Homozygous nonsense mutation in DSC3 resulting in skin fragility and hypotrichosis

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Keywords:	Desmosomes, Cadherins, Genetic Diseases, Bullous Disease, Hair Biology

Dear Mark,

Homozygous nonsense mutation in *DSC3* resulting in skin fragility and hypotrichosis

Alexandros Onoufriadis *et al.*

JID-2019-0619.R1

Thank you for your letter about our manuscript and for the thoughtful comments of your editorial team and reviewers.

I would be grateful if you would kindly consider our work for publication in the JID as a Letter.

We have now revised the manuscript based on those comments. Specific point-by-point responses and details of the changes are listed in a separate response to reviewers file.

I confirm that all the data in the manuscript are original and that the manuscript has not been submitted elsewhere, previously or concurrently. None of the manuscript contents have been published previously, not even in abstract form. All authors have read and approved all versions of the manuscript, its contents, and its submission to the JID.

As corresponding author, I confirm my willingness to pay page charges and online file fees, if the submission is accepted for publication.

Thank you for your further consideration of our revised manuscript.

Kind regards,

John

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Response to reviewers

JID-2019-0619.R1

Homozygous nonsense mutation in *DSC3* resulting in skin fragility and hypotrichosis

Alexandros Onoufriadis et al.

Thank you for your kind and constructive comments. Please find below detailed responses to the specific issues you raised.

Reviewer comments:

Reviewer: 1

Comments to the Author
In this paper, Onoufriadis et al confirms the existence of a congenital blistering disorder associated with hypotrichosis and caused by mutations in the DSC3 gene. In contrast with a previous publication, the blistering phenotype is here clearly evident both clinically as well as histologically and ultrastructurally.

This is a carefully conducted and important study.

P values reflecting statistical significance should be added to the graph on Fig. 2C.

Thank you for your comments; we have now added p values to Figure 2c.

Reviewer: 2

Comments to the Author
This report is a concise well-characterized case report with a novel rare nonsense mutation of DSC3 in patients with widespread blisters and hypotrichosis.

1. The patient showed nail, dental, and skeletal abnormalities or syndactyly. The patients' sister and mother also showed partial skeletal abnormality. This phenotype has something to do with the DSC3 mutation? Or some other mutations are responsible to this phenotype?

We suspect that this is unrelated to the DSC3 pathology and is likely to be a separate dominant mutation in another gene that is shared by the boy, his sister and mother. Thus far, however, we have been unable to identify such a mutation. We have now made this clear in the manuscript (end of third paragraph). Nevertheless, we still think it is worth mentioning these features until such a time as (a) possible concurrent second gene pathology has been found in this case or (b) more cases of DSC3 mutations have been reported which will then allow for a more accurate definition of the recessive DSC3 phenotype.

2. This patient shows trauma-induced blisters but no apparent oral erosions. Any possible explanation or hypothesis on this?

We checked the mouth carefully (especially given the recent report of DSG3 mutations published in the JID) but could not find any erosions or blisters. The absence of oral erosions for DSC3 mutations was also noted in the report by Ayub et al. We can only speculate that wild-type DSG3 is sufficient to compensate for the lack of DSC3. Moreover, our gene expression analysis in patient skin (Figure 2c) indicated up to 5-fold upregulation of other desmosomal cadherins and plakins and armadillo proteins which could contribute to a new compensation hypothesis.

3. The authors showed 2-5-fold increase of mRNA levels for other desmosomal components such as DSC1, DSG1, and DSG3. Is this also confirmed at protein level?

Unfortunately, we did not have any patient keratinocytes to check protein levels by Western blotting. As mentioned in the manuscript, we did perform immunofluorescence microscopy against a panel of desmosomal antibodies and keratins but did not find evidence for increased or altered staining for most antibodies tested. Indeed, the only difference we noted (apart from the lack of DSC3 immunostaining) was a slight reduction in PKP1 labeling intensity (c.f. the >4-fold increase in PKP1 gene expression in patient skin). We considered adding all these immunofluorescence microscopy images to the supplementary material but given that there were no differences between patient and control we would prefer just to retain the images within the Figure 1 montage that show patient and control staining for DSC3, PKP1 and DSP.

JID-2019-0619.R1

Homozygous nonsense mutation in *DSC3* resulting in skin fragility and hypotrichosis

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Short title: DSC3 genodermatosis

Abbreviations used:

DSC, desmocollin; DSG, desmoglein; DSP, desmoplakin; ExAC, Exome Aggregation Consortium; IF, immunofluorescence; JUP, junction plakoglobin; PKP, plakophilin; RT-PCR, real-time polymerase chain reaction; TEM, transmission electron microscopy; WES, whole-exome sequencing; WT, wild type

Funding sources:

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Word count: 1096

Figures: 2

References: 10

Keywords: desmosome, genetic disease, cadherin, skin fragility, hypotrichosis

To the Editor,

Desmosomes are intercellular junctions that are important in cell adhesion and maintaining epithelial integrity (Samuelov and Sprecher, 2015). The three main desmosomal protein groups are the cadherin desmogleins (DSG) 1-4 and desmocollins (DSC) 1-3, the armadillo plakophilins (PKP) 1-3 and plakoglobin (PG), and the plakin family member desmoplakin (DSP) (Getsios et al., 2004; Harmon and Green, 2013). Germline mutations in at least 11 desmosomal genes have implicated in a range of pathology involving skin, hair, and heart, or combinations thereof (Najor, 2018; also see Supplementary online Table S1 for summary).

In 2009, Ayub et al. identified a homozygous nonsense mutation (c.2129T>G; p.Leu710*) in *DSC3* in four siblings from a consanguineous Afghani family who presented with a new genodermatosis affecting hair and skin (Ayub et al., 2009). Clinically, eyebrows and eyelashes were absent, and scalp hair was sparse and fragile. In addition, generalized skin vesicles occurred which would burst periodically, releasing thin watery fluid. Histologic analysis of scalp showed slight follicular plugging but no epithelial fragility. The disorder was proposed as a new skin fragility genodermatosis, but others suggested that the data, as presented, were more consistent with keratosis pilaris (Payne, 2010). Moreover, the lack of clear clinical illustrations of any vesicles or skin biopsy data meant that there was insufficient evidence to formally classify loss of DSC3 as an inherited desmosomal skin fragility disorder (Fine *et al*., 2014).

