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A hallmark of the majority of monogenic disorders of keratinization is a thickening of the stratum corneum or hyperkeratosis, which can be the result of an acceleration of epidermopoiesis, with an increased production of corneocytes (proliferation hyperkeratosis), or of an impaired desquamation process in which too few corneocytes are shed at the skin surface (retention hyperkeratosis) [1]. During keratinization, the formation of filaggrin and involucrin plays an important role. Filaggrin is a basic protein normally present in the stratum corneum of the epidermis. Profilaggrin, the precursor of filaggrin, is synthesized at the level of the granular layer, where it is stored in keratohyalin granules [2, 3]. After dephosphorylation and proteolysis by specific enzymes, profilaggrin is processed into filaggrin, during cornification of keratinocytes [4-7] (Fig. 1). Involverin is normally present in the upper third of the epidermis of healthy skin. The protein becomes incorporated in the cornified envelope under the plasma membrane. Because involucrin is not related to keratins, neither immunologically nor biochemically, it is a distinct marker for terminal differentiation [8-10].

Tenascin is an extracellular matrix glycoprotein. In vitro studies suggest that proliferating epithelium induces the production of tenascin by mesenchymal cells [11]. In normal human skin, tenascin expression is found diffusely in the subepidermal dermis adjacent to basement membranes, around the eccrine sweat glands, and blood vessels [12]. An increased expression of tenascin has been observed in the upper dermis in hyperproliferative skin diseases such as psoriasis, basal cell carcinoma, Bowen’s disease and actinic keratosis [13]. In the present investigation we compared the expression of filaggrin, involucrin and tenascin in several monogenic disorders of keratinization with that observed in normal skin.

Disorders of keratinization comprise a large spectrum of distinct disease entities. Filaggrin and involucrin are major components of the keratinization process. On the other hand, the extracellular matrix molecule, tenascin, is expressed predominantly in disorders characterized by epidermal proliferation such as epidermal neoplasms and psoriasis. In the present investigation, the expression of filaggrin, involucrin and tenascin was assessed in various keratinization disorders. Filaggrin proved to be specifically absent or minimally expressed in patients with autosomal dominant ichthyosis vulgaris. In disorders of keratinization with increased epidermal proliferation, the expression of filaggrin, involucrin and tenascin was markedly increased, whereas the normoproliferative disorders of keratinization displayed normal expression of these markers for keratinization and extracellular matrix. (Key words: filaggrin, involucrin, monogenic disorders of keratinization, tenascin.)

Material and methods

Patients and biopsies

Four mm punch biopsies were taken from 48 patients with disorders of keratinization and healthy volunteers. These were embedded in Tissue Tek OCT compound, snap frozen in liquid nitrogen and stored at –80°C, until use. The groups comprised 7 patients with autosomal dominant ichthyosis vulgaris (ADIV), 6 patients with X-linked ichthyosis vulgaris (XRI), 12 patients with autosomal recessive lamellar ichthyosis and 13 patients with other monogenic disorders of keratinization.

Figure 1. Filaggrin expression in non-erythrodermic autosomal recessive lamellar ichthyosis is markedly increased compared with normal skin.
Table 1. Filagrin expression in keratinization disorders, expressed as a ratio of positive cell layers/total cell layers at the tip of a normal papilla and two different papillae.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Filagrin expression</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human skin</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Autosomal dominant ichthyosis vulgaris</td>
<td>4 ± 2.15</td>
<td>1</td>
</tr>
<tr>
<td>Non-epidermolytic recessive ichthyosis vulgaris</td>
<td>4 ± 2.15</td>
<td>1</td>
</tr>
<tr>
<td>X-linked ichthyosis vulgaris</td>
<td>4 ± 2.15</td>
<td>1</td>
</tr>
<tr>
<td>Non-epidermolytic recessive ichthyosis vulgaris</td>
<td>4 ± 2.15</td>
<td>1</td>
</tr>
<tr>
<td>Epidermolytic recessive ichthyosis vulgaris</td>
<td>4 ± 2.15</td>
<td>1</td>
</tr>
</tbody>
</table>

