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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.

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Increased pachyonychia congenita severity in patients with concurrent keratin and filaggrin mutations

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Summary

Pachyonychia congenita (PC), a rare autosomal-dominant keratin disorder caused by mutations in keratin genes *KRT6A/B*, *KRT16* or *KRT17*, is characterized by painful plantar keratoderma and hypertrophic nail dystrophy. Loss-of-function mutations in the filaggrin (*FLG*) gene underlie the most prevalent skin disorder of cornification, ichthyosis vulgaris (IV), which presents with generalized scaling and is also associated with atopic dermatitis. Recently, *FLG* mutations have been reported to increase phenotype severity of X-linked ichthyosis and alopecia areata. We report a parent–child trio in which the mother and the son have PC and the father has IV. Both the mother and the son are carriers for the *KRT16* mutation p.Leu132Pro. The son, who is much more severely affected than his mother, in addition carries the heterozygous *FLG* mutation p.R2447X, which was inherited from the father. This observation suggests that coinheritance of mutations in *KRT16* and *FLG* may aggravate the PC phenotype and that *FLG* could serve as a genetic modifier in PC.

Pachyonychia congenita (PC) is a rare autosomal-dominant ectodermal dysplasia which can be divided into two major subtypes.[1,2] Patients with PC-1 (OMIM 167200, Jadassohn–Lewandowsky syndrome) present with hypertrophic nail dystrophy, painful diffuse or focal symmetrical hyperkeratosis of palms and soles sometimes associated with erosions, follicular keratosis on the extensor surfaces of the extremities and oral leucokeratosis.[3] Individuals with PC-2 (OMIM 167210, Jackson–Lawler type) additionally show epidermoid cysts, neonatal teeth and leucokeratosis of the larynx and trachea.[4] At the molecular level, PC-1 is caused by dominant-negative mutations in keratin genes *KRT6A* and *KRT16* whereas PC-2 is due to mutations in *KRT6B* and *KRT17*.[5–7] Keratin 16 and 17 are expressed in differentiated epithelial structures such as nail beds, palmoplantar epidermis and mucosa, which comprise the affected tissues in PC-1.[2,6] Because of phenotype variations in patients carrying the same genotype, it was hypothesized that genetic or environmental modifiers could influence the genotype–phenotype relationship;[2,8] however, until now this has not been confirmed.

In 2006, loss-of-function mutations in the gene coding for filaggrin (*FLG*) were discovered as the molecular basis of ichthyosis vulgaris (IV), the most common hereditary disorder of cornification in humans. The same mutations are also strongly associated with atopic eczema.[9,10] Recently, it was reported that coinheritance of *FLG* mutations increases phenotype severity of X-linked ichthyosis[11] and alopecia areata.[12] We present the first case report of a patient with concurrent *KRT16* and *FLG* mutations showing an increased PC severity, indicating that *FLG* could serve as a genetic modifier in PC.

Case report

A 46-year-old Austrian woman (Figs 1a–d and 2) and her 20-year-old son (Fig. 1e–h) presented with typical PC-1 with onset at 1 year of age. Findings included hypertrophic dystrophy of the fingernails and toenails, painful diffuse symmetrical hyperkeratosis of the soles and mild follicular keratosis on the extensor surfaces of the extremities. Strikingly, the son was much more severely affected than his mother. In addition to the findings of his mother, he also showed palmar hyperkeratosis, plantar macerations and large blisters, oral leucokeratosis and palmar hyperlinearity (Fig. 1e–h).

Mother and son lived in the same household with similar environmental conditions. While the mother worked as a secretary, her son was a student. He had previously experienced a deterioration of his plantar calluses during increased physical activity (walking) when temporarily jobbing in a parcel service. At the time of the present study, with avoidance of physical activity, his cutaneous symptoms had returned to baseline. Even though the mother showed stronger symptoms of PC during childhood, she was never as severely affected as her son. Treatment consisted of topical emollients in both patients. In addition, 32 years previously the mother had received a