Here, we report an unrelated individual with a different homozygous nonsense mutation in *DSC3* who has unequivocal skin blistering and hypotrichosis. The proband is a 5-year-old boy born to consanguineous Egyptian parents (see Figure 1 for clinicopathologic features and Supplementary online Figures S1 and S2). At birth, his skin appeared normal but from 4 years of age, he started to develop blisters on his hands, feet, and knees, as well as at sites of trauma.

There was no scalp hair at birth, and although hair grew during infancy it was always sparse and easily pulled out. On examination, there were trauma-induced bullae and crusted erosions on the hands, knees, legs, and feet. No intra-oral fragility was noted. His scalp hair and eyebrows were sparse and thin, and there was also follicular hyperkeratosis on the scalp, trunk, and extremities. His skin was dry, and he had cracked lips with angular cheilitis. His nails showed areas of leukonychia and thinning with breakage. No abnormalities were detected on cardiac examination, chest X-ray, or echocardiography. Additional features included slight thinning and irregularities of dental enamel, an inverted left nipple, and incomplete syndactyly of the 2nd and 3rd toes of both feet and clinodactyly of the 4th toe of the right foot, although the partial syndactyly was also present in his sister (Supplementary online Figure S3) and mother and thus may reflect a separate autosomal dominant gene abnormality (currently unknown) rather than a consequence of the recessive *DSC3* pathology we report here.

Skin biopsy of rubbed normal skin revealed acanthosis and pan-epidermal widening of spaces between keratinocytes. Ultrastructurally, desmosomes had variable appearances: some showed near-normal morphology, but several were small with cell-cell detachment mostly occurring through the inner desmosomal plaques (Figure 1 and Supplementary online Figures S4 and S5). Following ethics committee approval and written informed consent, whole-exome sequencing was performed using genomic DNA extracted from blood samples from the proband, his sister, and both parents, in accordance with the Declaration of Helsinki principles. Candidate mutations were prioritized by filtering for rare variants with a frequency < 0.01 in public repositories such as the 1000 Genomes Project, Exome Aggregation Consortium (ExAC) and an in-house database (Supplementary online Tables S2-S4). We identified a homozygous loss-of-function mutation in *DSC3* (c.2180T>G; p.Leu727*); both parents and the unaffected sister were heterozygous for this variant (Sanger sequencing confirmation shown in Figure 2a; see Supplementary online Table S5 for primer details). This mutation is

similar in nature to the previously reported *DSC3* mutation, p.Leu710*, but while the latter is located within the transmembranous domain of DSC3, p.Leu727* sits within the intracellular region of the protein (Figure 2b).

Immunofluorescence microscopy in our patient's skin revealed a complete absence of DSC3 labeling, consistent with nonsense-mediated RNA decay. To assess the impact of the loss of DSC3 in the patient's skin, we also undertook quantitative RT-PCR for other desmosomal components. We observed 2-5-fold increases for *DSC1*, *DSG1*, *DSG3*, *PKP1*, *DSP*, *JUP* and keratins (*KRT1*, *KRT10*, *KRT5* and *KRT14*), with only *DSC2* and *DSG2* not showing much difference from controls (Figure 2c). At a protein level, however, immunostaining for these proteins (apart from DSC3) showed no major differences between patient and control, although the intensity of PKP1 labeling was slightly reduced in patient skin (see Supplementary online Table S6 for list of antibodies assessed).

One of the key phenotype distinctions between our patient and the pedigree report by Ayub et al. (2009) is the blistering. Our patient clearly shows widespread trauma-induced blisters which is more congruent with the findings in the conditional knockout mouse model (Chen et al., 2008). Regarding desmosomes and skin/mucosal fragility syndromes, pathogenic autosomal recessive mutations have now been demonstrated in *DSG1*, *DSG3*, *DSC3*, *DSP*, *JUP*, *PKP1* and *CDSN* (see Supplementary online Table S1). In addition, autosomal dominant mutations in *DSG1* occasionally can cause blisters (Lovgren et al., 2017). Interestingly, the heterozygous sister of our patient also reported occasional trauma-induced blisters and erosions (Supplementary online Figure S6) although neither (heterozygous) parent has any skin blistering.

The hypotrichosis and follicular papules in our patient are very similar to the cases described by Ayub et al. (2009). Hair shedding was also noted in the conditional *Dsc3* knockout mouse, in which acantholysis occurred in the keratinocytes around the telogen club hairs (Chen

et al., 2008). Autosomal recessive mutations in *DSC2*, *DSC3*, *DSG4*, *DSP*, *JUP* and *PKP1* have been shown to result in human hair abnormalities, as have autosomal dominant mutations in *DSP* and *CDSN* (see Supplementary online Table S1).

The presence of follicular papules in association with recessive loss-of-function *DSC3* mutations appears to be a notable finding (Ayub et al., 2009). Although a precise explanation for the papules is currently lacking, altered epidermal differentiation is likely. Our quantitative RT-PCR data provide some support for this possibility, although DSC3 is also known to interact with p53 and to have tumor suppressor gene function in inhibiting epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK) pathways (Cui et al., 2013). Whether or how DSC3 anomalies might be implicated in more keratosis pilaris warrants further exploration.

In summary, we present definitive clinicopathologic and molecular evidence that loss of DSC3 causes both skin fragility and hypotrichosis in humans and thereby expand genotype-phenotype correlation for desmosomal genodermatoses.

Data Availability Statement

Datasets related to this article can be found at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA561129, hosted at Sequence Read Archive (SRA) under the collection ID PRJNA561129.

Conflicts of interest

None declared.

Acknowledgments

This work was supported by the UK National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre (BRC) award to Guy's and St. Thomas' NHS Foundation Trust, in partnership with the King's College London and King's College Hospital NHS Foundation Trust. We would also like to thank the patients and their relatives who kindly contributed samples, as well as Patricia Lovell for excellent technical work on the transmission electron microscopy and Kenji Tomita for assistance with sample collection.

