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Bolling, Maria C.; Jan, Sabrina Z.; Pasmooij, Anna M. G.; Lemmink, Henny H.; Franke, Lude H.; Yenamandra, Vamsi K.; Sinke, Richard J.; van den Akker, Peter C.; Jonkman, Marcel F.

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Generalized Ichthyotic Peeling Skin Syndrome due to *FLG2* Mutations

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TO THE EDITOR

Peeling skin syndrome (PSS) is a group of recessive skin fragility genodermatoses with superficial peeling of the skin as the characteristic clinical feature that may be accompanied by ichthyosis and/or inflammation. Localized PSS is caused by mutations in *TGM5* and *CSTA* (Blaydon et al., 2011; Cassidy et al., 2005), whereas generalized PSS is caused by mutations in *CDSN* (Oji et al., 2010), *SERPINB8* (Pigors et al., 2016) and *CHST8* (Cabral et al., 2012). Recently, in a family with generalized ichthyotic PSS, a homozygous nonsense mutation in *FLG2* was found, thereby broadening the PSS genetic spectrum (Alfares et al., 2017). Here, we present two siblings with generalized peeling skin and ichthyosis with the same homozygous *FLG2* nonsense mutation and provide clues to the pathogenesis of the epidermal separation and abnormal cornification.

The index patient is an 11-year-old girl from consanguineous Moroccan parents (V:5) (Figure 1). At birth, she was erythrodermic, and afterward, dry skin was present with superficial peeling of the skin upon minor trauma, leaving a red denuded area. The lesions healed with hyperpigmentation without leaving scars. Warm and humid environment aggravated the peeling tendency. Over the knees and elbows, hyperkeratosis and ichthyosis were present. Hairs, nails, and mucosae were unaffected. The condition markedly improved during childhood. One older brother (V:4) was similarly affected and also had remarkable improvement with age.

The index patient was first analyzed with our targeted next-generation sequencing gene panel for skin fragility disorders, which includes *CSTA*, *CDSN*,

and *TGM5* (see Supplementary Materials online). Because no pathogenic or likely pathogenic variants were identified that could explain the phenotype, whole-exome sequencing followed by comprehensive gene network analysis was performed next, using the known PSS genes as bait (see Supplementary Materials). This uncovered a homozygous nonsense mutation in exon 3 of the *FLG2* gene (c.632C>G,p.(Ser211*), NM_001014342.2), encoding filaggrin 2. This variant was not yet described as a disease-causing mutation at that time, was identified only three times in 245,962 exomes and 30,956 genomes (Exome Aggregation Consortium), and was not reported in the dbSNP or ClinVar databases (<http://gnomad.broadinstitute.org/variant/1-152329630-G-C>). Sanger sequencing confirmed the homozygous mutation in the index patient (V:5) and her affected brother (V:4). Both unaffected parents were heterozygous carriers, and four other unaffected siblings were either heterozygous carriers or homozygous wild type, indicating co-segregation of the mutation with the phenotype. Altogether, these data strongly supported the *FLG2* p.(Ser211*) variant being the disease-causing mutation in this family.

Histopathology of perilesional skin (V:5) showed separation in the lower stratum corneum (SC) with parakeratosis (Figure 1g). Electron microscopy showed abnormal keratin bundles and keratohyalin granules in the stratum granulosum (SG). Corneocytes were swollen, interdigitating, and loosely packed and contained light grey globular inclusions with central abnormal vesicles (see Supplementary Materials).

Immunofluorescence microscopy (see Supplementary Table S1 online) of

perilesional skin showed absent staining with antibody Ab122001 (anti-filaggrin-2 residues 280–381), whereas SG and SC in control skin stained positively (Figure 2). Staining with antibodies Abx100795 and Mbs20059595 against the C-terminal part of filaggrin-2 showed a faint positive staining (see Supplementary Figure S2 online). Keratin 2 and corneodesmosin stainings were reduced in patient skin. The latter staining indicated swollen corneocytes (Figure 2, arrowhead). Desmocollin-1 and desmoglein-1 expression were reduced in SG, and desmoglein-1 staining was retained in the SC in perilesional skin at age 4 years, but all these staining results were normal at age 10 years (Figure 2). Loricrin and filaggrin-1 stainings were absent in perilesional skin at age 4 years but were near normal at age 10 years. Staining results for keratin 10, claudin-1, and desmoplakin were normal (see Supplementary Figure S3 online).