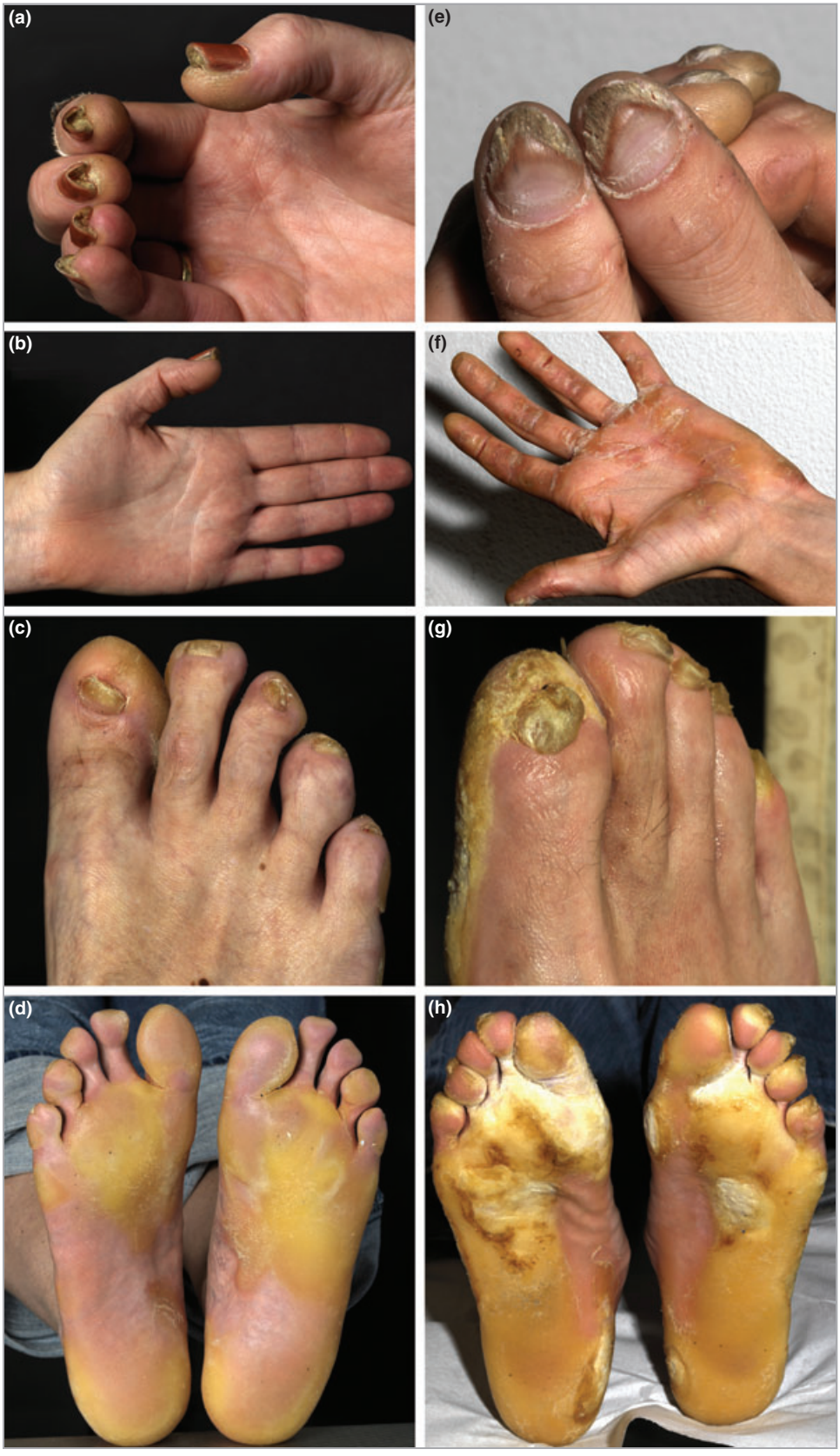

Fig 1. Phenotype of the index patient (a–d) and her son (e–h) showing the typical features of pachyonychia congenita type 1. Note the more severe phenotype in the son with involvement of the distal nail surfaces, palmar hyperkeratosis and hyperlinearity, plantar macerations, large blisters and calluses.

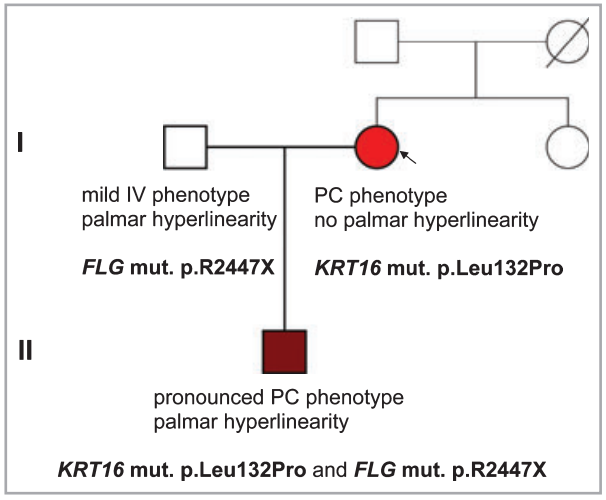

Fig 2. Pedigree of the family with phenotype and genotype characteristics. The index patient is marked with an arrow. PC, pachyonychia congenita; IV, ichthyosis vulgaris; mut., mutation.

course of oral etretinate (Ro-A-Vit 10-9359; Hoffmann-La Roche, Vienna, Austria) 25 mg twice daily for about 4 weeks, but treatment was discontinued because of increased plantar blistering and pain. Taken together, it was unlikely that environmental factors were solely responsible for the differences in phenotype severity between mother and son.