Results

The results of keratinization are expressed as a ratio of positive cell layers/total cell layers at the tip of a normal papilla and two different papillae. In patients with Drier’s disease, and even more marked in patients with Drier’s disease and even more marked in patients with Drier’s disease, filagrin expression was noted to be significantly decreased. No expression of filagrin was seen in one patient, while in the other patient, filagrin expression was restricted to one cell layer, and in the fourth patient, a sporadic expression was seen (Table 1).
Table II. The involucrin expression in keratinization disorders, expressed as mean ± standard deviation of the mean and the p-value compared with normal healthy skin. Expression was assessed by calculating the ratio of positive cell layers/total cell layers (%) of the living epidermis. This was performed at two sites: at the tip of a dermal papilla and between two dermal papillae (%)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Involucrin, tip dermal papilla (mean ± SD)</th>
<th>Involucrin, inter-papillary (mean ± SD)</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human skin</td>
<td>19 ± 1.0</td>
<td>9 ± 0.42</td>
<td>4</td>
</tr>
<tr>
<td>Autosomal dominant ichthyosis vulgaris</td>
<td>21 ± 3.7 (NS)</td>
<td>12.4 ± 1.9 (NS)</td>
<td>7</td>
</tr>
<tr>
<td>X-linked ichthyosis</td>
<td>18 ± 2.3 (NS)</td>
<td>11 ± 1.3 (NS)</td>
<td>6</td>
</tr>
<tr>
<td>Non-erythodermal autosomal recessive lamellar ichthyosis</td>
<td>14 ± 0.6 (p = 0.02)</td>
<td>8 ± 0.4 (NS)</td>
<td>6</td>
</tr>
<tr>
<td>Erythodermal autosomal recessive lamellar ichthyosis</td>
<td>44 ± 0 (p = 0.03, n = 3)</td>
<td>27 ± 0.9 (p = 0.03, n = 3)</td>
<td>4</td>
</tr>
<tr>
<td>Erythodermal ichthyosiformis congenita bulbosa (Brocq)</td>
<td>44 ± 3.8 (p = 0.02)</td>
<td>28 ± 1.9 (p = 0.02)</td>
<td>4</td>
</tr>
<tr>
<td>Ichthyosis bullosa of Siemens</td>
<td>50 ± 17.6</td>
<td>29 ± 10.5 (NS)</td>
<td>2</td>
</tr>
<tr>
<td>Collodion baby</td>
<td>29 ± 13.1</td>
<td>18 ± 7.5 (NS)</td>
<td>2</td>
</tr>
<tr>
<td>Epidermolytic naevus</td>
<td>69 ± 6.1 (p = 0.06)</td>
<td>67 ± 5.6 (p = 0.06)</td>
<td>4</td>
</tr>
<tr>
<td>Darrier’s disease</td>
<td>31 ± 6.8 (NS)</td>
<td>15 ± 4.5 (NS)</td>
<td>3</td>
</tr>
</tbody>
</table>

Involucrin expression in non-erythodermal autosomal recessive lamellar ichthyosis was decreased compared with healthy skin.

In contrast, in erythodermal autosomal lamellar ichthyosis, the number of involucrin positive cell layers was increased (Fig. 3). Involucrin staining in 3 out of 4 patients with erythodermal autosomal recessive ichthyosis showed a pronounced and significant increase compared with normal human skin (p = 0.03). The two patients with epidermolytic naevus and two patients with Darrier’s disease showed an increased involucrin expression. In one of the patients with Darrier’s disease we observed normal involucrin expression. In patients with bullous ichthyosis the increase was even more pronounced.

Tenascin expression was observed, not only as a discontinuous staining pattern adjacent to the interfollicular epidermis but also around capillaries, eccrine sweat glands and hair follicles. Although the expression in ADIV, X-linked ichthyosis and of the collodion babies was virtually normal, patients with lamellar ichthyosis, bullous ichthyosis, Darrier’s disease and epidermolytic naevi showed an increased and continuous staining pattern with the antitenascin antibody. In the 3 cases of non-erythodermal autosomal recessive lamellar ichthyosis, we observed diffuse tenascin staining, however 2 out of these 3 biopsies showed a continuous staining pattern adjacent to the basal lamina. Another biopsy in this group showed an increased but discontinuous tenascin expression (Fig. 4).

**Discussion**

In the present study, the expression of filaggrin, involucrin and tenascin was studied in disorders of keratinization and

![Image of involucrin expression in erythodermal autosomal recessive lamellar ichthyosis.](image-url)
the results were compared with the expression of these antigens in normal human skin.

In normal skin, filaggrin was observed in the stratum corneum and stratum granulosum with 8% expression in the epidermal layers at the tip of dermal papillae and 16% expression in the interpapillary epidermis (Table I). Although Kanitakis et al. [15] reported a decreased filaggrin expression in X-linked ichthyosis compared with healthy skin, in the present study such a decrease could not be confirmed. It must be kept in mind that the study of Kanitakis et al. [15] was performed on paraffin-embedded sections, while we used frozen sections. The markedly decreased expression of filaggrin in autosomal dominant ichthyosis vulgaris is in agreement with the finding that in ADIV, the granular layer is thin or absent and is in keeping with the observations of Kanitakis et al. [15].