Our results show that that biallelic *FLG2* nonsense mutations are associated with generalized ichthyotic PSS with a level of separation in the lower SC and that, in conjunction with in vitro observations by Pendaries et al. (2015), filaggrin-2 is essential for proper cornification and integrity of the SC.

Filaggrin-2, like filaggrin-1, belongs to the S100 fused-type protein family (Wu et al., 2009). The 248-kDa precursor protein, produced by granular keratinocytes, consists of an N-terminal S100 domain/EF hand domain, which is a putative calcium binding site, followed by A- and B-repeat domains separated by a spacer. The gene consists of two small exons followed by a large third exon encoding the filaggrin repeats (see Supplementary Figure S4 online). The repeats are thought to be proteolytically cleaved into smaller subunits, like filaggrin-1, and deposited in the

Abbreviations: PSS, peeling skin syndrome; SC, stratum corneum; SG, stratum granulosum

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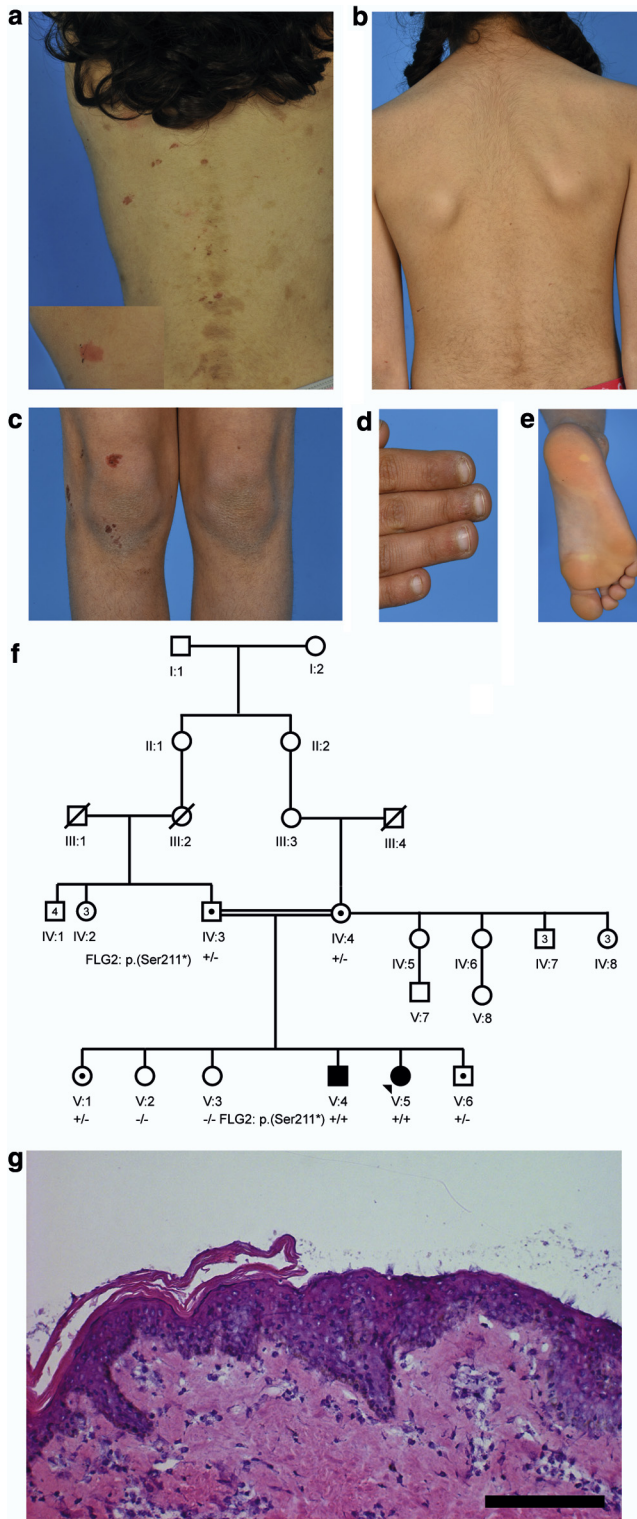