Genetic analyses revealed a heterozygous dominant missense mutation in the keratin 16 gene, namely p.Leu132Pro, in both the mother and her son. The histology of plantar skin showed acanthosis and compact hyperkeratosis with focal parakeratosis consistent with the diagnosis of PC. Except for the more prominent hyperkeratosis in the son compared with the mother, which is consistent with the clinical picture, no further histological differences could be detected. Staining for KRT16 revealed expression throughout the epidermis in both patients (Fig. 3d–f). While there was strong immunohistochemical staining for FLG using a mouse monoclonal antibody (Novocastra, Newcastle upon Tyne, U.K.) in the mother and in a control individual, the son showed decreased FLG expression with a reduced stratum granulosum layer (Fig. 3a–c).

The 68-year-old father displayed features consistent with moderate IV, characterized by fine scaling on the extensor surfaces of the extremities, keratosis pilaris on the upper arms and palmoplantar hyperlinearity (Figs 2 and 4). A screen for the common European *FLG* mutations R501X, 2282del4, R2447X and S3247X, using a TaqMan allelic discrimination

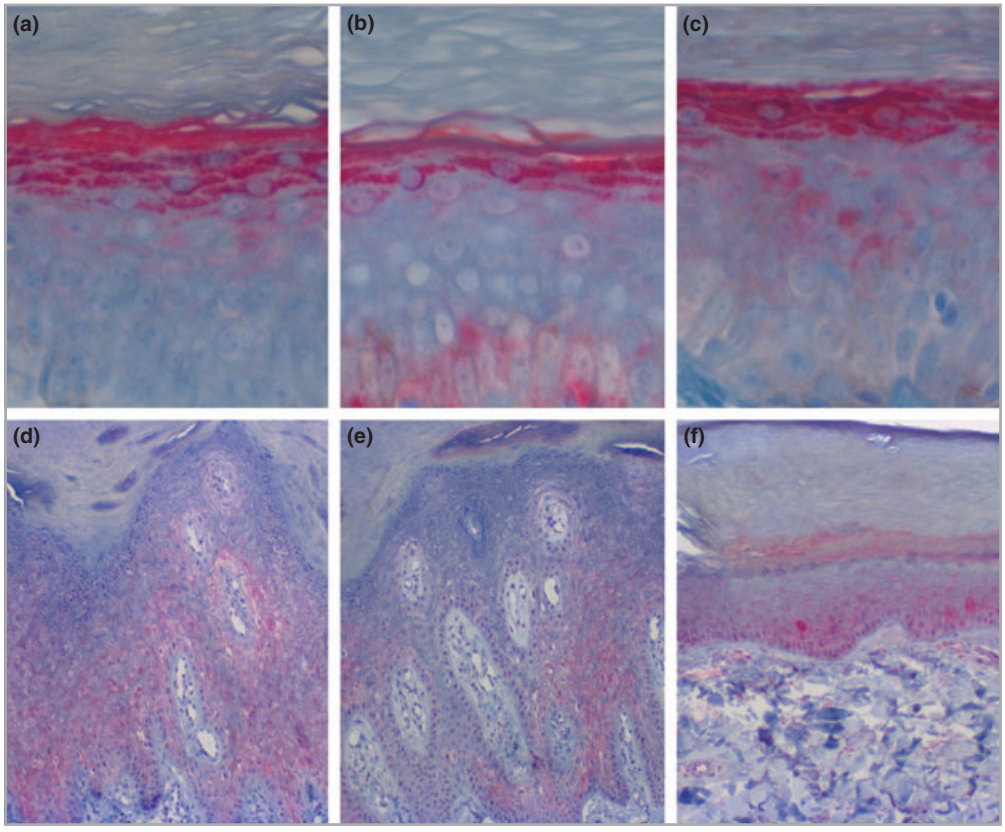

Fig 3. Immunohistochemical staining for filaggrin (FLG) (a–c) and keratin 16 (d–f) using monoclonal antibodies. Skin sections from the mother (a) and from a control (c) exhibit a normal FLG staining of the granular layer whereas the *FLG* heterozygote patient (b) shows decreased FLG expression. While there is a regular suprabasal keratin pattern in the control (f), both patients with pachyonychia congenita (d,e) show more diffuse epidermal staining. Original magnification × 100.

