# Filaggrin 2 Deficiency Results in Abnormal Cell-Cell Adhesion in the Cornified Cell Layers and Causes Peeling Skin Syndrome Type A

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Peeling skin syndromes form a large and heterogeneous group of inherited disorders characterized by superficial detachment of the epidermal cornified cell layers, often associated with inflammatory features. Here we report on a consanguineous family featuring noninflammatory peeling of the skin exacerbated by exposure to heat and mechanical stress. Whole exome sequencing revealed a homozygous nonsense mutation in *FLG2*, encoding filaggrin 2, which cosegregated with the disease phenotype in the family. The mutation was found to result in decreased *FLG2* RNA levels as well as almost total absence of filaggrin 2 in the patient epidermis. Filaggrin 2 was found to be expressed throughout the cornified cell layers and to colocalize with corneodesmosin that plays a crucial role in maintaining cell-cell adhesion in this region of the epidermis. The absence of filaggrin 2 in the patient skin was associated with markedly decreased corneodesmosin expression, which may contribute to the peeling phenotype displayed by the patients. Accordingly, using the dispase dissociation assay, we showed that *FLG2* downregulation interferes with keratinocyte cell-cell adhesion. Of particular interest, this effect was aggravated by temperature elevation, consistent with the clinical phenotype. Restoration of corneodesmosin levels by ectopic expression rescued cell-cell adhesion. Taken together, the present data suggest that filaggrin 2 is essential for normal cell-cell adhesion in the cornified cell layers.

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## INTRODUCTION

Peeling skin syndrome (PSS) refers to a complex group of autosomal recessive disorders of cornification featuring superficial detachment of the epidermal cornified cell layers with no mucosal fragility (Kose et al., 2012; Levy and Goldsmith, 1982).

Two major clinical types of PSS have been reported: localized (acral) and generalized PSSs. Generalized PSS is further subdivided into a noninflammatory (type A) form and an inflammatory (type B) form. This latter type also features itching, atopic diathesis, allergic reactions, and failure to thrive (Kose et al., 2012).

These three clinical groups of PSS correspond to five genetic subtypes of the disease. Localized or acral PSS (PSS2; Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 609796: http://www.ncbi.nlm.nih.gov/omim/) can be caused by mutations in two genes: *TGM5* (Cassidy et al., 2005), encoding transglutaminase 5, which catalyzes the formation of γ-glutamyl-ε-lysine isopeptide bonds between epidermal differentiation-associated proteins, and *CSTA* (Blaydon et al., 2011; Krunic et al., 2013) (PSS4; Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 607936: http://www.ncbi.nlm.nih.gov/omim/), encoding cystatin A, a cysteine protease inhibitor. Type B generalized PSS results from mutations in *CDSN* (PSS1; Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 270300: http://www.ncbi.nlm.nih.gov/omim/), encoding corneodesmosin (CDSN) (Oji et al., 2010), a component of the

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*Abbreviations: CDSN, corneodesmosin; PSS, peeling skin syndrome; siRNA, small interference RNA*

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desmosomal plaques in the upper epidermal layers. CDSN is incorporated into desmosomes in the cornified layers (Garrod and Chidgey, 2008) and has been shown to play a critical role in cell-cell adhesion in both humans and animal models (Ishida-Yamamoto and Igawa, 2015; Matsumoto et al., 2008). Additional conditions that also feature inflammatory generalized peeling include Netherton syndrome (Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 256500: http://www.ncbi.nlm.nih.gov/omim/), also associated with CDSN deficiency, and severe skin dermatitis, multiple allergies, and metabolic wasting (SAM; Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 615508: http://www.ncbi.nlm.nih.gov/omim/) syndrome (Samuelov and Sprecher, 2014). Type A generalized PSS encompasses two major phenotypes: PSS type 5 (Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 617115: http://www.ncbi.nlm.nih.gov/omim/), which features late-onset peeling over the hands, feet, and knees as well as palmoplantar keratoderma and is caused by mutations in the *SERPINB8* gene, which encodes a serine protease inhibitor (Pigors et al., 2016); and PSS type 3 (Online Mendelian Inheritance in Man, OMIM. Johns Hopkins University, Baltimore, MD. MIM Number: 616265: http://www.ncbi.nlm.nih.gov/omim/), which manifests with superficial and generalized peeling without pruritus or any associated signs (Cabral et al., 2012).

