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Ultrastructural Identification of Basic Abnormalities as Clues to Genetic Disorders of the Epidermis

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The present article discusses specific, directly gene-dependent ultrastructural markers of dominantly inherited epidermal disorders that serve as clues to their underlying molecular genetic abnormalities. These are epidermolysis bullosa simplex Koebner and Weber-Cockayne with rupture or non-assembly of basal cell keratins and point mutations in keratins 5 and 14. Clumping of basal cell keratins is pathognomonic of EB Dowling-Meara and caused by mutations in hot spots of the rod domain of K 5 and K 14. Clumps and aggregates of basal keratins occur side by side in the same cell and thus do not indicate specific different types of mutations. Similar clumping of suprabasal keratins in bullous CIE Brocq and in palmoplantar keratoderma Voerner have been assigned to identical types of mutations in the same critical position of the rod domain in K 1, K 10, and K 9, respectively.

Highly unusual tubular keratins are pathognomonic of another dominant palmoplantar keratoderma type the genetic basis of which still awaits elucidation. Shell formation of (low molecular weight?) keratins in ichthyosis hystrix Curth-Macklin is not linked to the keratin gene clusters on chromosomes 12 and 17 and might be related to regulatory genes of keratin expression. Suprabasal shells in congenital reticular ichthyosiform erythroderma do not consist of keratins but resemble glycoprotein networks. Finally, the keratohyalin abnormality in ichthyosis vulgaris was the clue for the identification of a filaggrin deficiency, at the same time giving evidence to the heterogeneity of keratohyalin proteins. Key words: keratin filament abnormalities/keratin gene mutations/EB simplex group/dominant types of keratinization disorders. J Invest Dermatol 103:6S-12S, 1994

Dermatology has been a morphologically oriented specialty in human medicine from the very beginning. With most skin changes directly visible to the eyes, morphologic observation, investigation, and nosologic distinction have always been important tools in dermatology, in contrast to many other medical disciplines where more indirect methods have to be applied and tissue samples are not so easily available. Therefore, with the introduction of electron microscopy (EM) to the biomedical sciences in the late 1950s, ultrastructural investigations of normal and diseased skin were enthusiastically taken up in dermatology. Until the end of the 1970s, most of the common dermatologic conditions had been characterized by their EM features.

Other than these, genetic skin diseases are still continuing to be a source of surprise and of possibilities to encounter with unexpected or yet unknown pathogenetic pathways or basic abnormalities. From our systematic ultrastructural investigations performed in Heidelberg on large series of patients suffering from genodermatoses such as ichthyoses (inherited ichthyoses) or mecanobullous disorders (epidermolysis bullosa) it became evident that heterogeneity is much larger than expected from the clinical features and light microscopic histopathology alone. Based on their peculiar and often highly specific ultrastructural features various types of genodermatoses could be delineated as distinct nosologic entities [1-5].

It is the aim of this article to show that such peculiar ultrastructural features may serve as a clue to the elucidation of the underlying molecular genetic event.

IDENTIFICATION OF PRIMARY, DIRECTLY GENE-DEPENDE ULTRASTRUCTURAL MARKERS

When tissue samples of a disease entity are investigated by EM many of the morphologic features, though perhaps interesting morphologically and from the point of view of skin biology, are unspecified and/or of secondary nature. This is often the case with blistering disorders, such as epidermolysis bullosa, where the initial separation in the specific plane above, within, or underneath the dermo-epidermal junction is rapidly followed by secondary changes that start before the blister becomes clinically visible: translocation of cell and tissue elements, remodeling processes, inflammatory reactions with invasion of inflammatory cells to the blister cavity or to the epidermis, and wound repair mechanisms [6].

