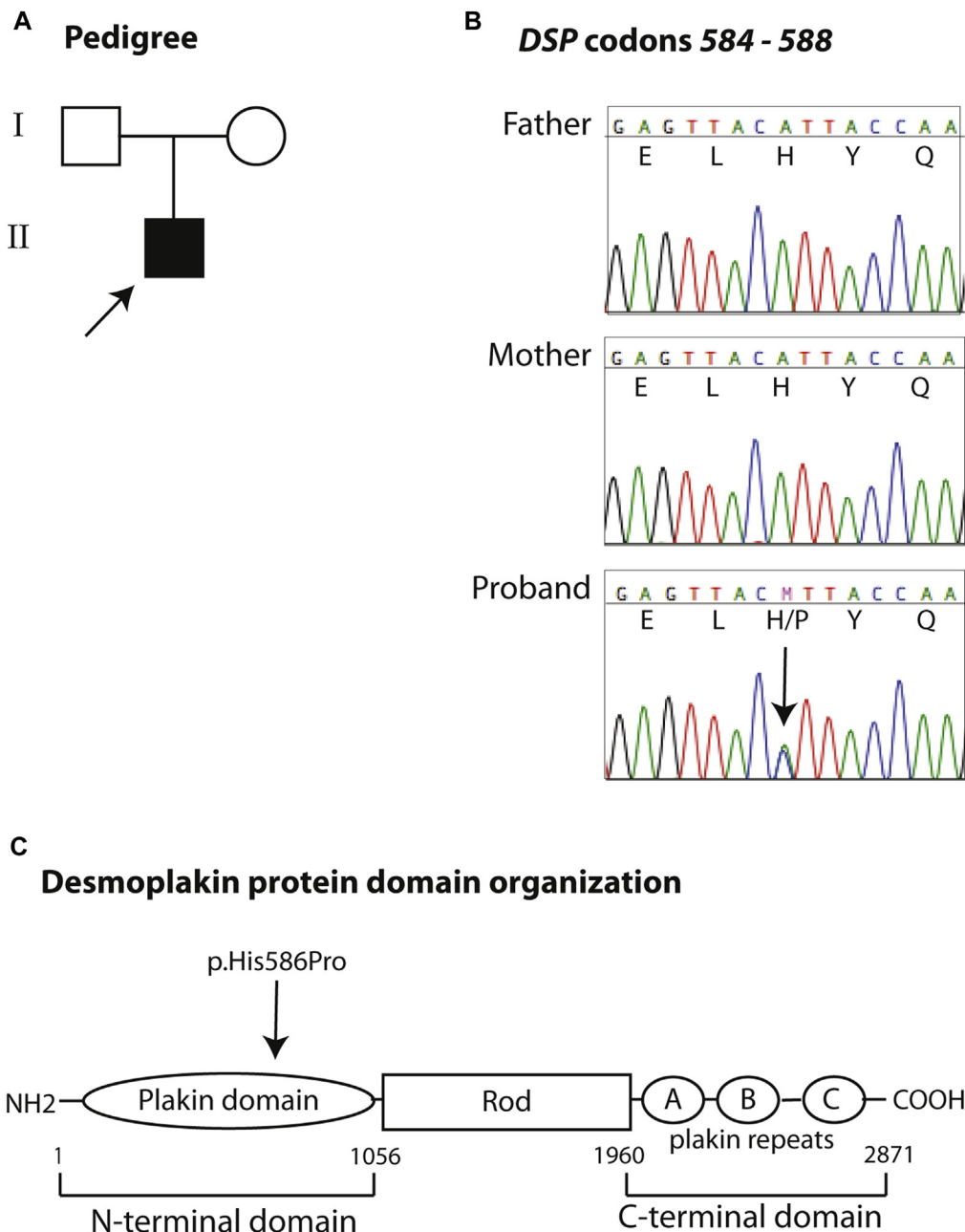


FIG 3. Molecular genetics. **A**, Pedigree of the family in this study. The *arrow* indicates the proband. **B**, Sequence of the *DSP* exon 14 spanning codons 584 to 588. Both parents are wild-type for this region, and the proband has the heterozygous missense mutation c.1757A>C (indicated by the *arrow*), predicting the amino acid substitution p.His586Pro at the protein level. **C**, Primary structure of desmoplakin. Amino acid boundaries of the 3 major domains of the protein are indicated. The p.His586Pro mutation lies within the plakin domain in the N-terminal head of desmoplakin.

corneodesmosomes, and abnormalities in the postsecretory maturation (Fig 5, C) and organization of secreted lamellar body contents (Fig 5, B, and E-G).