CRediT statement

Conceptualization, A.O., N.A., H.B., and J.A.M.; Investigation, A.O., N.A., H.B., A.G., P.L., L.L., A.M., E.K., J.E.M., and J.A.M.; Formal Analysis, A.O. and A.M.; Resources, N.A., H.B., M.P., and M.A.S; Supervision, J.A.M; Writing – original draft, A.O., J.Y.W.L., and J.A.M; Writing – review & editing A.O. and J.A.M.

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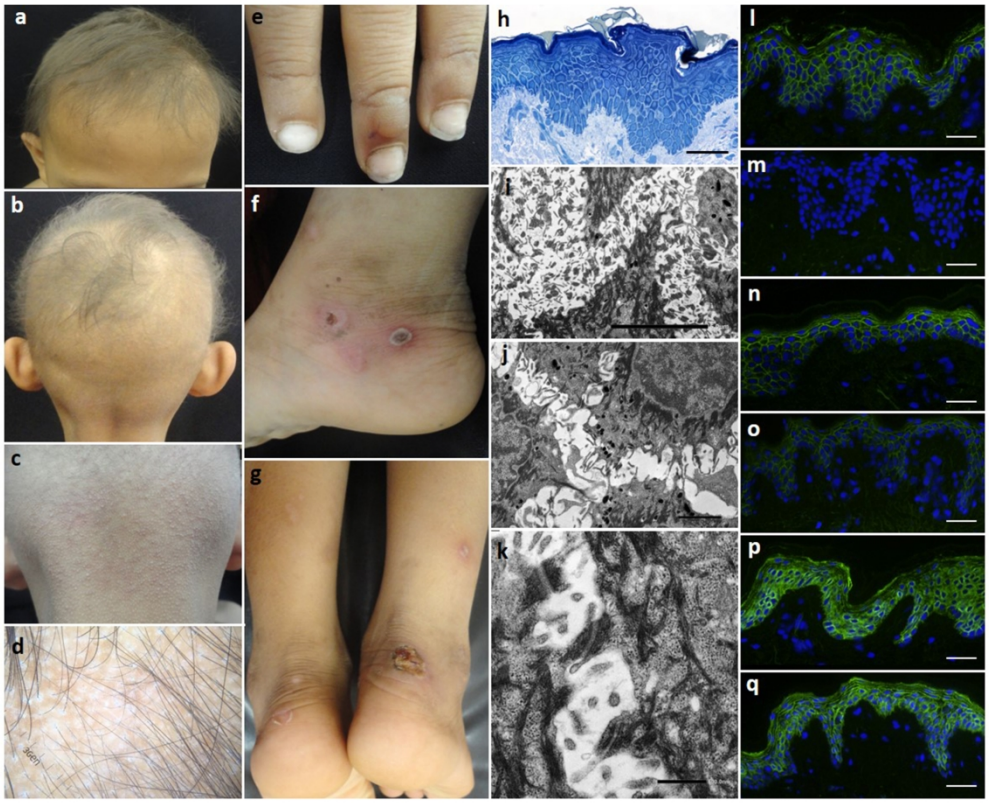
Figure legends

Figure 1. Clinicopathologic features of the 5-year-old boy with this desmosomal genodermatosis. (**a**, **b**) scalp hypotrichosis; (**c**) follicular papules on occiput; (**d**) dermatoscopy reveals empty follicles, white dots and a predominance of single follicle hair units; (**e**) tense blister on the middle finger and leukonychia; (**f**) inflammatory blisters on the medial aspect of the ankle; (**g**) erosions and crust son the heels. Additional clinical images are presented in Supplementary online Figures S1 and S2; (**h**) light microscopy shows acanthosis and pan-epidermal widening of spaces between keratinocytes (Richardson's stain; bar = 50 μm); (**i**) transmission electron microscopy shows desmosome detachment between keratinocytes (bar =

5 μm); (**j**) desmosome numbers are reduced in some areas with intermediate filaments retracted from the keratinocyte cell peripheries (bar = 2 μm); (**k**) desmosomes are present between some cells although many are small and lack mid-line dense plates (bar = 500 nm). Additional TEM images are available in Supplementary online Figures S4 and S5; (**l**) DSC3 labeling in normal control skin epidermis showing pan-epidermal cell membrane staining (bar = 50 μm); (**m**) in patient skin, there is a complete absence of DSC3 immunoreactivity (bar = 50 μm); (**n**) PKP1 labeling in normal control skin showing keratinocyte cell membrane staining from the suprabasal layer upwards (bar = 50 μm); (**o**) in patient skin PKP1 staining is slightly reduced in intensity but the distribution is similar (bar = 50 μm); (**p**) DSP labeling in normal control skin showing pan-epidermal cell membrane staining (bar = 50 μm); (**q**) DSP staining in patient skin is similar to control (bar = 50 μm).

Figure 2. The inherited skin/hair pathology result from a homozygous nonsense mutation in *DSC3* that is associated with upregulation of other cutaneous desmosomal cadherin and plaque protein gene expression. (**a**) Segregation analysis and sequence chromatograms of the c.2180T>G; p.Leu727* *DSC3* mutation. (**b**) Schematic of human DSC3 and its domains. Locations of the DSC3 mutations identified in this study and in the Ayub et al. (2009) study are indicated with dark and light purple arrows respectively. EC, extracellular cadherin; EA, extracellular anchor; TM, transmembrane; IA, intracellular anchor. (**c**) Quantitative RT-PCR analysis for gene expression of desmosomal components was performed using cDNA derived from skin punch biopsies of the patient and two unrelated healthy controls. *GAPDH* mRNA levels were used as endogenous control. Plots represent the mean ± SD of two independent experiments, each carried out in triplicate. All significance values were calculated using Welch Two Sample t-test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ns, not significant.