From the genetic point of view it is crucial to identify those abnormalities that are directly related to the molecular genetic event, which means that they represent ultrastructural markers, ie structural defects of primary gene products, and to distinguish these from more indirectly-caused secondary changes [7]. For example, for the many genetic types of epidermolysis bullosa these ultrastructural markers are not represented by the plane of separation, a feature that is group-specific but not genotype-specific. Instead, they concern structural defects of cell constituents or products, the abnormalities of which preceed blister formation and must be investigated in prelesional non-blistered skin. In junctional EB, the ultrastructural marker is a more or less pronounced hypoplasia of hemidesmosomes, with the most constant feature of the lack of their subbasal dense plates in the lamina rara [6]; recent molecular genetic studies have related this marker to akinin and k-laminin.
In very rare cases of EBS Koebner (in our Heidelberg material three of 44) basal cells are entirely devoid of keratin filaments. The basal cell layer therefore stands against the completely normal suprabasal cells as an exceptionally clear layer (see Fig 22.21 in [6]). As soon as the cells enter into terminal differentiation and keratinization, normal keratin filaments appear in the cytoplasm of the first suprabasal layer, corresponding in diameter, contrast, and intracellular arrangement to those of healthy skin (Fig 1). Basal cells of these three Koebner cases express actin filaments and develop normal desmosomes and hemidesmosomes. In one of these three cases, desmosome-associated keratin filaments were quite normal as well, although the remainder of the cytoplasm was free of keratins. This indicates that a special subset of keratins seems to be associated with desmosomes and hemidesmosomes, different from the normal basal cell keratin pair K 5 and 14. Cases like these three Koebner-type patients seem to be due to keratin gene mutations of either keratin 5 or 14 that prevent filament assembly entirely. As a consequence, the smallest mechanical stress results in cytolysis and blister formation in these defective basal cells that are devoid of their normal protective cytoskeleton. Two of the three cases were newborn babies that showed an exceptionally severe, generalized blistering after birth.

In most other Koebner (and Weber-Cockayne) cases the type(s) of mutation must be different, as they do not prevent filament assembly but seem to result in unstable keratin filaments that undergo breakdown rapidly. Many Koebner cases have a pronounced temperature threshold indicating that thermal instability of preformed keratins has to be considered also.

In our material there was no principal difference in the site of initial blister formation in the subnuclear cytoplasm of basal cells in either type of EBS, Koebner, and Weber-Cockayne, with a total of 60 cases [6]. Pearson [24] stressed that blisters in the Weber-Cockayne type may be suprabasal in contrast to Koebner type basal blistering. As to our experience, in ridged skin, blisters become clinically evident at a later time of their development because of the thickness of the overlying horny layer and therefore later stages are most often biopsied as compared to the Koebner type. It can, however, not be excluded that two different sub-types of EBS of palms and soles (Weber-Cockayne type) might exist, with one type concerning basal cell keratins 5 or 14, and another type concerning suprabasal keratins such as keratin 9, the ridged skin-type keratin [25–28], and therefore appearing at a higher epidermal level. The kind of mutations, regardless of the keratin genes involved, should be comparable to each other, and different from the mutations that lead to disorders like EB herpetiformis Dowling-Meara, bullous ichthyosiform erythroderma (epidermolytic hyperkeratosis), or Voerner type palmoplantar keratoderma (see below), characterized by clumping of keratins [29,30].

Mutations in the genes coding for keratins 5 and/or 14 that induce blistering disorders such as the Koebner type have been assigned to regions in the central parts of the rod domain. In contrast, mutations inducing keratin clumping are obviously located in so-called hot spots within the highly conserved N- or C-terminal end portions of the rod domain [30–33]. A missense mutation in the K 14 rod domain was found in a family with a recessive subtype of EBS Weber-Cockayne [34]. However, one large Koebner sibship showed linkage to the long arm of chromosome 1 [35].