Immunohistochemistry

Immunofluorescence analysis revealed aberrant desmoplakin staining, appearing as large accumulations or aggregates (Fig 6, A and B). Striking reductions in both desmoglein 1 and keratin 10 staining were observed (Fig 6, E-H). What *DSG1* remained

appeared in aggregates. Plakoglobin distribution appeared less perturbed, with largely plasma membrane-associated staining punctuated by fluorescence-bright regions that colocalized in some cases with desmoplakin aggregates (Fig 6, C and D).

DISCUSSION

Intercellular junction complexes are a diverse group of organelles that function to provide adherence and communication between individual cells, as well as contributing to the

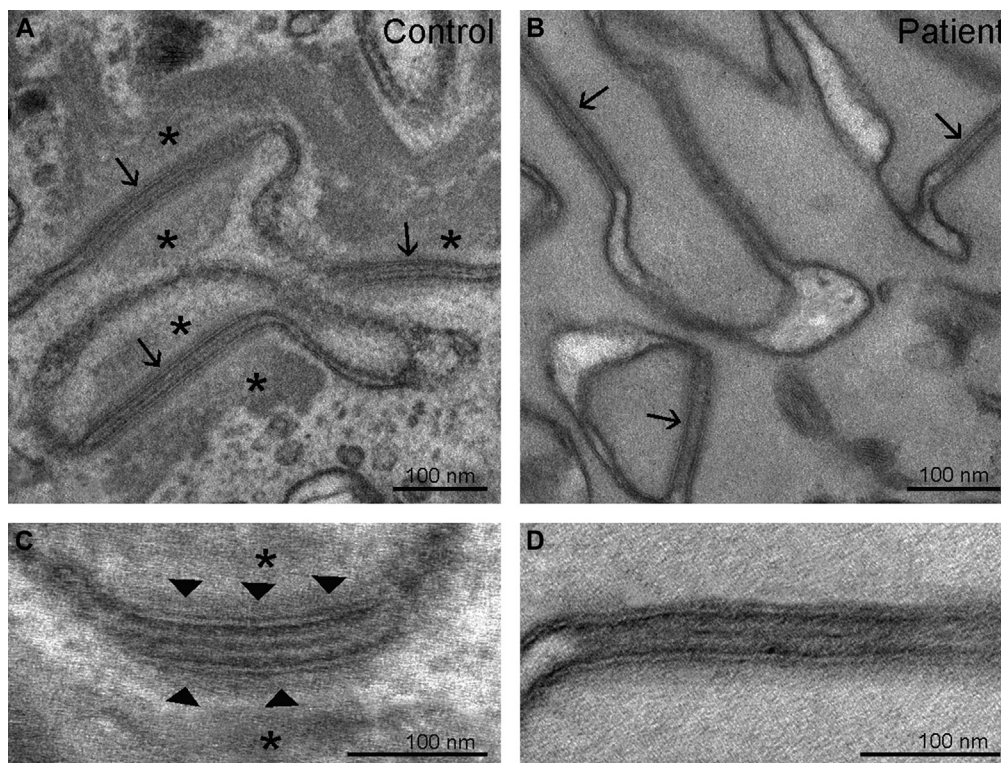


FIG 4. Ultrastructural abnormalities. **A**, Abundant keratin filaments (*asterisks*) anchored to desmosomes (*arrows*) in the cytoplasm of a spinous cell in normal human epidermis. **B**, Note the scarcity of keratin filaments at the same level in the proband. **C**, Common desmosomal structure with normal inner dense plaque (*arrowheads*) in a human control sample. **D**, In contrast, a poorly formed inner dense plaque lacking evidence of keratin filament attachment was observed in the proband.