PSS3 was found in one family to be caused by a mutation in *CHST8* encoding a carbohydrate sulfotransferase, N-acetylgalactosamine-4-O-sulfotransferase 1 (Cabral et al., 2012) (although this mutation may represent a neutral polymorphism; Fiete et al., 2017). Two recent studies identified the same homozygous variant in *FLG2* encoding filaggrin 2, which cosegregated with PSS3 (Alfares et al., 2017; Bolling et al., 2018). The functional significance of this variant has not been studied.

Here we show in an additional family with PSS3 that loss of expression of filaggrin 2 underlies PSS3 and destabilizes epidermal cell-cell adhesion.

## RESULTS

### Clinical features

We studied two siblings born to consanguineous parents of Arab Moslem origin who displayed noninflammatory type A PSS since birth. All other family members lacked cutaneous manifestations although atopic diathesis was seen among first-degree cousins of the affected children. Peeling, mostly involved the limbs, was occasionally seen on the trunk with sparing of the face and palmoplantar skin. It was most prominent during the summer time, after exposure to elevated ambient temperature, and was also triggered by minor trauma to the skin (Figure 1a). Skin lesions resolved leaving residual discolored skin (Figure 1b). Of note, the disease manifestations were much more prominent during childhood (Supplementary Figure S1 online) and improved with age. Skin biopsy revealed subcorneal separation with little or no dermal inflammatory infiltrate (Figure 1c). Electron microscopy demonstrated intraepidermal separation within the lower corneal layers (Figure 1d) and a reduction in the number of keratohyalin granules, most of which were deformed and irregularly shaped (Supplementary Figure S2 online).

## MUTATIONAL ANALYSIS

After having excluded by direct sequencing pathogenic mutations in the coding sequences of *CDSN*, *CHST8*, and *CSTA*, which have been associated with various forms of PSS (Cabral et al., 2012; Krunic et al., 2013; Oji et al., 2010), DNA samples extracted from individuals I-3 and II-8 (Figure 2b) were subjected to whole exome sequencing. A hitherto unreported homozygous transversion in *FLG2*, c.1065T>A (p.Y355*), was identified in affected individual II-8. The mutation was validated using direct sequencing (Figure 2a) and a PCR-restriction fragment length polymorphism assay (Figure 2b). The mutation was found to cosegregate with the disease phenotype in the family: the two patients (individuals II-7 and II-8) were found to carry the mutation in an homozygous state, whereas all other family members were found to carry the mutation in a heterozygous state or to be homozygous for the wild-type allele except for healthy individual II-9 who carries mutation c.1065T>A in a homozygous state in her peripheral blood leukocytes as a result of the fact that she received a bone marrow transplantation from patient II-7 as a treatment for thalassemia major.

The mutation was excluded using the PCR-restriction fragment length polymorphism assay from a cohort of 151 population-matched nonaffected individuals (not shown). Further confirming its pathogenicity, we could not identify the mutation in available public exome databases including gnomAD, ESP, 1000 Genomes, Ensembl, UCSC, and HGMD totaling more than 130,000 individual *FLG2* sequences.

### Consequences of mutation c.1065T>A in *FLG2*

Mutation c.1065T>A is predicted to result in the generation of a premature termination codon, which in turn is likely to lead to nonsense-mediated mRNA decay. To ascertain this possibility, we used quantitative real-time PCR to assess the effect of the mutation at the RNA level. We found out that *FLG2* RNA levels were reduced by more than 80% in the skin of the patients as compared with normal skin (Figure 3a). Accordingly, filaggrin 2 protein expression was dramatically reduced in the stratum granulosum and absent in the stratum corneum in patients' skin as compared with normal skin (Figure 3b).

We then investigated the effect of filaggrin 2 deficiency on the expression of a number of biologically relevant proteins in the skin of the patients. CDSN has been shown to play a vital role in cell-cell adhesion within the cornified cell layers (Matsumoto et al., 2008; Oji et al., 2010). CDSN expression was found to be decreased by immunofluorescence in the epidermis of our patients in contrast with normal expression of other proliferation, epidermal differentiation, or cell-cell adhesion proteins such as E-cadherin, filaggrin, loricrin, keratin 10, and keratin 14 (Figure 4).

Further supporting the pathogenicity of the mutation, three-dimensional skin equivalents generated from the patient's keratinocytes show abnormal differentiation (Supplementary Figure S3 online). Epidermal thickness and more specifically live keratinocyte layers were thinner in