Epidermolysis Bullosa Herpetiformis Dowling-Meara
This dominant EB type is much more severe than all other types of the EB simplex group. Blisters become rapidly hemorrhagic, show peripheral spreading and rapid central healing (in a characteristic herpetiform pattern that is most pronounced in children of about 2–3 years of age), induce postbullous pigmentation and leave scars and milia in sites of more severe inflammatory reaction. Further, pronounced nail dystrophies (often onychogryphotic) and palmoplantar hyperkeratosis ("tylosis") complicate the clinical image, as does mucous membrane involvement [3,21]. Before being classified by means of their highly specific ultrastructural features [3,6], such patients have therefore been misclassified, mostly as dystrophic EB, until the end of the 1970s.
Figure 1. EBS Koebner. Lack of keratins in basal cells, normal keratin synthesis in suprabasal cells. Desmosomes and hemidesmosomes normal. M, melanocyte; N, nuclei; arrows, actin filament bundles: open arrow, dermo-epidermal junction. Bar, 5 μ.

Figure 2. EBH Dowling-Meara. Clumps and aggregates of keratins occur side by side in the same basal cells. Clumps show great variation in density and substructure and partly are associated with less aggregated keratins (arrow). Both, clumps and aggregates are also retained during mitosis (note metaphases M1 and M2 with chromosomes C). N, nuclei. Bar, 5 μ.

Figure 3. PPK with tubular keratins (tonotubules). Densely aggregated tubular keratins in longitudinal and cross sections from the mid-epidermis of palmar skin. Cross sections show the origin from filaments by rolling up in a supercoil fashion (arrows). Bar, 0.5 μ.

Figure 4. Ichthyosis hystrix Curth-Macklin. Keratin filaments in two perinuclear shells. Note higher density and contrast of the marginal keratin filaments. D, desmosome. Bar, 0.5 μ.

Figure 5. Congenital reticular ichthyosiform erythroderma. Perinuclear shells consist of a network of fine interlacing filaments distinctly different from keratin filaments but reminiscent of glycoprotein networks. N, part of nucleus, G, glycogen. Bar, 0.5 μ.
Clinically being so different from classical EBS cases, it seems confusing to use the term EBS only because of the intraepidermal plane of blistering. EB simplex is a clinical description that comprises blisters healing without scar formation and other sequelae, lack of nail dystrophies, and lack of milia. All this is not true for the Dowling-Meara type. EB herpetiformis characterizes one of the most distinctive clinical features of this unusual intraepidermal type of EB, and therefore should be helpful for the clinician, to identify such cases.

The Dowling-Meara type is not only clinically unusual, its pathomorphogenesis shows a quite peculiar and distinctive abnormality separating it from all other types of EB. Blister formation is preceded by a specific aggregation of basal cell keratins: affected basal cells show round clumps of fine filaments or even of a granular central texture with sharp margins, as well as all types of tight to more loose aggregations of keratins, often in form of curled strands [3,6]. This special pattern was first identified by us in Heidelberg from a large number of patients from Germany and Norway and has since been confirmed by others [36,37]. Kitajima et al [38] recently claimed that clumps and aggregated keratins, respectively observed in two unrelated cases, should indicate two distinctly different keratin gene mutations. In most of our Heidelberg Dowling-Meara cases (n = 76), clumps and aggregates occurred side by side in one and the same cell (Fig. 2, see also Figs. 22,23–25 in [6]) clearly indicating that both aggregates and true clumps occur simultaneously in consequence of one and the same mutation.

Once formed, these keratin clumps and aggregates are not broken down when the cells differentiate (either in blister roofs or in nonblistered perilesional skin) but are preserved in the suprabasal cells, by the way indicating the persistence of low-molecular weight basal keratins (5 and 14) in the suprabasal layers during further differentiation [25,39]. Downregulation of their synthesis must not necessarily mean break-down of the preformed proteins even if they are no longer recognized by most of their antibodies under normal conditions. In contrast, in the outermost margin of the spreading lesion, clumping of keratins is exclusively located in basal cells whereas the suprabasal cells are normal, indicating that basal cell keratins are affected by the respective individual gene mutation. Keratinocytes in culture were shown to similarly express the specific clumping of keratins [40].