integrity of larger tissues. These intercellular junctions include desmosomes, adherens junctions, tight junctions, and gap junctions.¹⁸ Desmosomes are intercellular attachment and anchoring sites for the intermediate filament cytoskeleton.¹⁹ Electron microscopic studies have characterized their ultrastructure as containing the intermediate filament-associated inner plaque, an electron-dense outer plaque, the plasma membrane, and the electron-dense midline in the extracellular domain.¹⁸

Desmosomes are composed of several transmembrane and intracellular molecules. The transmembrane proteins facilitating intercellular adhesion are desmosomal cadherins, desmogleins, and desmocollins.¹⁷ Intracellular domains are attached to the intermediate filaments through a range of linker molecules, including desmoplakin, plakoglobin, and plakophilins.¹⁷

Desmosomal structures are widely expressed in epithelia, including the intestinal mucosa, gall bladder, uterus and oviduct, liver, pancreas, stomach, salivary and thyroid glands, and epithelial cells of the nephron, but they are most abundant in tissues that experience mechanical stress, such as the skin and myocardium.¹⁸ Not only do desmosomes provide resistance to the strong mechanical forces applied to skin and cardiac muscle and therefore contribute to tissue cohesiveness, they also have a role in cell signaling and skin barrier functionality.^{17,19} These important functions explain, in part, the diverse range of disease phenotypes observed in patients with desmosomal diseases. Genetic abnormalities in desmosomal components result in a variety of skin and cardiac diseases.

Desmoplakin is an obligate component of functional desmosomes and is highly expressed in the heart, epidermis,^{18,19} and hair follicles.²⁰ Desmoplakin associates with intermediate filaments through its C-terminus and interacts with plakophilins and plakoglobin through its N-terminus to target the desmoplakin-keratin complex to the desmosomal inner plaque.^{21,22} Desmoplakin haploinsufficiency has been reported to cause autosomal dominant type II striate PPK without any other skin, hair, or extracutaneous features.²³ This clinical phenotype suggests that a 50% expression level of desmoplakin is sufficient for epidermal functioning in most body sites but not for the palms and soles, which are subject to considerable mechanical stress.¹⁷ Recessive mutations in desmoplakin result in severe phenotypes. Carvajal syndrome is characterized by striate PPK, woolly hair, and left ventricular cardiomyopathy.²⁴ Skin fragility/woolly hair syndrome is also caused by recessive mutations in *DSP*. Reported cases included compound heterozygosity for a nonsense/missense combination of mutations. The clinical phenotype described in the reports was a focal and diffuse PPK, hyperkeratotic plaques on the trunk and limbs, and varying degrees of alopecia but no apparent cardiomyopathy. Heterozygous carriers of these mutations displayed no phenotypic abnormalities.¹² Lethal acantholytic epidermolysis bullosa has been reported in a neonate presenting as complete alopecia, neonatal teeth, nail loss, extensive skin erosion, and neonatal death. The infant had compound heterozygosity for a recessive nonsense and frameshift *DSP* mutation, resulting in deletion of the intermediate filament-binding sites in the desmoplakin tail domain.²⁵

