Ichthyosis, Follicular Atrophoderma, and Hypotrichosis Caused by Mutations in *ST14* Is Associated with Impaired Profilaggrin Processing

Thomas Alef[1,2], Serena Torres[1], Ingrid Hausser[3], Dieter Metze[4], Ümit Türsen[5], Gilles G. Lestringant[6] and Hans Christian Hennies[1,7]

Congenital ichthyosis encompasses a heterogeneous group of disorders of cornification. Isolated forms and syndromic ichthyosis can be differentiated. We have analyzed two consanguineous families from the United Arab Emirates and Turkey with an autosomal recessive syndrome of diffuse congenital ichthyosis, patchy follicular atrophoderma, generalized and diffuse nonscarring hypotrichosis, marked hypohidrosis, and woolly hair (OMIM 602400). By genome-wide analysis, we found a homozygous interval on chromosome 11q24–q25 and obtained a LOD score of 4.0 at D11S910. We identified a homozygous splice-site mutation in the Arab patients and a frame-shift deletion in the Turkish patient in the gene suppression of tumorigenicity-14 (*ST14*). The product of *ST14*, matriptase, is a type II transmembrane serine protease synthesized in most human epithelia. Two missense mutations in *ST14* were recently described in patients with a phenotype of ichthyosis and hypotrichosis, indicating diverse activities of matriptase in the epidermis and hair follicles. Here we have further demonstrated the loss of matriptase in differentiated patient keratinocytes, reduced proteolytic activation of prostasin, and disturbed processing of profilaggrin. As filaggrin monomers play a pivotal role in epidermal barrier formation, these findings reveal the link between congenital disorders of keratinization and filaggrin processing in the human skin.

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INTRODUCTION

The epidermal barrier function is crucial for protecting the organism against the environment and to preventing the body exposed to the air from dehydrating by excessive transepidermal water loss. The barrier function is mainly localized to the outer layers of the epidermis, in which the keratinocytes undergo terminal differentiation finally resulting in the formation of the cornified cell envelope and horny lamellae characteristic of the stratum corneum. Recent studies have demonstrated that disturbance of the epidermal barrier function is involved in various genetic cornification disorders, particularly autosomal recessive congenital ichthyoses (OMIM 242300, 601277, 604777, 606545). Autosomal recessive congenital ichthyosis, characterized by generalized scaling of the skin and erythema (Traupe, 1989), is clinically and genetically heterogeneous and can be caused by mutations in more than six different genes, including *TGM1*, *ALOX12B*, and *ALOXE3*. Transglutaminase 1, encoded by *TGM1*, is involved in the formation of the cornified cell envelope. The lipoxygenases encoded by *ALOX12B* and *ALOXE3* are components of the epidermal 12-lipoxygenase pathway, their biological role for the epidermal barrier formation, however, remains to be clarified. Mouse models revealed that both transglutaminase-1 and 12*R*-lipoxygenase deficiencies caused ichthyosiform skin and severe barrier dysfunction (Matsuki *et al.*, 1998; Kuramoto *et al.*, 2002; Epp *et al.*, 2007; Moran *et al.*, 2007). Congenital ichthyosis may also be associated with hair abnormalities, as in Netherton syndrome (OMIM 256500) caused by mutations in *SPINK5*, which codes for the (lymphoepithelial kazal-type-related) serine protease inhibitor LEKTI (Chavanas *et al.*, 2000). Mouse lines with a *Spink5* knockout presented a lethal ichthyosis phenotype and an impairment of barrier function demonstrating the important role of LEKTI as a regulator of protease activity in the epidermis (Descargues *et al.*, 2005; Hewett *et al.*, 2005).