Non-erythrodermic autosomal recessive lamellar ichthyosis, the erythrodermic autosomal recessive lamellar ichthyosis, the bullous congenital ichthyosiform erythroderma of Brocq, and Darier’s disease are characterized by hypergranulosis. Kanitakis et al. [15] observed a similar increase of filaggrin expression in erythrodermic autosomal recessive lamellar ichthyosis and Darier’s disease. However, they did not investigate filaggrin expression in non-erythrodermic autosomal recessive lamellar ichthyosis and bullous ichthyotic erythroderma of Brocq.

The biopsies of the patients with ichthyosis bullosa of Siemens, collodion baby and erythrokeratodermia variabilis showed a markedly increased staining of filaggrin; however the small number of patients does not permit statistical analysis. In normal human skin, involucrin expression has been observed around the inner root sheath, the infundibulum, the sebaceous duct, the acrosyringium, in the granular layer and in the upper cell layers of the stratum spinosum [16, 17]. These observations are confirmed by our investigations. Involutcin expression in normal human skin appeared to be 9% at the tip of a dermal papilla and 19% interpapillarily. The percentage staining of anti-filaggrin and anti-involucrin seemed to be quite similar. However, filaggrin expression was observed in the stratum granulosum and corneum, while involucrin expression was observed in the stratum granulosum and a part of the stratum spinosum. It must be kept in mind that in our study, a quantitative evaluation method was used, while in most of the other investigations a descriptive method was used.

Expression of involucrin in ADIV and X-linked ichthyosis was comparable with the expression in normal skin and in line with the observations of Kanitakis et al. [17]. Involutcin expression in the cases of collodion babies was variable. This variability is possibly due to the fact that collodion baby is probably heterogeneous. The underlying disease may be non-erythrodermic autosomal recessive lamellar ichthyosis, erythrodermic autosomal recessive lamellar ichthyosis or autosomal dominant ichthyosis vulgaris. Moreover, 10% of collodion babies do not develop ichthyosis in later life. From the present and earlier observations it seems apparent that its expression correlates with the degree of epidermal turnover; [18]. In the hyperproliferative keratinization disorders (lamellar ichthyosis, ichthyosis bullosa of the Brocq and Siemens type, collodion baby, epidermolytic naevus, Darier’s disease, psoriasis), involucrin expression is increased. Recruitment of cycling epidermal cells is activated by protein kinase C. The activation of protein kinase C results in increased TGFα expression, IL-8 production, and involucrin expression of keratinocytes [19]. TGFα induces keratinocyte proliferation. The association between hyperproliferation of the epidermis and increased involucrin expression is intriguing.

In normal human skin, expression of tenascin can be observed around hair follicles, eccrine sweat glands, capillaries and, more sparsely, in the upper dermis adjacent to the basal membrane [12, 13]. In the present study, the tenascin expression in normal skin was comparable with earlier observations [11-13]. Recently, an increased expression of tenascin has been reported in other hyperproliferative skin diseases as psoriasis, basal cell carcinoma, Bowen’s disease and keratosis solars [13]. The non-erythrodermic autosomal recessive lamellar ichthyosis (NEARLI) showed a variable tenascin expression. An explanation for this variability might be the variable degree of concomitant dermal inflammation in our NEARLI group. Schalkwijk et al. [20] noticed in 6 out of 7 patients with NEARLI, absent or sparse staining. The present findings suggest a relationship between epidermal proliferation, inflammation and tenascin expression. An increased epidermal turnover is accompanied by a high tenascin expression adjacent to the basal membrane in the upper dermis. The source of tenascin is not clear. In vitro experiments suggest that proliferating epithelium induce the production of tenascin by mesenchymal cells [11]. In an investigation on the wound healing process in rat epithelium, an increased tenascin expression under the migrating proliferating epidermis was found [11]. It is attractive to hypothesize that the expression of tenascin by activated epithelium is of significance for the maintenance of the hyperproliferative state.

The present investigation suggests that filaggrin staining might be of help in the diagnosis of autosomal dominant ichthyosis vulgaris. A markedly decreased filaggrin expression proved to be a selective change in this disorder of keratinization. Involucrin and tenascin expression were not involved specifically in one single disease but were rather compatible with the degree of epidermal proliferation. Therefore, the present study suggests that increased epidermal proliferation, and increased expression of tenascin and involucrin are associated processes in so-called hyperproliferative ichthyotic conditions.
References