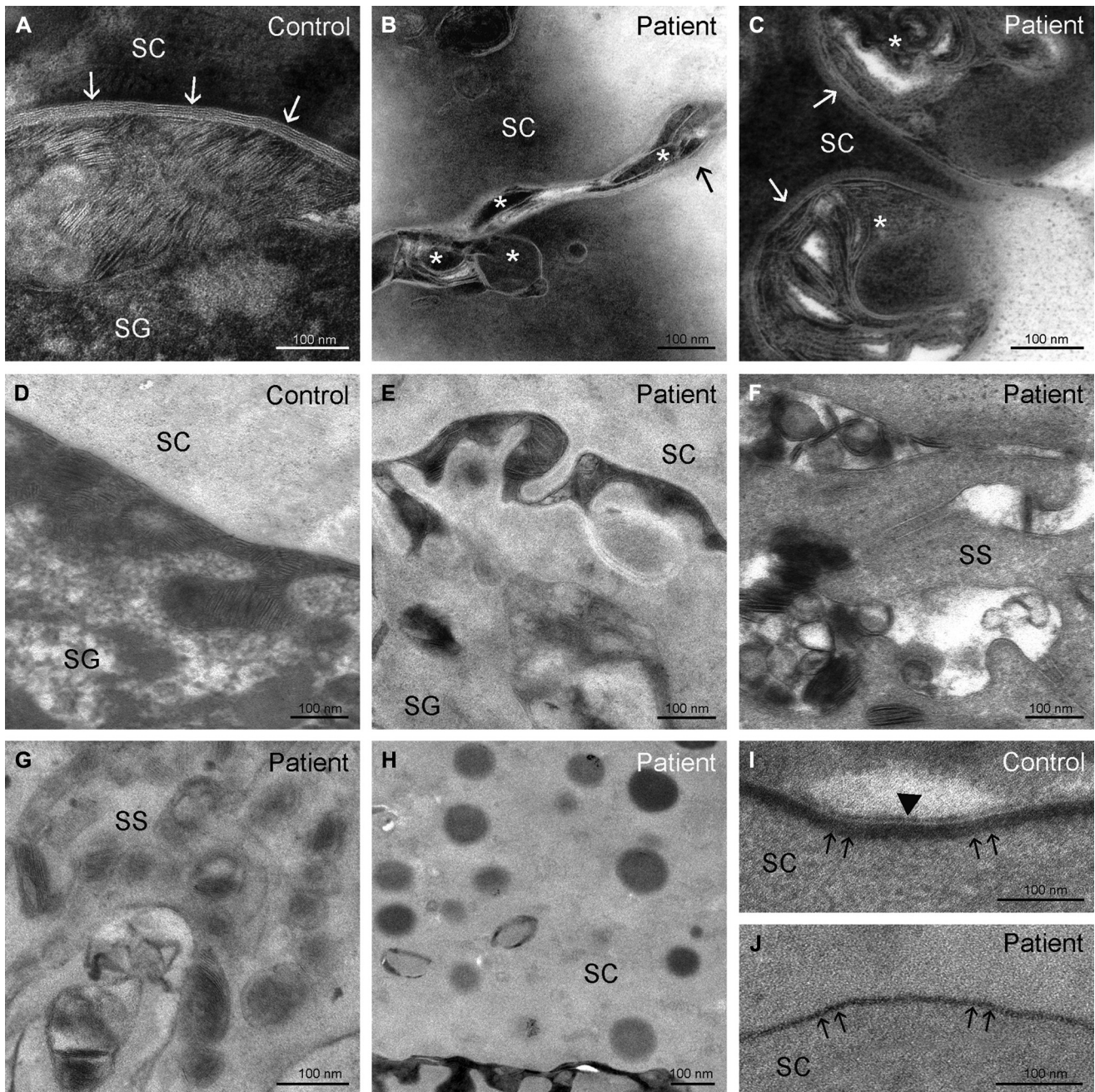


FIG 5. Stratum corneum (SC) ultrastructural abnormalities. **A**, Mature lamellar bilayers (arrows) and normal postsecretory lipid processing in the upper portion of the stratum granulosum (SG)-SC interface in normal human skin. **B** and **C**, In addition to areas of processed lamellar bilayer arrays (arrows), the patient sample shows abnormal lamellar bilayer organization with disruption by nonlamellar domains (Fig 5, *B*, asterisks), delayed lipid processing, and incompletely processed lamellar material (Fig 5, *C*, arrows and asterisks, respectively). **D**, Normal lamellar body secretion with homogenous extracellular bilayers in a healthy human. **E** and **F**, Inhomogeneous lamellar body secretion with foci of vesicular contents at the SG-SC interface and premature secretion in the stratum spinosum (SS) in the patient. **G**, Predominantly abnormal, ellipsoid-shaped lamellar bodies with aberrant internal structures in the patient. **H-J**, Non-membrane-bound droplets throughout the SC, containing electron-dense material, possibly lipids (Fig 5, *H*). In contrast to the normal regular cornified envelope (double arrows) and corneocyte lipid envelope (Fig 5, *I*, arrowhead), note the thinning of the cornified envelope (double arrows) and absence of the corneocyte lipid envelope in the patient (Fig 5, *J*).