Figure 1. Clinical features of the index patient. (a) The patient at age 4 years showing the back with superficial peeling (insert), crusts and hyperpigmented maculae. (b–e) The patient at age 10 years showing (b) marked improvement, (c) mild hyperkeratosis on the knees with mild ichthyosis, (d) periungual peeling, and (e) mild plantar hyperkeratosis on pressure points. (f) Pedigree of the family described. Index patient is indicated by arrowhead. Squares indicate males, circles indicate females, blackened symbols indicate affected individuals, and symbols with black dots indicate carriers of the *FLG2* mutation. *FLG2* carrier status is indicated below each individual: $-/-$, homozygous wild type; $+/-$, heterozygous carrier; $+/+$, homozygous carrier. (g) Histopathology of a perilesional skin biopsy sample of the index patient showed skin separation in the lower stratum corneum with parakeratosis. Scale bar = 50 μ m.

SC. The function of filaggrin-2 is largely unknown, but because of its similarity with filaggrin-1, it was thought to play a role in epidermal barrier function. Recently, filaggrin-2 was shown to be down-regulated in atopic dermatitis skin (Trzeciak et al., 2017), and heterozygous *FLG2* nonsense mutations were associated with more persistent atopic dermatitis in African Americans (Margolis et al., 2014). Neither heterozygosity nor homozygosity for the *FLG2* mutation was associated with atopy in our family or the family described by Alfares et al. (2017), although dry skin was noted in carriers. A filaggrin-2 knockdown skin model (Pendaries et al., 2015) showed parakeratosis, down-regulation of loricrin, and abnormal vesicles in the SC, all of which we also observed in our patient (see Supplementary Figures S1 and S2).

Processed filaggrin monomers are known to be responsible for proper keratin filament bundling (Lonsdale-Eccles et al., 1982). Keratin 2 was reduced in our patient. Filaggrin-2 possibly renders corneocytes vulnerable because of inadequate keratin compaction during the early phase of cornification (see Supplementary Figure S1). An interaction between filaggrin-2 and the desmosomal proteins desmocollin-2 and/or desmoglein-2 has not been reported before. However, using the Functional Human Gene Network (Franke et al., 2006), *FLG2* gene expression was found to be co-regulated with the gene expression of *DSC1* and *DSG1* (<http://molgenis27.target.rug.nl/gene/ENSG00000143520>). The reduction in and altered distribution of corneodesmosin, desmocollin-1, and desmoglein-1, together with the clinical data, indicate that filaggrin-2 is also necessary for proper cell-cell adhesion in the lower SC. The exact biochemical mechanisms of these findings remain elusive, as well as an explanation for the remarkable improvement of the phenotype during childhood.

In conclusion, evidence is accumulating that loss of filaggrin-2 due to biallelic mutations in *FLG2* is associated with a generalized ichthyotic form of PSS. Our data indicate that filaggrin-2 is important for proper integrity and mechanical strength of the SC.