Molecular genetic studies have identified various point mutations in the highly conserved N- and C-terminal segments of the rod domain of both K 5 and K 14 in patients with the Dowling-Meara type [29,30,32,41–43]. Experimentally, a similar exchange of amino acids in the same critical position produced a Dowling-Meara-like phenotype in transgenic mice and induced keratin clumping in cultured human keratinocytes [42].

In spite of this exciting and convincing molecular genetic evidence, some basic problems of the mode of action of these mutant genes remain unsolved: clumping of basal cell keratins of the Dowling-Meara type is not a persistent feature: samples of clinically unaffected skin distant from developing or spreading lesions or in phases of remission have normal basal cells with normal keratin filaments and do not express the specific clumping. Therefore, some additional factor(s) is (are) required to express the mutant gene condition in prelesional and lesional skin. Temperature, mechanical stress, or some diffusible factors related to inflammation (peripheral spreading of the lesions) could be such co-factors of expression. However, some of the Dowling-Meara patients clear up during fever [21], and blisters often can not be provoked by friction or mechanical stress in clinically normal skin. Furthermore, the Dowling-Meara type characteristically improves, often dramatically, with age, and clumping of keratins as well as blister formation itself are no longer demonstrable in most of the adults that were more or less severely affected as small children. Therefore, larger series of patients and their specific individual mutations have to be compared to find out why such mutations do not always, at any time and location, induce their profound disturbance of the basal cell keratin network. Some such co-factors might also be studied in keratinocyte cultures.

### Table I. Comparison of EB Herpetiformis Dowling-Meara (EBH-DM) and Bullous Congenital Ichthyosiform Erythroderma Brocq (Bullous CIE, Epidermolytic Hyperkeratosis)

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<th>EBH Dowling-Meara</th>
<th>Bullous CIE Brocq</th>
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<td>Keratin clumping</td>
<td>Basement</td>
<td>Suprabasal</td>
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<td>Keratins</td>
<td>5 and 14</td>
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<td>Types of mutations</td>
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<td>Clinical consequences</td>
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<td>congenital ichthyosis</td>
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<td>Blisters</td>
<td>Intrabasal</td>
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<td>Duration of blisters</td>
<td>Years or throughout</td>
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<td>lifetime</td>
<td>after birth,</td>
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<td>persisting</td>
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<td></td>
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<td>Perfectly normal</td>
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<td>Hyperkeratosis</td>
<td>Palms and soles only</td>
<td>Mild or lacking</td>
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<td>Nails</td>
<td>Dystrophic</td>
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<tr>
<td>Inflammatory reaction</td>
<td>Strong</td>
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<tr>
<td>Mucous membranes</td>
<td>Affected</td>
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**Bullous Congenital Ichthyosiform Erythroderma (CIE) Brocq (Epidermolytic Hyperkeratosis)** Also, in this dominant genodermatosis, the basic abnormality is represented by clumping of keratins restricted, however, to suprabasal instead of basal cell layers. The consequences lead to EB on one hand and to a congenital type of ichthyosis on the other hand. Blisters with shedding of the entire epidermis persist over years in the Dowling-Meara type but are perinatally expressed only and superficial in Brocq's ichthyosis. Cytolysis and blistering predominate in fetal and perinatal skin in bullous CIE, to be replaced gradually by developing hyperkeratosis. For details see Table I.