Our case is the first reported case of SAM syndrome caused by a *DSP* mutation. The recently reported families with SAM syndrome had biallelic loss-of-function mutations in *DSG1*.^{7,8} Desmoglein 1 is a member of the desmosomal cadherins and strongly expressed

in the granular and spinous layers of the epidermis^{18,19} and hair follicles.²⁰ Heterozygous mutations in *DSG1* cause type I striate PPK. Homozygous *DSG1* mutations were reported to cause the severe phenotype of SAM syndrome.⁷ The first reported cases were

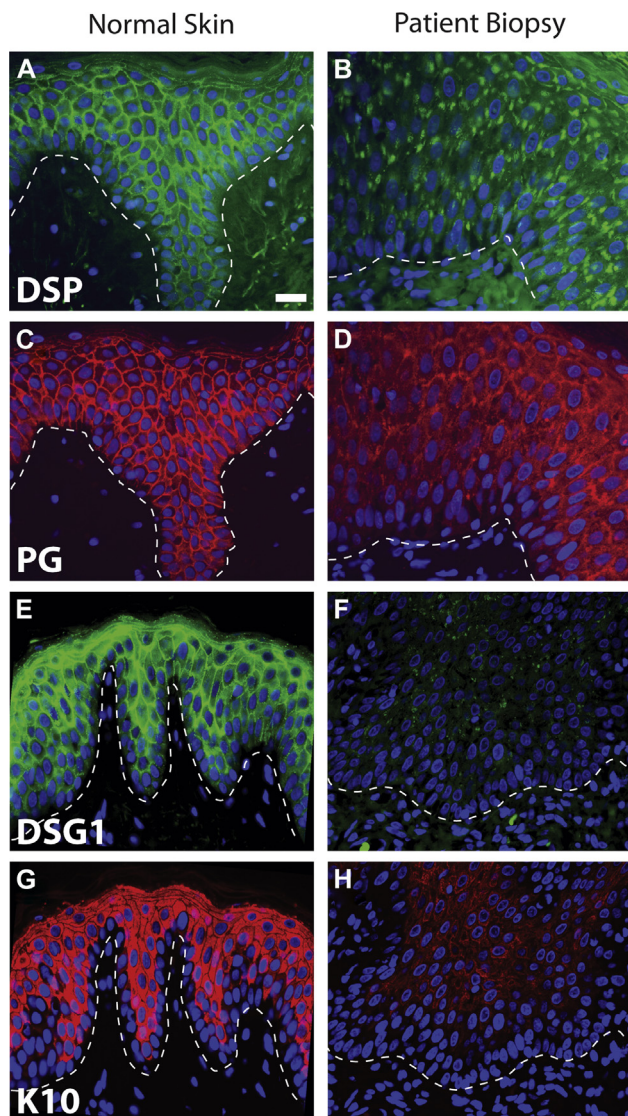


FIG 6. Immunostaining. Paraffin-embedded sections of control biopsy material from patients and control subjects were stained for key desmosome components and interacting epidermal proteins (control epidermis: **A, C, E, and G, left panels**; proband: **B, D, F, and H, right panels**). Fig 6, **A and B**, Staining for desmoplakin (*DSP*; green). Note the reduction in staining in the proband's skin plus the accumulation of desmoplakin in the cytoplasm. Fig 6, **C and D**, Plakoglobin staining (*PG*; red) is weaker in the patient's skin and appears less intense at the cell borders. Fig 6, **E and F**, Desmoglein 1 expression (*DSG1*; green) is drastically reduced in the patient's epidermis. Fig 6, **G and H**, Staining for keratin 10 (*K10*), a major component of the intermediate filament cytoskeleton in suprabasal keratinocytes, which was greatly reduced in the proband. Nuclei were visualized with 4',6-diamidino-2-phenylindole. The dashed line in each image indicates the location of the dermal-epidermal junction. Calibration bar = 20 μ m.