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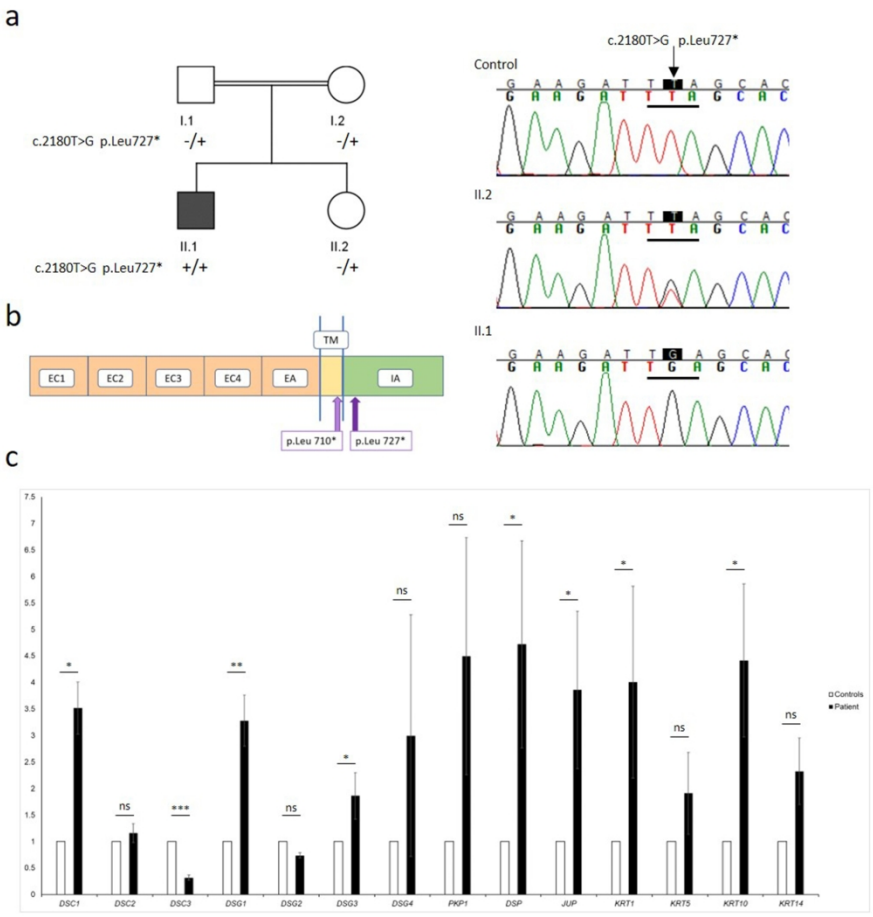

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196x194mm (300 x 300 DPI)

Supplementary online material

Homozygous nonsense mutation in *DSC3* resulting in skin fragility and hypotrichosis

Alexandros Onoufriadis[1], Noha Ahmed[2], Hagar Beser[2], Alyson Guy[3], Patricia Lovell[3], Lu Liu[3], Alexandros Marantzidis[1], Evangelia Kesidou[1], Maria Papanikolaou[1], Michael A. Simpson[4], Jemima E. Mellerio[1], John Y. W. Lee[1], John A. McGrath[1]

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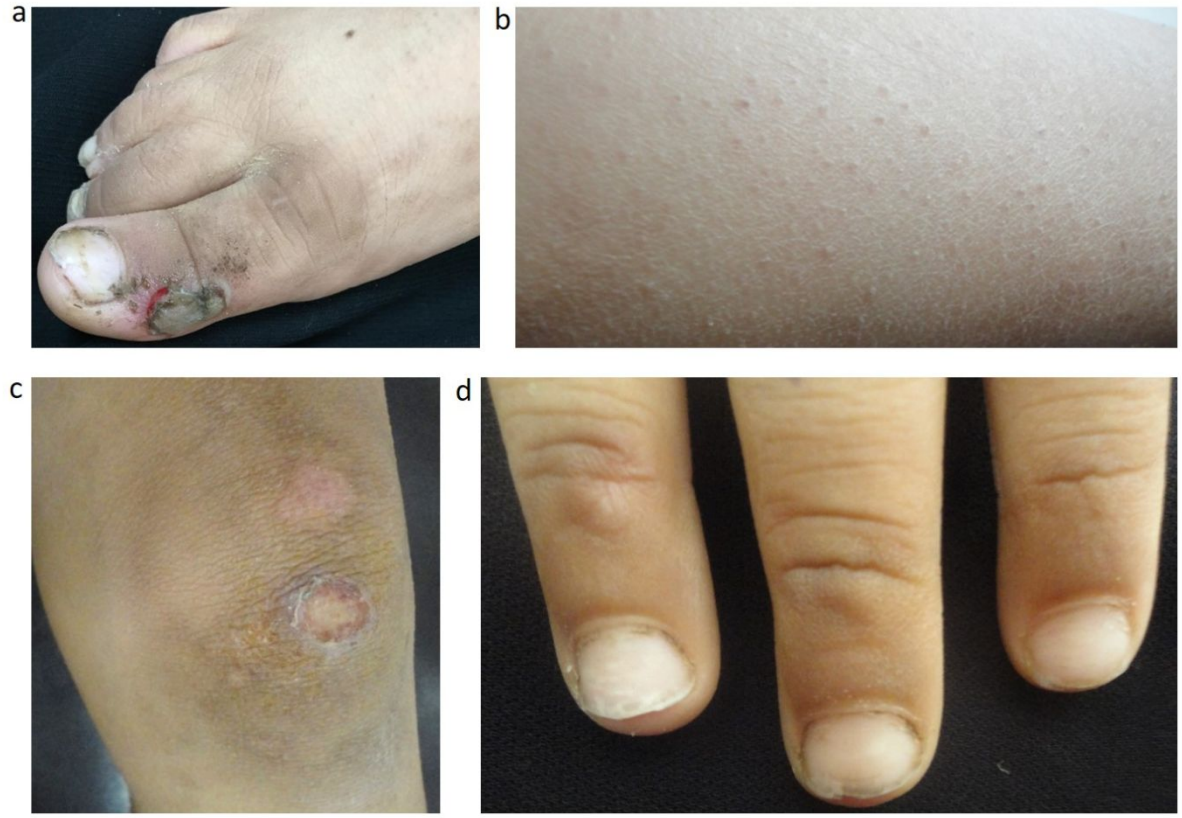

Figure S1. Additional clinical findings in the affected boy. (**a**) blister on the right great toe; (**b**) keratosis pilaris-like papules on the upper outer arm; (**c**) crusted trauma-induced erosions, hyperpigmentation and mild scarring on the left knee; (**d**) leukonychia and fragile distal nail plates.