Recently, two missense mutations in the suppression of tumorigenicity-14 (matriptase) gene (*ST14*) were identified in

[1]*Division of Dermatogenetics, Cologne Center for Genomics, University of Cologne, Cologne, Germany;* [2]*Institute of Genetics, University of Cologne, Cologne, Germany;* [3]*Department of Dermatology, University of Heidelberg, Heidelberg, Germany;* [4]*Department of Dermatology, University of Münster, Münster, Germany;* [5]*Department of Dermatology, University of Mersin, Mersin, Turkey;* [6]*Division of Dermatology, Tawam Hospital (ret.), Al Ain, United Arab Emirates and* [7]*Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany*

Correspondence: Dr Hans Christian Hennies, Division of Dermatogenetics, Cologne Center for Genomics, University of Cologne, Zülpicher Str. 47, 50674 Köln, Germany. E-mail: h.hennies@uni-koeln.de

Abbreviations: ARIH, autosomal recessive ichthyosis with hypotrichosis; IFAH, congenital ichthyosis, follicular atrophoderma, hypotrichosis and hypohidrosis; ST14, suppression of tumorigenicity-14

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two families with autosomal recessive ichthyosis with hypotrichosis (ARIH, OMIM 610765; Basel-Vanagaite *et al.*, 2007; Avrahami *et al.*, 2008). Matriptase is a member of the type II transmembrane serine proteases (List *et al.*, 2006a; Uhland, 2006). It is specifically synthesized in terminally differentiating keratinocytes and in matrix, precortex, and cortex cells and the shaft of the anagen hair. It is characterized by a complex life cycle, including autoactivation by proteolytic cleavage, shedding from the membrane, and regulation through the hepatocyte growth factor activator inhibitor-1. *St14* hypomorphic mice demonstrated low activity of matriptase and a phenotype resembling ARIH (List *et al.*, 2007a). Analysis of their epidermis showed reduced prostasin activation and profilaggrin processing.

Here we describe the analysis of two families with congenital ichthyosis, follicular atrophoderma, hypotrichosis, and hypohidrosis (IFAH, OMIM 602400) (Lestringant *et al.*, 1998; Tursen *et al.*, 2002). After a whole-genome linkage scan, we identified ablating mutations in *ST14* as the cause for the disease. The mutations resulted in an impairment of profilaggrin processing, demonstrating the link between congenital ichthyosis and loss of epidermal barrier function, characterized by the lack of filaggrin units.

RESULTS

Phenotype

The first family was a consanguineous family of Bedouin ancestry from the United Arab Emirates with five affected siblings (Figure 2a). The patients have been described earlier by one of us (Lestringant *et al.*, 1998), and a follow-up is given here with particular respect to the phenotype of ARIH published recently (Basel-Vanagaite *et al.*, 2007). The patients presented with congenital ichthyosis, follicular atrophoderma, hypotrichosis, and hypohidrosis. They ranged in age from 4 to 18 years at the time of first examination. Ichthyosis was present at birth, and there were no collodion babies. In all cases scaling was diffuse and involved the great flexures and scalp (Figure 1a). It spared face, elbows, knees, hands, and feet. Keratosis was always severe with possible episodes of transient variations: It might present either with large, light or dark brown adherent plate-like scales, up to 20 mm in diameter, occupying a shallow depression of the skin and of variable thickness, ranging from thin epidermal films to 3–4 mm thick keratotic ''plugs'', or with an intense powdery scaling, especially on the trunk and flexor aspects of the limbs. The scales were mostly distributed following a random linear pattern. In no case was there erythema or itching.

Follicular atrophoderma, a rare skin anomaly consisting of enlarged funnel-shaped depressions of the pilosebaceous orifices, was congenital and present in all patients (Figure 1b). These ''ice-pick'' marks appeared in patches on the dorsal aspects of hands and feet, first phalanges of fingers and toes, wrists, and around elbows and knees. In all patients, the facial skin was involved by ill-defined pitting giving a fine vermiculate or ''orange peel'' appearance. Around the wrists, elbows, and knees, there was a zone of ichthyosis progressively transformed into follicular atrophoderma.