from 2 families with consanguineous healthy parents.^{7,8} Their described phenotype was congenital erythroderma; skin erosions and scaling; yellowish papules and plaques at the periphery of the palms, along the fingers, and over weight-bearing areas of the feet; and hypotrichosis. From early infancy, they had markedly increased IgE levels, severe food allergies, and recurrent infections with severe metabolic wasting.⁷ Minor cardiac developmental defects were noted in 2 patients, which is in keeping with the association between desmosomal diseases and cardiac conditions.⁷ Two

of the patients also had esophageal involvement, which was similar to our patient.⁷ The fourth reported case, the only child of healthy unrelated parents, had a milder phenotype with PPK, dermatitis, and multiple allergies but had normal hair and was otherwise well.⁸ Samuelov et al⁷ demonstrated that *DSG1* deficiency was associated with increased expression of genes encoding the allergy-related cytokines thymic stromal lymphopoietin, IL-5, and TNF. It is notable that our patient and other patients with SAM syndrome had esophageal involvement; our patient had separation and detachment of the esophageal superficial squamous cells, and another patient with SAM syndrome had typical eosinophilic esophagitis.⁷ Similar to SAM syndrome, eosinophilic esophagitis is characterized by allergic inflammation of the esophageal mucosa, immune sensitization to foods, and impaired esophageal barrier function.²⁶ Recent data have suggested a functional role for *DSG1* and its dysregulation in the pathophysiology of eosinophilic esophagitis. Furthermore, the loss of *DSG1* expression might potentiate allergic inflammation through induction of proinflammatory mediators.²⁶

Our patient showed a marked reduction in desmoglein 1 protein expression, as shown by immunohistochemical staining of the skin (Fig 6), despite not having a *DSG1* mutation. This reduction in expression might be due, at least in part, to disruption of stable complexes comprising both *DSP* and *DSG1*. The primary structure of desmoplakin has 3 distinct regions: the 1056-amino-acid N-terminal domain, an 890-residue central coiled-coil domain, and a 925-residue C-terminal intermediate filament binding domain (Fig 3, C).¹⁴ Yeast two-hybrid assays and coimmunoprecipitation experiments showed that desmoplakin's N-terminal 584-amino-acid region is necessary and sufficient to target desmoplakin to the inner desmosomal plaque through its association with armadillo proteins.^{14,21} It is likely that replacement of histidine with proline in close proximity to this region disrupts protein conformation and possibly armadillo protein binding, leading to defects in *DSP* localization and function. More recently, the microtubule plus tip protein end-binding protein 1 was identified as a binding partner for this same region of desmoplakin.²⁷ In addition, the observed loss of *DSG1* that occurs secondary to desmoplakin defects might be a causative factor in decreasing keratin expression because *DSG1* suppresses the extracellular signal-regulated kinase signaling required to promote differentiation, including expression of genes important for barrier formation.^{28,29} Thus *DSG1* not only maintains adhesion in the upper epidermis but also instructs an early epidermal differentiation program on stratification.²⁹ The attenuation of differentiation might also lead to loss of expression of other epidermal proteins, therefore further exacerbating the barrier defect. Because *DSG1* expression or localization is affected in all cases of SAM syndrome, it is unclear whether it is the loss of *DSG1* or *DSP* at intercellular junctions that might be causative in the disease.