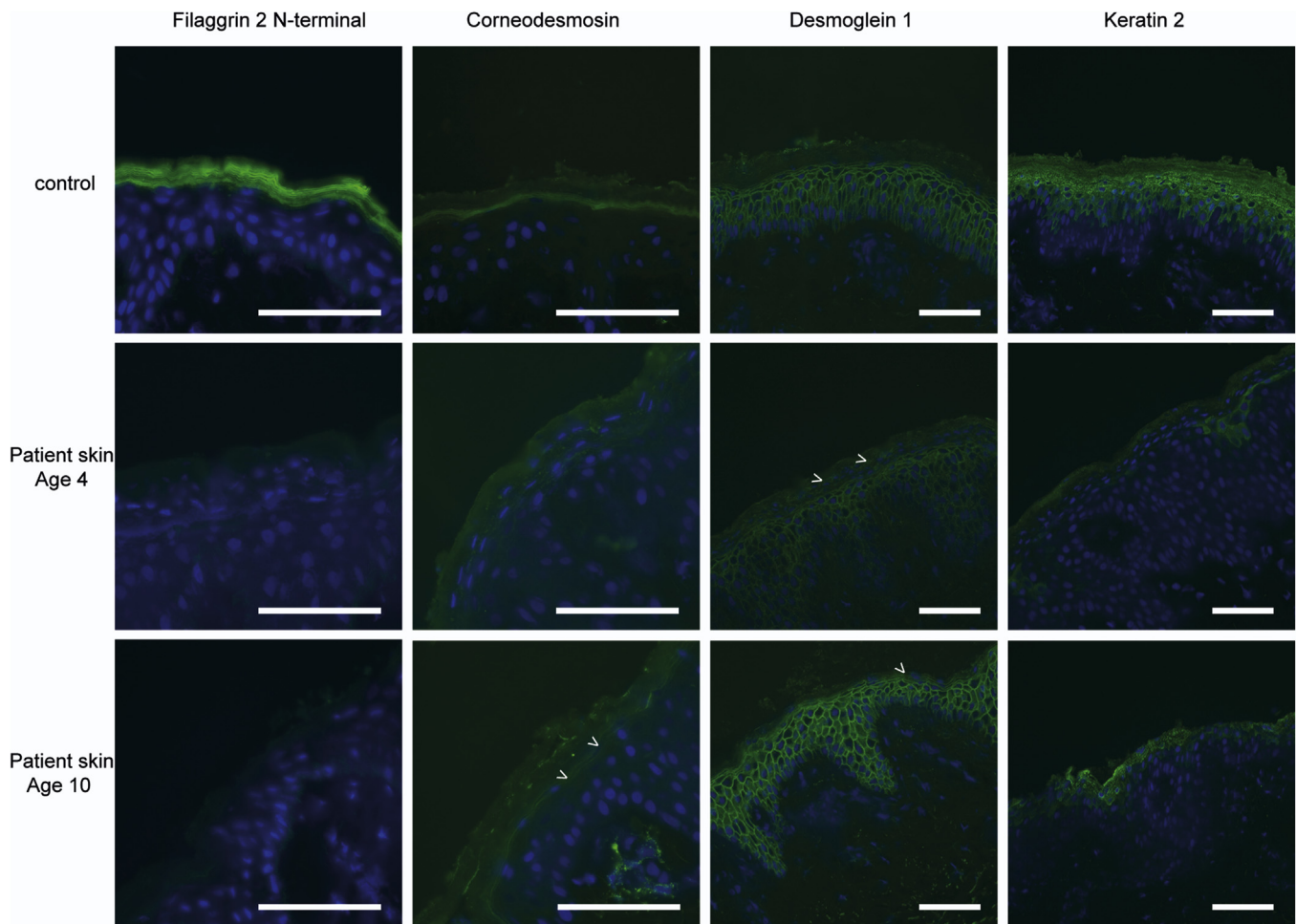


Figure 2. Immunofluorescence microscopy of patient skin. Filaggrin-2 was absent in patient skin when stained with N-terminal antibody Ab122001 (directed against amino acids 280–381), slightly C-terminal of the mutation (amino acid 211). Corneodesmosin was absent at age 4 years and reduced at age 10 years. Note the thicker corneocytes (arrowheads), also observed in electron microscopy (see [Supplementary Figure S1](#) online). Desmoglein-1 was reduced and retained in the SC (arrowheads) at age 4 years, but results were normal at age 10 years. Keratin 2 staining was strongly reduced and visible in the SC only at age 4 years and was overall reduced at age 10 years. Scale bars = 50 μ m.

The medical ethical committee of the University Medical Center Groningen, The Netherlands, gave approval for studies on human material initially obtained for diagnostic purposes. Studies were performed according to the Declaration of Helsinki principles. Written informed consent for publication and use of their photographs was obtained from the patients and their caregivers.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Maria C. Bolling^{1,3}, Sabrina Z. Jan^{2,3}, Anna M.G. Pasmooij¹, Henny H. Lemmink², Lude H. Franke²

Vamsi K. Yenamandra¹, Richard J. Sinke², Peter C. van den Akker^{2,3} and Marcel F. Jonkman^{1,3,*}

¹University of Groningen, University Medical Center Groningen, Department of Dermatology, Center for Blistering Diseases, Groningen, The Netherlands; and ²University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, The Netherlands

³These authors contributed equally to this work.

*Corresponding author e-mail: m.f.jonkman@umcg.nl

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2018.01.038>.