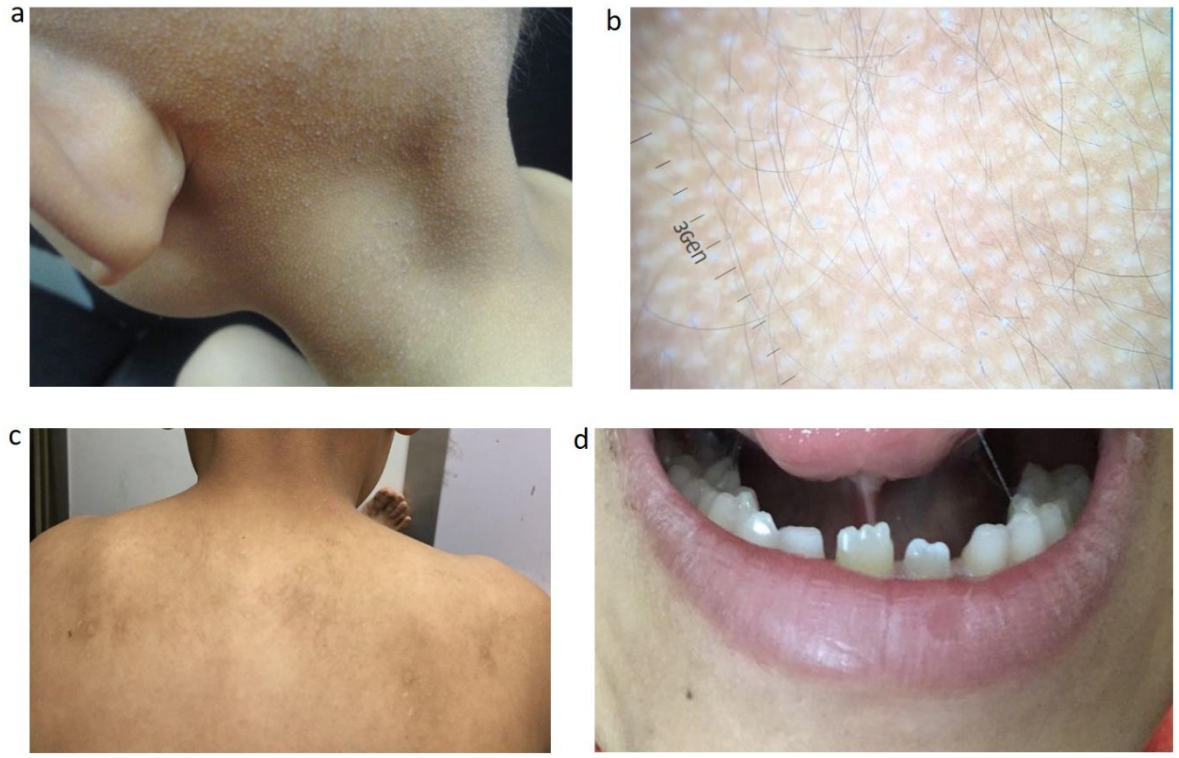

Figure S2. Additional clinical findings in the affected boy. (**a**) hypotrichosis and follicular papules on occipital scalp; (**b**) dermatoscopy reveal thin sparse hairs and pallor surrounding the follicles; (**c**) post-inflammatory hyperpigmentation on the upper back; (**d**) irregular tooth grooving.

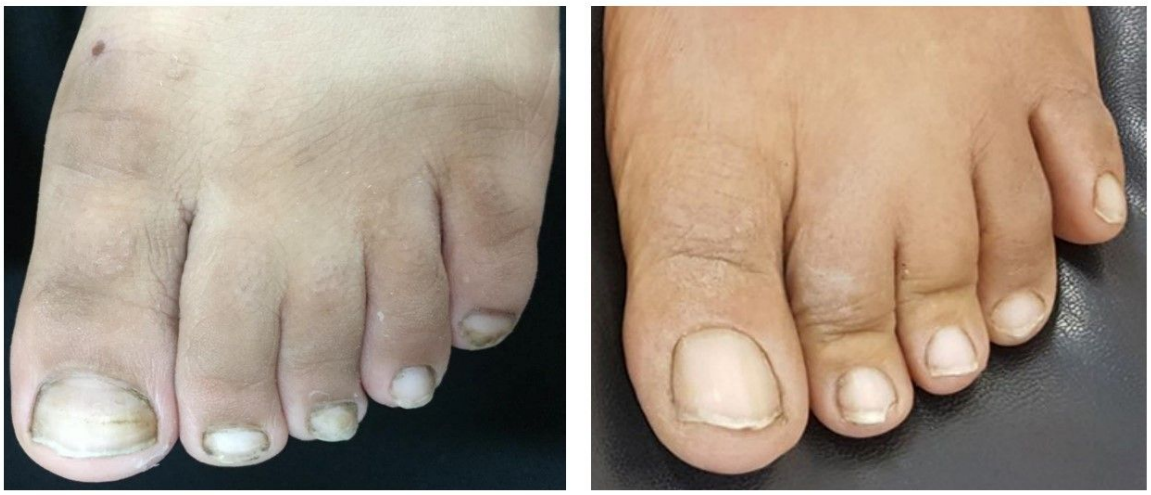

Figure S3. Additional clinical findings in the affected boy and unaffected sister. Both individuals have partial syndactyly of the second and third toes. The mother has similar findings (not shown).