Although *FLG* mutations are the most significant mutations associated with AD, many other genes involved in skin barrier function have been implicated, including SNPs in the *SPINK5* gene.^{30,31} Homozygous or compound heterozygous loss-of-function mutations in the *SPINK5* gene result in the autosomal recessive disorder Netherton syndrome, with severe AD and allergy.¹ Furthermore, proteomic profiling of skin from patients with AD has found that multiple other proteins related to the skin barrier were expressed at significantly lower levels in lesional compared with nonlesional sites of patients with AD. These

proteins included filaggrin 2, corneodesmosin, desmoglein 1, desmocollin 1, and transglutaminase 3.³⁰ Nonlesional AD skin might also partially share the lesional skin phenotype. It has been shown that nonlesional skin has reduced expression of filaggrin and filaggrin-like proteins,³² as well as increased expression of immune genes.³³

The pathomechanisms of AD are complex and include interplay between epidermal structural abnormalities and immune dysregulation. However, there are several lines of evidence supporting the role of an aberrant skin barrier in the development of atopic diseases and allergy. The compromised skin barrier might allow enhanced allergen exposure to the cutaneous immune system, enhanced T_H2 responses, and development of allergies. Alternatively, or in addition, the structural protein-deficient epithelium might be proinflammatory and primed for the development of allergy.³⁴

We report a fifth case of SAM syndrome and the first case caused by desmoplakin mutations. SAM syndrome substantiates the role of heritable skin barrier defects, particularly in desmosomal proteins, in the pathogenesis of atopic disease. The pathomechanisms of SAM syndrome require further investigation and might provide valuable insights into the development of atopic diseases.

We thank the patient and his parents for their involvement in this study. We also thank Eddy Maher, Charlotte Keith, and Mary Glancy, South East Scotland Cytogenetics Laboratory, Edinburgh, United Kingdom, for aCGH analysis.

Clinical implications: Three families with SAM syndrome have been reported, all with mutations in *DSGI*. We report a fifth case, the first linked to a *DSP* mutation. In patients with SAM syndrome, mutations in *DSGI*, *DSP*, and other desmosomal proteins should be considered.