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Genome-Wide Association of *PVT1* with Vitiligo



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TO THE EDITOR

Vitiligo is a common skin disease in which depigmented patches of skin and overlying hair result from autoimmune destruction of melanocytes in the involved regions, with elevated frequencies of several other autoimmune diseases (Picardo and Taïeb, 2018). We previously carried out three genome-wide association studies (GWASs) of vitiligo in subjects of European-derived white ethnic origin (EUR) and thereby identified and confirmed 48 genetic loci that are significantly associated with vitiligo (Jin et al., 2010a, 2010b, 2012, 2016). Most of these loci harbor genes that are involved in regulation of immune cells, apoptosis, and melanocyte function (Spritz and Andersen, 2017). In addition to these loci, in our previous GWAS we detected provisional association of vitiligo with another locus, *PVT1*. However, for technical reasons this association could not be verified by independent replication (Jin et al., 2016). Here, we report the results of a genetic association study of *PVT1* in an independent set of EUR vitiligo patients and control individuals. We detected highly significant association of *PVT1* with vitiligo, both in this replication study and in a meta-analysis that combines the data from the replication study with the previous GWAS data. These findings establish *PVT1* as a vitiligo susceptibility locus.

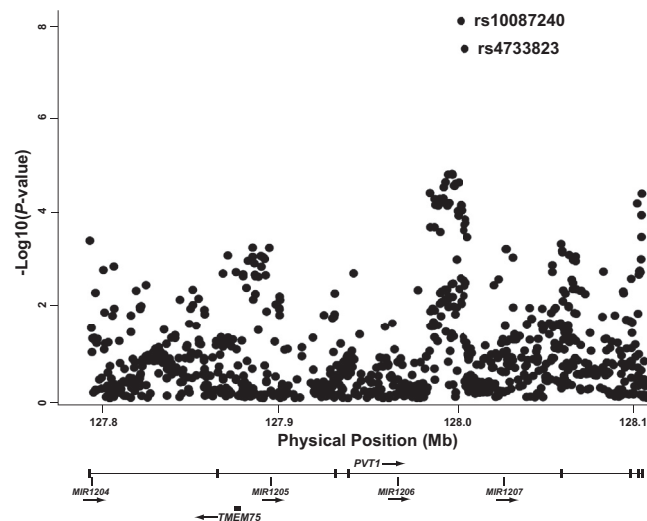


Figure 1. Association of vitiligo with nucleotide polymorphisms in the *PVT1* region of chromosome locus 8q24.21. Results from the previous GWAS (Jin et al., 2016) showing polymorphisms (black dots) in the *PVT1* region of chromosome locus 8q24.21. $-\log_{10}(P\text{-values})$ derived from Cochran-Mantel-Haenszel meta-analysis combining data from GWAS1, GWAS2, and GWAS3 are plotted against GRCh38/hg38 positions on chromosome 8 in mega base pairs. Single-nucleotide polymorphisms rs10087240 and rs4733823 are indicated. A genomic map is shown; arrows denote transcriptional orientations. GWAS, genome-wide association study; Mb, mega base pair; MIR, microRNA.

The three GWASs of vitiligo in EUR subjects have been described previously (GWAS1, GWAS2, GWAS3; Jin et al., 2010a, 2012, 2016). Briefly, subjects included 2,853 unrelated EUR vitiligo patients and data from 37,405 unrelated EUR control individuals obtained from the Database of Genotypes and Phenotypes. Meta-analysis of data from the three GWASs (Jin et al., 2016)

identified apparent association of vitiligo with two single-nucleotide polymorphisms (SNPs) located within the *PVT1* gene at chromosome locus 8q24.21 (Figure 1), rs10087240 and rs4733823, both surpassing the standard P -value less than 5.0×10^{-8} criterion for genome-wide significance (rs10087240: $P = 7.74 \times 10^{-9}$, odds ratio = 1.18; rs4733823: $P = 3.04 \times 10^{-8}$, odds ratio = 1.17). Association of vitiligo with both SNPs was supported by GWAS1 and GWAS3, but not by GWAS2 (Table 1), likely because of the low odds ratio and small sample size in GWAS2. As shown in Table 1,

Abbreviations: EUR, European-derived whites; GWAS, genome-wide association study; SNP, single-nucleotide polymorphism

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