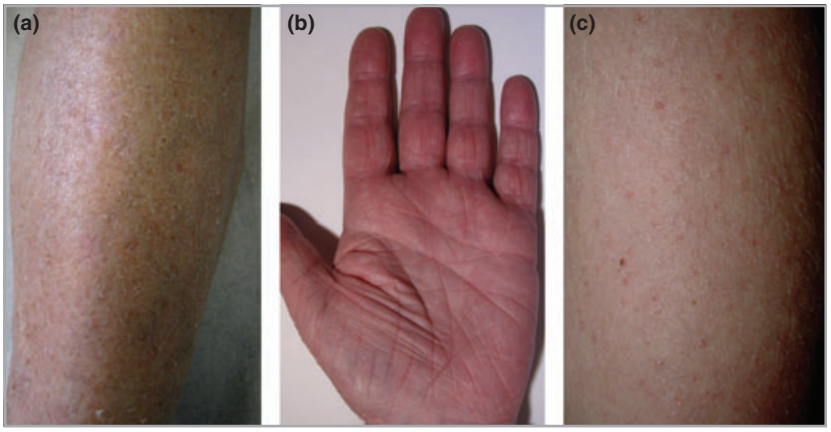

Fig 4. Phenotype of the father presenting with characteristic features of ichthyosis vulgaris. (a) Fine scaling on the extensor surfaces of the lower extremities. (b) Hyperlinearity of the palms. (c) Keratosis pilaris on the upper arms.

assay as described previously,[13] identified the heterozygous mutation p.R2447X in the son and his father.

In summary, while the index patient carried the p.Leu132-Pro mutation in *KRT16* and her husband the p.R2447X mutation in *FLG*, their son inherited both mutations. Past medical history and recent laboratory findings of both patients with PC were unremarkable. A detailed family history did not reveal other family members affected by PC and the examination of the mother's father and sister did not show any cutaneous features. There was no history or current evidence for atopic dermatitis, allergic rhinitis or asthma in the family.

Discussion

Because patients with PC due to keratin mutations can show phenotype variation even when carrying the same genotype, it was hypothesized that the genotype–phenotype relationship is influenced by additional environmental or genetic modifiers.[2,8] The present case report suggests that concomitant *FLG* mutations can act as genetic modifiers of the PC phenotype. It appears unlikely that the more pronounced PC-1 phenotype of the son is due to environmental effects, because both individuals live in the same household with similar working and leisure conditions. Although we cannot completely exclude the possibility that environmental trauma may play a superimposing role in phenotype severity, in this family it does not sufficiently explain the striking phenotype differences. The fact that the mother throughout life, including childhood and adolescence, was never affected as severely as her son, points to a modifying genetic factor. Based on this observation we hypothesize that the phenotype variation in this family can be ascribed to the *FLG* genotype, i.e. concomitant mutations p.Leu132Pro in *KRT16* and p.R2447X in *FLG* in the son. This assumption is substantiated by two recent reports, describing that coinheritance of *FLG* mutations can aggravate phenotype severity in other cutaneous disorders.[11,12]

The epidermal keratins are intermediate filament proteins that form the cytoskeletal network of keratinocytes. Mutations in *KRT16* result in accumulation of defective keratins within the cytoplasm, aberrant intermediate filament organization, increased epithelial fragility and hyperproliferation of mechanically stressed ectodermal structures, i.e. nails, soles and palms, clinically presenting as PC. At the ultrastructural level, suprabasal keratinocytes in PC are characterized by densely aggregated keratin filament bundles predominantly in the perinuclear region, and sparing the cell periphery.[6] The dominant-negative mutation p.Leu132Pro, that was found in this family, is located in the 1A domain of the type I keratin 16 protein, which is a recognized mutation hotspot in keratin genes and has been reported previously.[14] FLG has been proposed to play a key role in epidermal barrier formation because it is involved in the transformation of keratinocytes into corneocytes during cornification. *FLG* mutation p.R2447X in repeat 7 of exon 3, which was detected in our patients, results in a lack of the precursor protein profilaggrin, the main constituent of the keratohyaline granules.[13] Keratins and FLG interact during terminal keratinocyte differentiation. FLG monomers, proteolytically cleaved from the precursor profilaggrin, facilitate keratin intermediate filament aggregation into macrofibrils. In addition, FLG associates with the cornified envelope, a layer of cytoplasmic proteins coating the inner cell membrane, during corneocyte maturation.[9,15] Either pathway could contribute to the more pronounced PC phenotype in individuals with abnormalities in both *FLG* and *KRT16*.

In conclusion, the observation that coinheritance of mutations in keratin and *FLG* aggravates the phenotype of PC, warrants studies in larger patient cohorts addressing whether *FLG* is a genetic modifier of this disease.

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