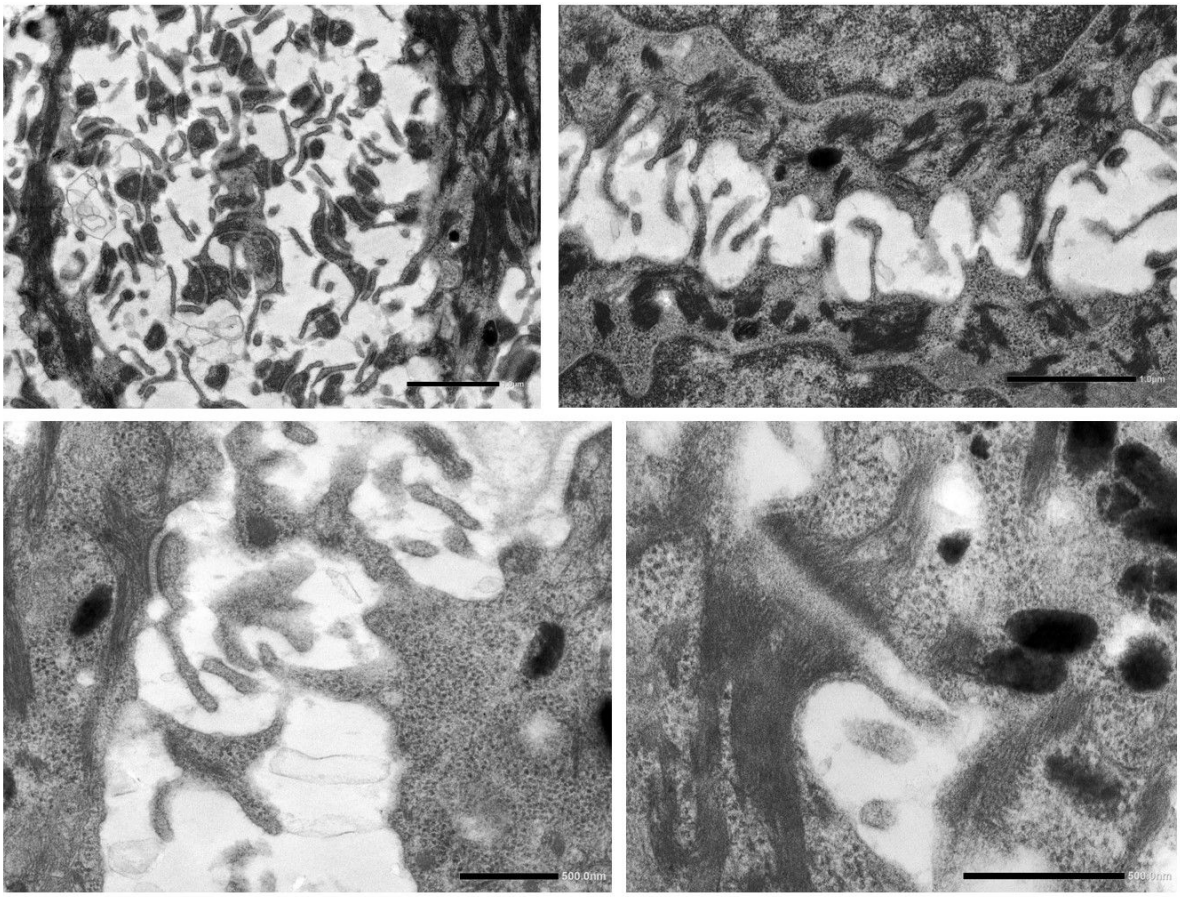

Figure S4. Transmission electron microscopy of the affected boy's skin. At low magnification the desmosomes have a detached, pinched off appearance. Higher magnification shows the plan of cleavage is mostly within the desmosomal inner plaque rather than in the extracellular space. Some desmosomes have poorly formed inner plaques and lack mid-line dense plates.

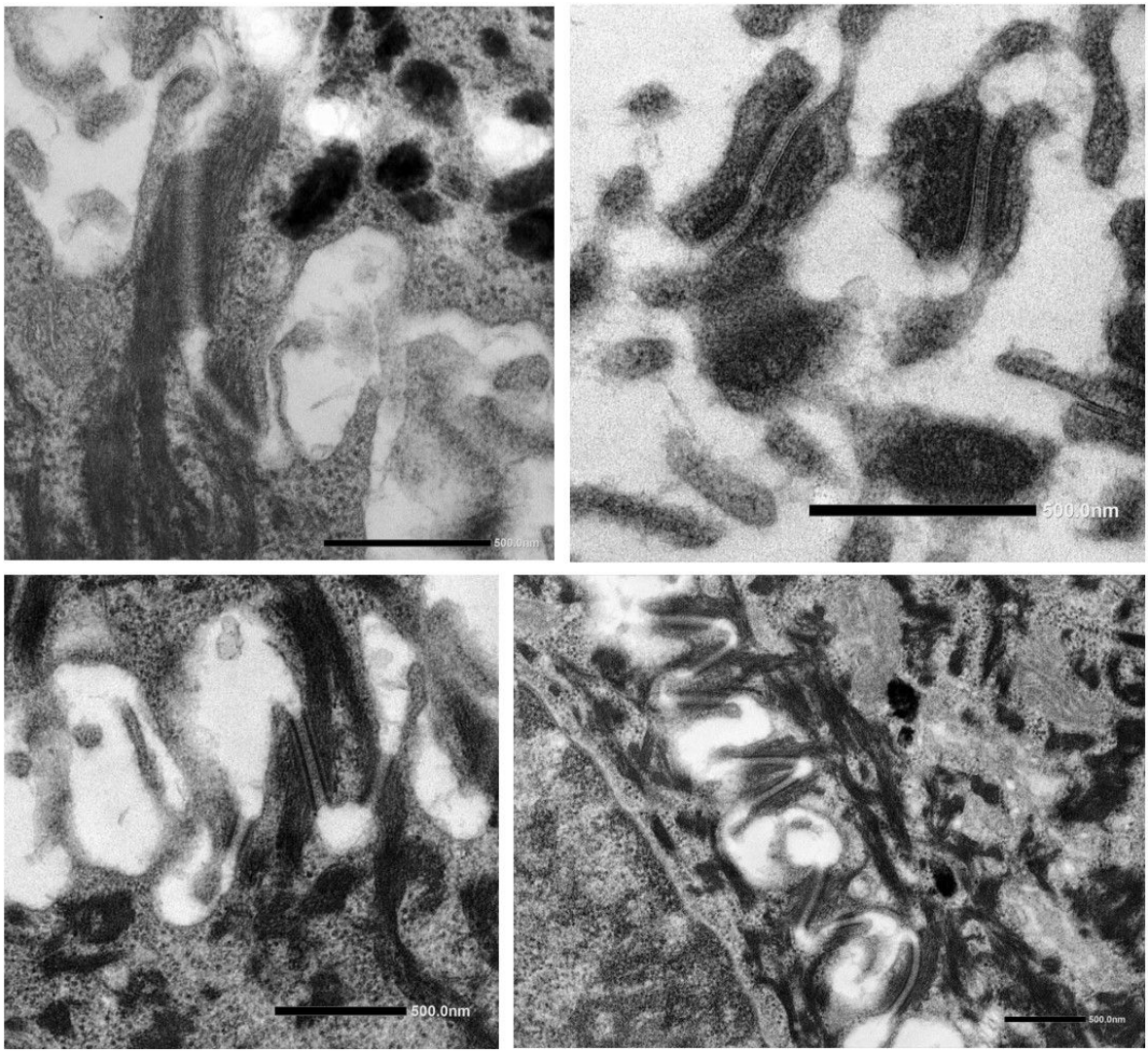

Figure S5. Transmission electron microscopy of the affected boy's skin. Although some desmosomes lack clear laminated plaques and plates, both intracellularly and extracellularly, several desmosomes within the spinous layer have near normal appearing desmosomes, in terms of number, size and ultrastructural morphology.