Ultrastructurally the basal cells are perfectly normal in bullous CIE in fetal, perinatal, and postnatal skin. As soon as the cells enter terminal differentiation, suprabasal cells form clumped keratin with sharp margins and finely filamentous textures as in the Dowling-Meara type and, during further terminal keratinization, increasing amounts of looser aggregates and irregular conglomerates. Histopathology textbooks stress coarse and abnormal keratohyalin granules, but keratohyalin is ultrastructurally normal. Its deposition in the granular cells is only secondarily disturbed because its spreading is not possible as in normal cells. Ultrastructurally it is evident that the basic abnormalities again concern the keratin filament system and not the keratohyalin proteins. Increased amounts of flaggrin in cases of bullous CIE were assumed to be responsible for the normal keratin aggregation [44]. By immunoelectron microscopy, however, clumped keratins are not specifically labeled by an anti-flaggrin antibody, neither in the Dowling-Meara type nor in Brocq's bullous CIE, clumps appearing much earlier in both cases than the first signs of profilaggrin synthesis and keratohyalin deposition [45]. This clearly indicates that profilaggrin/flaggrin is not contributing to the clumping of keratins in these disorders. The demonstration of keratin gene mutations has eventually confirmed the early conclusions from the ultrastructural abnormality of the keratin filament system as a primary cause of both dominant disorders [25,29,32,46]. Most mutations identified so far in bullous CIE occur in mutational hot spots in the highly conserved terminal segments of the rod domain in keratin 10 and 1 [46–52].

Clinically, the expression of bullous CIE and its severity are highly variable from family to family, indicating that more types of
mutations, perhaps also concerning keratins other than 1 and 10, may be expected.

The underlying mutations belong to the most frequent among genodermatoses and do not only occur in the germ line but in somatic cells as well. Many of the bullous CIE patients are solitary representatives, representing dominant new mutations, and nevi of the epidermolytic verrucous nevus type from small and circumscribed to more or less widespread, uni- or bilateral verrucous lesions occur. Even focal isolated clones with the specific keratin clumping, clinically too small to become visible, may be detected by chance in histopathologic or even ultrastructural sections.

**Ichthyosis Bullosa Siemens** Similarly inherited autosomal-dominantly, this type of ichthyosis [53] is extremely rare. Traupe et al. [54] stressed lack of erythroderma and superficial erosions as the major difference to Brocq's bullous CIE. As to our personal experience with now 41 cases of Brocq's bullous CIE and two other cases of ichthyosis bullosa Siemens the major difference is the late occurrence of keratin clumping in the superficial Malpighian layers, often only in the granular cells. Not only the basal, but also suprabasal cells and their keratin networks are normal in ichthyosis bullosa Siemens, and the amount and degree of clumping in the granular cells is much less than in comparable layers of bullous CIE skin samples. Spreading of keratohyalin granules also is not disturbed (secondarily) to the same degree, and the overlying cornified layers are less dyskeratotic. Moreover, there may be areas in the superficial epidermis that do not express clumping and seem to be more or less undisturbed.

It is obvious that keratins such as K 1 and K 10 are not very likely to be candidates for the causal mutant event. Keratin 9 may be late appearing with a patchy distribution in the uppermost living epidermis, apart from its major site of expression in palms and soles [27]. Keratins like this one, or other only late expressed keratins, should be regarded candidates for carrying the mutation(s) of ichthyosis bullosa Siemens.

**Palmoplantar Keratoderma Voerner Type** Only two dominant types of PPK are characterized by morphologically distinct abnormalities that do not only allow a clear diagnosis but also served (or may still serve) as clues for the elucidation for the underlying molecular genetic defect.

The Voerner type (epidermolytic PPK) with diffuse or irregular hyperkeratoses and an inflammatory borderline to the unaffected non-ridged skin is clinically very similar to the Unna type. Both are dominant disorders. In contrast to merely quantitative changes in the Unna type, the Voerner type is characterized by the same suprabasal clumping of keratins as found in bullous CIE, but the amount of keratin filaments, corresponding to this location, is higher, and bicomponent keratohyalin is found in the upper granular layer as in normal ridged skin [6].

The Voerner type has been shown to map to 17q12-q21 by linkage analysis, close to the gene cluster of the type I keratins [55,56]. Recently the first mutations could be localized in the gene for keratin 9 [57,58] on chromosome 17, specific for ridged skin [27,28] and therefore the first candidate gene for the Voerner type. The similarity of the kind of mutation to those found in bullous CIE and EBH Dowling-Meara parallel the ultrastructural similarities of the aberrant gene products, the clumped keratins.