REFERENCES

- Chavanas S, Bodemer C, Rochat A, Hamel-Teillac D, Ali M, Irvine AD, et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000;25:141-2.
- Smith FJ, Irvine AD, Terron-Kwiatkowski A, Sandilands A, Campbell LE, Zhao Y, et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006;38:337-42.
- Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
- Sandilands A, Smith FJ, Irvine AD, McLean WH. Filaggrin's fuller figure: a glimpse into the genetic architecture of atopic dermatitis. *J Invest Dermatol* 2007;127:1282-4.
- Israeli S, Zamir H, Sarig O, Bergman R, Sprecher E. Inflammatory peeling skin syndrome caused by a mutation in CDSN encoding corneodesmosin. *J Invest Dermatol* 2011;131:779-81.
- Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol* 2009;129:1892-908.
- Samuelov L, Sarig O, Harmon RM, Rapaport D, Ishida-Yamamoto A, Isakov O, et al. Desmoglein 1 deficiency results in severe dermatitis, multiple allergies and metabolic wasting. *Nat Genet* 2013;45:1244-8.
- Has C, Jakob T, He Y, Kiritsi D, Hausser I, Bruckner-Tuderman L. Loss of desmoglein 1 associated with palmoplantar keratoderma, dermatitis and multiple allergies. *Br J Dermatol* 2015;172:257-61.
- Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 2012;9:357-9.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytzky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010;20:1297-303.
- McLaren W, Pritchard B, Rios D, Chen Y, Flicek P, Cunningham F, et al. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. *Bioinformatics* 2010;26:2069-70.
- Whittock NV, Wan H, Morley SM, Garzon MC, Kristal L, Hyde P, et al. Compound heterozygosity for non-sense and mis-sense mutations in desmoplakin underlies skin fragility/woolly hair syndrome. *J Invest Dermatol* 2002;118:232-8.
- Dusek RL, Getsios S, Chen F, Park JK, Amargo EV, Cryns VL, et al. The differentiation-dependent desmosomal cadherin desmoglein 1 is a novel caspase-3 target that regulates apoptosis in keratinocytes. *J Biol Chem* 2006;281:3614-24.
- Choi HJ, Weis WI. Crystal structure of a rigid four-spectrin-repeat fragment of the human desmoplakin plakoin domain. *J Mol Biol* 2011;409:800-12.
- Serrano L, Sancho J, Hirshberg M, Fersht AR. Alpha-helix stability in proteins. I. Empirical correlations concerning substitution of side-chains at the N and C-caps and the replacement of alanine by glycine or serine at solvent-exposed surfaces. *J Mol Biol* 1992;227:544-59.
- Smith FJ, Del Monaco M, Steijlen PM, Munro CS, Morvay M, Coleman CM, et al. Novel proline substitution mutations in keratin 16 in two cases of pachyonychia congenita type 1. *Br J Dermatol* 1999;141:1010-6.
- Ishida-Yamamoto A, Igawa S. Genetic skin diseases related to desmosomes and corneodesmosomes. *J Dermatol Sci* 2014;74:99-105.
- Brooke MA, Nitou D, Kelsell DP. Cell-cell connectivity: desmosomes and disease. *J Pathol* 2012;226:158-71.
- Petrof G, Mellerio JE, McGrath JA. Desmosomal genodermatoses. *Br J Dermatol* 2012;166:36-45.
- Kurzen H, Moll I, Moll R, Schäfer S, Simics E, Amagai M, et al. Compositionally different desmosomes in the various compartments of the human hair follicle. *Differentiation* 1998;63:295-304.
- Kowalczyk AP, Bornslaeger EA, Borgwardt JE, Palka HL, Dhaliwal AS, Corcoran CM, et al. The amino-terminal domain of desmoplakin binds to plakoglobin and clusters desmosomal cadherin-plakoglobin complexes. *J Cell Biol* 1997;139:773-84.
- Kowalczyk AP, Hatzfeld M, Bornslaeger EA, Kopp DS, Borgwardt JE, Corcoran CM, et al. The head domain of plakophilin-1 binds to desmoplakin and enhances its recruitment to desmosomes. Implications for cutaneous disease. *J Biol Chem* 1999;274:18145-8.
- Whittock NV, Ashton GH, Dopping-Hepenstal PJ, Gratian MJ, Keane FM, Eady RA, et al. Striate palmoplantar keratoderma resulting from desmoplakin haploinsufficiency. *J Invest Dermatol* 1999;113:940-6.
- Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, et al. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum Mol Genet* 2000;9:2761-6.
- Jonkman MF, Pasmooij AM, Pasmans SG, van den Berg MP, Ter Horst HJ, Timmer A, et al. Loss of desmoplakin tail causes lethal acantholytic epidermolysis bullosa. *Am J Hum Genet* 2005;77:653-60.
- Sherrill JD, Kc K, Wu D, Djukic Z, Caldwell JM, Stucke EM, et al. Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis. *Mucosal Immunol* 2014;7:718-29.
- Patel DM, Dubash AD, Kreitzer G, Green KJ. Disease mutations in desmoplakin inhibit Cx43 membrane targeting mediated by desmoplakin-EB1 interactions. *J Cell Biol* 2014;206:779-97.
- Harmon RM, Simpson CL, Johnson JL, Koetsier JL, Dubash AD, Najor NA, et al. Desmoglein-1/Erbin interaction suppresses ERK activation to support epidermal differentiation. *J Clin Invest* 2013;123:1556-70.
- Getsios S, Simpson CL, Kojima S, Harmon R, Sheu LJ, Dusek RL, et al. Desmoglein 1-dependent suppression of EGFR signaling promotes epidermal differentiation and morphogenesis. *J Cell Biol* 2009;185:1243-58.
- Leung DY. New insights into atopic dermatitis: role of skin barrier and immune dysregulation. *Allergol Int* 2013;62:151-61.
- Kabesch M, Carr D, Weiland SK, von Mutius E. Association between polymorphisms in serine protease inhibitor, kazal type 5 and asthma phenotypes in a large German population sample. *Clin Exp Allergy* 2004;34:340-5.
- Pellerin L, Henry J, Hsu CY, Balica S, Jean-Decoster C, Méchin MC, et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. *J Allergy Clin Immunol* 2013;131:1094-102.
- Suarez-Farinas M, Tintle SJ, Shemer A, Chiricozzi A, Nograles K, Cardinale I, et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol* 2011;127:954-64, e1-4.
- McAleer MA, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. *J Allergy Clin Immunol* 2013;131:280-91.