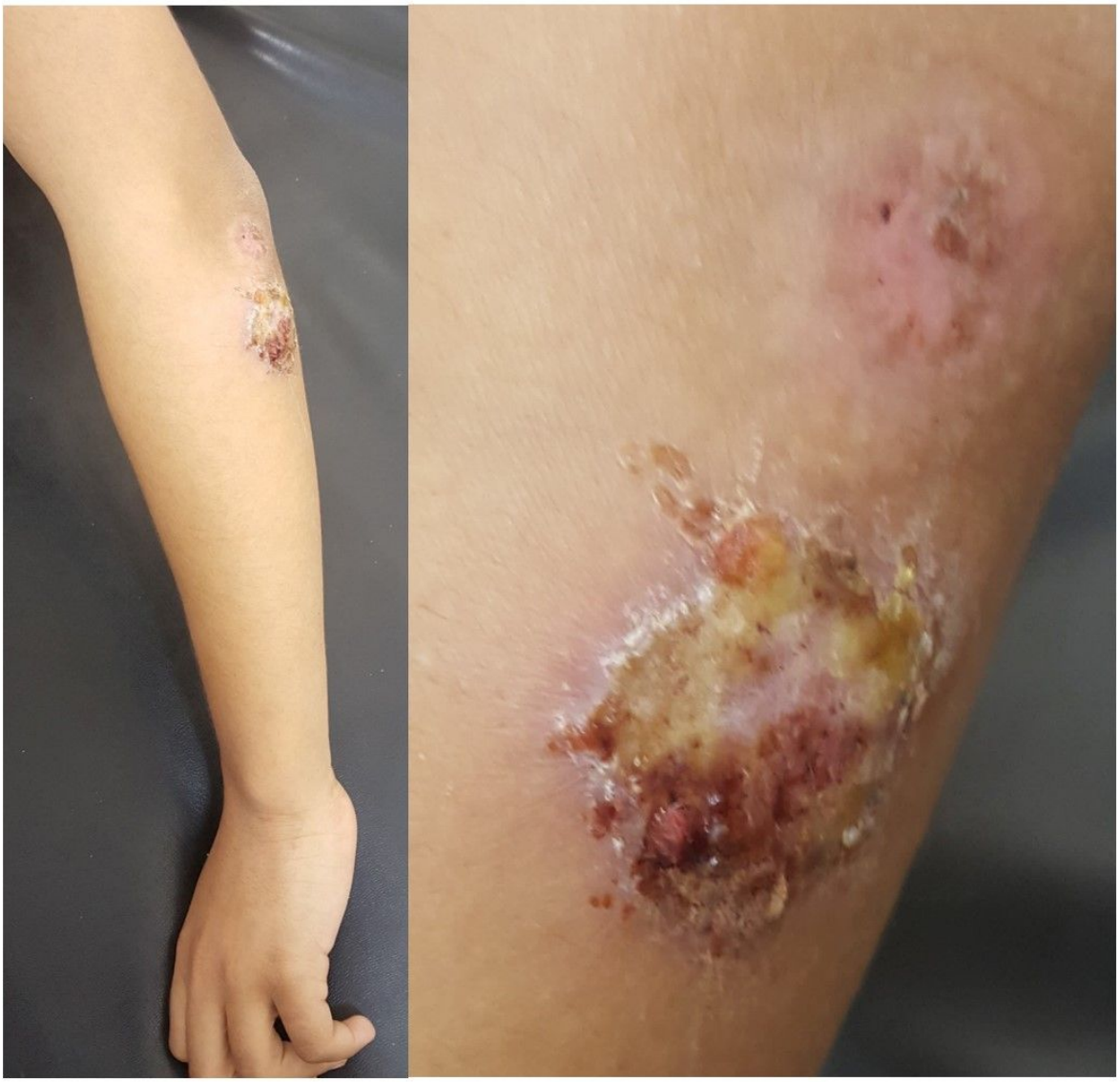

Figure S6. Skin features in the heterozygous sister. Trauma-induced erosion on the left elbow; no other skin fragility was evident.

Supplementary Table S1. Inherited desmosomal diseases.

Gene	Inheritance	Phenotype	MIM	Reference
DSC3	AR	Hypotrichosis and recurrent skin vesicles	613102	(Ayub et al., 2009)
DSC2	AR	Arrhythmogenic right ventricular cardiomyopathy with mild PPK and woolly hair	610476	(Simpson et al., 2009)
	AD	Arrhythmogenic right ventricular dysplasia 11	610476	(Heuser et al., 2006, Syrris et al., 2006)
DSG1	AR	Severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome	615508	(Samuelov et al., 2013)
	AD	Striate PPK	148700	(Rickman et al., 1999)
DSG2	AD	Arrhythmogenic right ventricular dysplasia 10	610193	(Pilichou et al., 2006)
DSG3	AR	Oral and laryngeal mucosal blistering	-	(Kim et al., 2019)
DSG4	AR	Hypotrichosis 6	607903	(Kljuic et al., 2003)
DSP	AR	Dilated cardiomyopathy with woolly hair and keratoderma	605676	(Norgett et al., 2000)
	AR	Lethal acantholytic epidermolysis bullosa	609638	(Jonkman et al., 2005)
	AR	Skin fragility-woolly hair syndrome	607655	(Whittock et al., 2002)
	AD	Arrhythmogenic right ventricular dysplasia 8	607450	(Rampazzo et al., 2002)
	AD	Dilated cardiomyopathy with woolly hair, keratoderma and tooth agenesis	615821	(Norgett et al., 2006)
	AD	Striate PPK	612908	(Armstrong et al., 1999)
JUP	AR	Naxos disease	601214	(McKoy et al., 2000)
	AR	Lethal congenital epidermolysis bullosa		(Pigors et al. 2011)
	AD	Arrhythmogenic right ventricular dysplasia 12	611528	(Asimaki et al., 2007)
PKP1	AR	Ectodermal dysplasia/skin fragility syndrome	604536	(McGrath et al., 1997)
PKP2	AD	Arrhythmogenic right ventricular dysplasia 9	609040	(Gerull et al., 2004)

CDSN	AD	Hypotrichosis 2	146520	(Levy-Nissenbaum et al., 2003)
	AR	Peeling skin syndrome 1	270300	(Oji et al., 2010)

AD = autosomal dominant; AR = autosomal recessive

Supplementary references.