**Palmoplantar Keratoderma With Tonotubules** This most exciting dominant type of PPK has been identified only three times as yet and seems to represent a rare and unique mutation. Clinically resembling the Unna and the Voerner type with diffuse palmoplantar hyperkeratoses and a sharply delineated livid-red margin, it is ultrastructurally highly specific. The first case was reported by us in 1985 (Anton-Lamprecht I, Werner I, SCUR Florence), the second 1 year later (Mahrle G, Küchmeister B, SCUR Paris). Meanwhile, a third case was analyzed in Heidelberg (Anton-Lamprecht I, Kalst I, unpublished), all three from dominant pedigrees and with identical ultrastructural peculiarities [6,59].

Basal cells are normal. Highly unusual tubular keratins are synthesized from the first suprabasal layer. They originate as isolated groups of tonotubules in hexagonal array and increase steadily by the rolling up of newly formed keratin filaments in a supercoil fashion, with fountain-like arrangement and formation of large clumps of tonotubules in the later stages (Fig. 3). Keratohyalin granules, bicomponent in the upper granular layer as typical for ridged skin, are deposited mainly between the dense tubular aggregates and only rarely within them. Keratohyalin changes are unspecific and secondarily induced by the tubular keratin abnormality.

Samples of clinically normal non-ridged skin (upper arm) of our first patient revealed normal epidermal ultrastructure and differentiation, corresponding to the exclusively palmoplantar expression of the keratinization abnormality. The suprabasal keratins 1 and 10 therefore are unlikely to carry the mutation. Instead, keratin 9, the ridged-skin keratin, is the first candidate to search for the mutation. The underlying keratin gene mutation must be quite different from those identified in the Voerner type, in bullous CIE and EBH Dowling-Meara. It seems to be unique among the mutations of the commonly known keratinization disorders, as the ultrastructural expression of tonotubules is unique and has not been observed in this form in any other inborn error of keratinization, with the exception of Richner-Hanhart's syndrome where low numbers of hollow tubules were formed at the borderline of densely aggregating (but not clumping) keratins in prickle and granular cells of ridged skin samples [60].

**Ichthyosis Hystrix Curth-Macklin** This severe, generalized, hystrix-like ichthyosis, described for the first time in two brothers in 1954 [61], is a very rare dominant mutation, as compared to bullous CIE. Because of some histopathologic similarities, it was thought to be a variant of Brocq's ichthyosis that lacks tendency to blister formation. It was only by the EM investigation of Curth's original family including one of the affected males, his affected daughter and his (unaffected) mother that the peculiar keratinization abnormality became evident, which is distinct different from that of bullous CIE [1,6,62]. About the same time an isolated case with the same specific abnormality was described [63], further cases were identified in Finland [64]. Our Heidelberg material now comprises a total of six cases, including one more localized variant with the same abnormality in the affected skin area.

The basic abnormality is again one of the keratin filament system [1,6,62-64]. Basal cells are normal. From the first suprabasal layer keratin filaments aggregate into unbroken perinuclear shells with sharply delineated margins to the inner perinuclear cytoplast with mitochondria, rough endoplasmic reticulum, and ribosomes, and to the peripheral cytoplast where additional keratins are gradually joining the shells and keep contact with desmosomes (Fig 4). Keratohyalin granules are deposited to the keratin filament shells mainly from the inner cytoplast. Up to 30% of all suprabasal cells are binuclear with one shell enclosing both nuclei, indicating a disturbed cell separation after mitosis [1,6,62]. Patients with ichthyosis hystrix Curth-Macklin respond excellently to oral retinoic acid therapy.

The underlying abnormality with shells and binuclear cells remains unchanged [6,62,64]. Application of anti-keratin antibodies revealed expression of fetal keratins in the basal cells and persistence of basal cell keratins in the suprabasal layer [65]; this is in good accordance to the low overall amount of keratins in the shells and to the persistence of the shells throughout keratinization with only lateral addition of larger keratin filaments at the outer margin of the shells, indicating that the shells probably consist mainly of lower molecular weight keratins.