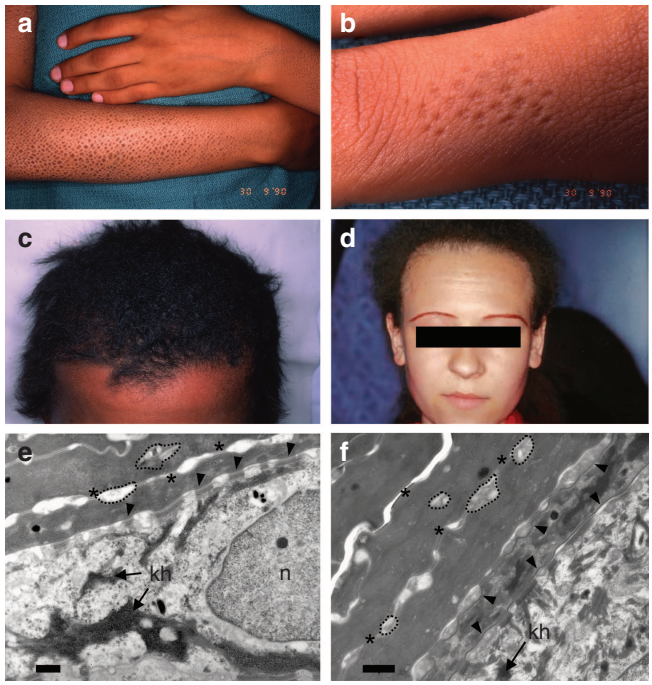

Figure 1. Clinical picture and ultrastructural analysis of patients with IFAH. (**a**–**c**) Patients from the Arab IFAH family. (**a**) Dark brown diffuse scaling on the right forearm and follicular atrophoderma on the wrist, hand, and fingers of IV:1 (Figure 2a). (**b**) Close-up of the phalanx dorsum showing follicular atrophoderma. (**c**) Unruly, curly scalp hair with the typical receding frontal hairline (IV:3). (**d**) The Turkish patient IV:1 (Figure 2b) showing light, woolly hair, hypotrichosis of the eyebrows, and the receding frontal hairline. (**e**–**f**) Ultrastructural analysis of the upper stratum granulosum and the lower stratum corneum. (**e**) Patient IV:1 from the Arab family, (**f**) patient IV:1 from the Turkish family. Dotted lines mark inter- and intralamellar deposits of lamellar body contents in the lower lamellae of the stratum corneum. Amounts of keratohyalin (kh) were reduced. Asterisks denote corneodesmosomes, arrow heads marginal bands of corneocytes (cornified envelopes); n, nucleus; scale bars = 1 μm.

Generalized, diffuse and nonscarring hypotrichosis was the third constant finding. It was present at birth, and boys, who presented with little head hair and next to no eyelashes and eyebrows, were more affected than girls. Hypotrichosis was improving with age. At the time of first examination, the younger siblings IV:3, IV:5, and IV:7 presented with sparse, unruly, and lusterless hair on the scalp, bald patches, and recessing frontal hair line (Figure 1c), whereas the two oldest siblings had nearly normal head hair with recessing frontal hairline only. Similarly eyebrows, at first wiry and limited to the very inner region, were progressively straightening and extending outward, and eyelashes, at first sparse and limited to the upper eyelids, were progressively growing on the lower ones. Facial and body hair were absent in IV:3, IV:5, and IV:7 but IV:1 had developed few hair on both ends of her upper lip and in her armpits at 18 years of age, and IV:2 at 22 years had sparse hair on his upper lip, with a tuft at both ends of it and on his whiskers. There were occasional vellus and crooked hair growing out of a pit of follicular atrophoderma.

Hypohidrosis was assessed clinically in IV:1, IV:2, and IV:3. Sweating could be seen only on the nose, eyebrows, palms, and soles even at 45 °C ambient temperature. There