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Supplementary Table S2. Exome sequencing coverage and mapping statistics

Individual	**I:1**	**I:2**	**II:1**	**II:2**
total_reads	64744805	64061480	60062152	56610031
mapped_to_target_reads	32328759	31788083	30139635	28779443
percentage	49.93	49.62	50.18	50.84
mapped_to_target_reads_plus_150bp	38779651	38321838	36205590	34591373
percentage	59.9	59.82	60.28	61.1
mean_coverage	58.47	57.29	54.43	52.05
accessible_target_bases	33323618	33323618	33323618	33323618
accessible_target_bases_1x	33193719	33157740	33182543	33145582
percentage	99.61	99.5	99.58	99.47
accessible_target_bases_5x	32996751	32957007	32981121	32929504
percentage	99.02	98.9	98.97	98.82
accessible_target_bases_10x	32571204	32498107	32504965	32316666
percentage	97.74	97.52	97.54	96.98
target_bases_20x	30357293	30091632	29840396	28834259
percentage	91.1	90.3	89.55	86.53

Supplementary Table S3. Variant calling for exome sequenced individuals

Individual	**I:1**	**I:2**	**II:1**	**II:2**
all_variants	28098	28651	27921	28228
het_variants	17589	18352	16944	17676
hom_variants	10509	10299	10977	10552
coding_variants	24456	24955	24309	24626
het_coding_variants	15376	16074	14810	15481
hom_coding_variants	9080	8881	9499	9145
splice_variants	3642	3696	3612	3602
het_splice_variants	2213	2278	2134	2195
hom_splice_variants	1429	1418	1478	1407
nonsynonymous_SNVs	11159	11401	11060	11275
het_nonsynonymous_SNVs	7035	7391	6751	7079
hom_nonsynonymous_SNVs	4124	4010	4309	4196
synonymous_SNVs	12060	12336	12077	12119
het_synonymous_SNVs	7573	7927	7370	7634
hom_synonymous_SNVs	4487	4409	4707	4485
stoploss_SNVs	8	10	9	9
het_stoploss_SNVs	6	7	6	7
hom_stoploss_SNVs	2	3	3	2
stopgain_SNVs	97	97	90	86
het_stopgain_SNVs	75	74	67	64
hom_stopgain_SNVs	22	23	23	22
deletions	280	254	262	262
het_deletions	187	160	158	171
hom_deletions	93	94	104	91
insertions	229	245	220	234
het_insertions	128	132	112	126
hom_insertions	101	113	108	108
frameshift_deletions	98	92	94	89
het_frameshift_deletions	60	55	54	51
hom_frameshift_deletions	38	37	40	38
frameshift_insertions	64	72	59	71
het_frameshift_insertions	26	31	23	32
hom_frameshift_insertions	38	41	36	39
ts_tv_ratio	2.9	2.89	2.92	2.86
het_ts_tv_ratio	2.93	2.89	2.96	2.87
hom_ts_tv_ratio	2.85	2.88	2.86	2.86

Abbreviations: het, heterozygous; hom, homozygous; SNV, single nucleotide variant; ts, transition; tv, transversion.

Supplementary Table S4. Summary of whole exome filtering process

Individual	**II:1**
Total variants	27437
Variants with MAF<0.01 in public and in-house exome databases	1200
Homozygous variants	52
Homozygous nonsynonymous, splice-site, or insertion/deletion variants	34
Variants that are shared by parents in a heterozygous state	8
Variants that are not shared by the unaffected sibling	8
Nonsense variants	1 (*DSC3*)

Abbreviations: MAF, minor allele frequency.

Supplementary Table S5. Primer sequences used for co-segregation analysis

Oligonucleotide	**Sequence 5' to 3'**
DSC3_F	GGGAAACCATGCTTAGTGGA
DSC3_R	TGACAGACAATATCTATGCCTATGAA

Abbreviations: F, forward; R, reverse

Supplementary Table S6. Antibodies used for immunofluorescence microscopy

Antibody to / clone	Antibody type	Dilution for use	Source	Product code	Batch/lot number
Desmocollin 1 / Dsc1	Rabbit polyclonal	1 in 50	Sigma Aldrich	HPA012891	C113886
Desmocollin 2	Mouse polyclonal	1 in 20	abcam	ab72792	GR3262682-2
Desmocollin 3 / Dsc3-U114	Mouse monoclonal	Neat (Fix and Triton X-100 pre-treatment)	Progen Biotechnik	65193	Lot 306071
Desmoglein 1 / 27B2	Mouse monoclonal	1 in 10	abcam	ab12077	N/A
Plakoglobin (gamma Catenin) / 15F11	Mouse monoclonal	1 in 250	abcam	ab11506	GR2939-2
Cytokeratin 10 / LHP1	Mouse monoclonal	1 in 100	VECTOR	VP-C408	N/A
Cytokeratin 1 / 34BB4	Mouse monoclonal	1 in 50	VECTOR	VP-C398	N/A
Plakophilin 1 / PP1-5C2	Mouse monoclonal	Neat (Fix and Triton X-100 pre-treatment)	Progen Biotechnik	65160	701061
Cytokeratin 14 / LL002	Mouse monoclonal	1 in 100	ThermoFisher Scientific	MA511599	TH2627104
Cytokeratin 5 / XM26	Mouse monoclonal	1 in 50	abcam	ab17130	GR217807-1
Desmoplakin 1 / DP 2.17	Mouse monoclonal	1 in 10	Progen Biotechnik	61024	Lot 001251