Linkage analysis of the large sibship of Curth and Macklin [66] did not disclose convincing evidence of close linkage to any of the genetic markers available at that time. Recently, linkage analysis with polymorphic loci of chromosomes 12 and 17 excluded genes of the keratin type II and type I gene clusters as the underlying genetic defect of the disease [67]. Thus, this unique mutation has still to be identified. It might concern genes regulating the differen-
Congenital Reticular Ichthyosiform Erythroderma Only three patients with this rare keratinization disorder have been identified so far [4,68,69]. All three are isolated cases, thus, the mode of inheritance remains open. Our patient [4] is the only affected one in a large sibship of ten, rendering a dominant mutation more likely.

Also in this type of keratinization disorder, perinuclear shells and a high proportion of binuclear cells, indicating a disturbance of cell separation after mitosis as in the Curth-Macklin type, are specific features. However, unlike the latter, the shells do not consist of keratin filaments, but of a three-dimensional network of fine interlacing filaments (Fig 5) of unknown biochemical composition [4,6]. An attempt to identify its nature with antikeratin antibodies did not disclose specific abnormalities [62]. Ultrastructurally this network is reminiscent of glycoprotein networks. Normal appearing keratin filaments are laterally added to this network at the outer margin of the shells, and deposition of keratohyalin granules in swollen granular cells is similar to that in the Curth-Macklin type. The defective cells are rich in glycogen, and amyloid deposits are found in the papillary dermis of involved areas [4,6,62].

Clinically the disorder is not hystrix-like [4]. It obviously starts with an erythrodermic ichthyosiform scaling [68] and in the second decennium gradually changes into the reticular skin pattern with islands of normal skin enclosed within the confluent keratotic areas [69]. The underlying mutation has still to be identified. A related, possibly allelic mutation might be responsible for the keratinization disturbance in another solitary case where low amounts of analogous filamentous networks are formed [5].

DISORDERS OF KERATOHYALIN PROTEINS

Autosomal Dominant Ichthyosis Vulgaris Autosomal dominat ichthyosis vulgaris (ADJ) is the only keratinization disorder with a primary, directly gene-dependent abnormality of keratohyalin [6,70]. In contrast to many other conditions such as psoriasis, dermatitis, erythrodermic exfoliations, or wound healing, where keratohyalin synthesis and ultrastructure are disturbed secondarily in the context of suppressed keratinization, ADJ shows a quite stable and pathognomonic ultrastructure. In contrast to light microscopic claims, a granular layer is not entirely missing in non-ridged skin by EM, tiny keratohyalin granules are demonstrable in the course of terminal differentiation with a specific crumbliness appearance, indicating that a major component part of keratohyalin is missing in ADJ [6,70].

Profilaggrin, as the major histidine-rich protein of keratohyalin granules, was the first candidate for the underlying molecular biochemical abnormality. The gene has been mapped to 1q21 [71]. Stimulated by a suggestion by us to study filaggrin expression in AD1, Sybert et al [72] subsequently confirmed lack of filaggrin biochemically.

In ridged skin of ADI patients keratohyalin granules are clearly present, consisting of one component only, in contrast to normal ridged skin with bicomponent keratohyalin granules in the upper granular layer. It is the late-appearing component, that is synthesized in ADI ridged skin [73].

In spite of the presence of keratohyalin in both locations, anti-filaggrin does not bind to these keratohyalin granules by immunoelectron microscopy in non-ridged and ridged ADI skin [45,73], confirming the lack of profilaggrin and filaggrin in ADI. In the bicomponent keratohyalin granules of normal ridged skin only the earlier appearing major component is labeled specifically by anti-filaggrin [73], giving further evidence of the heterogeneity of keratohyalin proteins. Another important consequence is that filaggrin is not an indispensable prerequisite for filament aggregation, as the ultrastructural keratin pattern of ADI horn cells is normal [45,70,73]. The underlying mutations of the gene encoding for profilaggrin in ADI patients have yet to be identified.

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