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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.
The molecular basis of inherited disorders of keratinization

M Giles S Dunnill

Inherited disorders of keratinization can result in a wide variety of clinical features. The skin is usually affected with blistering or ichthyosis, but other body systems may be involved. The severity of these disorders varies greatly. This article reviews the advances in the molecular pathology of these disorders, and shows how this has benefited our understanding of cell biology.

Keratinization is a term used to denote terminal differentiation of epithelia. The process relates to a number of epithelial proteins, glycoproteins and intercellular lipids, but as the name suggests, keratin is the principal molecule involved. Clinically, these diseases include ichthyotic disorders where the skin is dry, scaly, thickened or flaky. Hair, nails and mucous membranes may also be affected. Skin blistering may be the main feature.

This review will therefore start with an introduction to keratin filament biology and molecular genetics, and go on to describe the diseases which result from mutations within the family of keratin genes. Diseases resulting from disorders of other molecules involved in terminal differentiation will also be discussed.

KERATIN FILAMENTS: THE MAIN STRUCTURAL COMPONENTS OF EPITHELIAL CELLS

The keratins are a family of intermediate filament proteins which are expressed within the cytoplasm of epithelial cells (Lane, 1993). They all share the same basic molecular structure with a central alpha-helical rod domain, and non-helical head and tail domains (Figure 1). They are expressed in pairs: type I or acidic keratins, and type II or basic keratins. Keratin pairs associate as heterodimers, with the central rod domains forming a coiled coil structure. These dimers in turn aggregate in large numbers to form filaments visible microscopically within the cell cytoplasm (Figure 2). Different subtypes of keratin pairs are expressed in a tissue-specific fashion, some being specific for hair follicles, nails or mucous membranes or particular regions of the body such as palmpoplantar skin. They may also be expressed within specific layers of the epidermis or in response to wound healing (Figures 3 and 4).

Figure 1. Schematic diagram of the basic molecular structure of keratin. The boxes represent the alpha-helical domains. The lines represent non-helical regions. The shaded areas within the 1A and 2B helical domains represent the helix initiation peptide and helix termination peptide respectively. These amino acid sequences are highly conserved within different keratins, and also within keratins of different species. V1, V2 = variable domains. L1, L12, L2 = linker domains.

Figure 2. Electron micrograph of a basal keratinocyte. Keratin filaments within the basal cell are visible as tonofilaments within the cytoplasm and appear to interact with the plasma membrane of the cell in the region of the basement membrane. F = keratin filaments; H = hemidesmosomes; BM = epidermal basement membrane; D = dermis (magnification x 30,000).
The main function of keratin filaments within cells is thought to be structural. Thus the cytoplasmic keratin filament network is often referred to as the cytoskeleton. Direct evidence for this was not available until the discovery in 1991 that patients carrying genetic mutations within a basal cell-specific keratin suffered from skin blistering resulting from cytolyis of the epidermal basal layer.

**Figure 3.** Keratin expression in epithelium. The expression pattern of different keratins is shown. K5/K14 and K1/K10 are expressed in all squamous epithelia. K9 is confined to palmpoplantar epithelium. K6a/K16 are constitutively expressed in palmpoplantar epithelium, mucosa, nail bed and hair follicles. They are also expressed during the wound healing response. K17 is limited to palmpoplantar and appendageal epithelium. K4/K13 are expressed in mucosa only. K2e is expressed in cornified epithelium. Only keratins discussed in this article are shown.

**Figure 4.** Indirect immunofluorescence of skin using antibodies to (a) keratin 5 and (b) keratin 10. Anti-keratin 5 antibodies stain the cytoplasm of basal keratinocytes, demonstrating expression at this level. There is no expression suprabasally. There is some autofluorescence in the stratum corneum (a). Anti-keratin 10 antibodies stain suprabasal cells only with no evidence of expression in the basal layer. Dashed line = basement membrane.

**Figure 5.** Dowling-Meara variant of epidermolysis bullosa simplex. Clustered blisters occur spontaneously on an erythematous base or in response to minor mechanical trauma.

**Figure 6.** Electron micrograph of a basal keratinocyte in a patient with Dowling-Meara epidermolysis bullosa simplex. The keratin filaments in this cell are clumped and do not have the normal tonofilament arrangement. Blistering arises where cytolyis of these basal keratinocytes occurs. C = keratin filament clumps; BM = basement membrane; N = nucleus; D = dermis (magnification x 8000).

**THE EFFECT:**

**CRITICAL REVIEW**

The first keratins described were Dowling-Meara epidermolysis bullosa simplex (EBS) (Cook et al. 1992). This is characterized by mucous met mechanical trauma and have son 5). Affected individuals with blistering can be detected through the Ultrastruc
tual examination of basal epiderm
tal cells. Basal cells are thought to occur through the generic level, gen
eic substitution with highly conseq
tion in the phenotype. Thus the p sequences were of vital interest to clini
can in many patients with the disease and for many different keratin filaments.
THE EFFECTS OF MUTATIONS IN CRITICAL REGIONS OF KERATINS

The first keratin gene mutations to be described were in patients suffering from Dowling–Meara epidermolysis bullosa simplex (EBS) (Coulombe et al, 1991; Lane et al, 1992). This autosomal dominant disease is characterized by blistering of the skin and mucous membranes in response to minor mechanical trauma. Blisters are often clustered and have some inflammatory element (Figure 5). Affected children present at or soon after birth with blisters or erosions, and the condition can be life-threatening in the neonatal period due to sepsis.

Ultrastructurally, keratin filaments of the basal epidermal keratinocytes lack normal filament organization and appear clumped (Figure 6). Basal cells may lyse and blister formation occurs through the basal layer. At the molecular level, genetic mutations resulting in amino acid substitutions were detected within the highly conserved helix initiation or helix termination peptides of keratins K5 and K14. Thus the prediction that the amino acid sequences within the helix boundary peptides were vital for filament assembly was borne out by clinical and ultrastructural observations in patients with disruptive mutations. This pattern of mutation has now been demonstrated in many different keratin genes with similar cytoskeletal consequences.

MUTATION-PHENOTYPE CORRELATION

EBS variants: different keratin K5/K14 mutations

Autosomal dominant Webbe–Cockayne EBS is characterized by a much milder clinical course than the Dowling–Meara variant. Children may not manifest the disease until they are fully mobile, and blistering is largely confined to sites of maximum friction such as the palms and soles. Ultrastructurally, there is basal cell cytolsis in blistered skin, but no evidence of keratin filament clumping.

The disease is caused by missense mutations in keratins K5 and K14, but not within the helix boundary peptides. Mutations so far described have been within the L12 linker region and less highly conserved regions of the rod domain (Rugg et al, 1993). The functional implication is that these regions are less important for filament assembly.

EBS–Koebner is a rare autosomal recessive variant characterized by generalized epidermal blistering occurring spontaneously or after minor friction. Ultrastructural examination of blistered skin shows basal cell cytolsis, but intact basal keratinocytes are remarkable for a complete absence of keratin filaments. Molecular analysis has revealed homozygous premature termination codon mutations in keratin K14 (Rugg et al, 1994). The result is a complete absence of keratin 14 on immunostaining, and although keratin 5 is present, it is unable to form filamentous structures.

Epidermolytic hyperkeratosis results:
keratin K1 and K10 mutations

Epidermolytic hyperkeratosis (bullous ichthyosiform erythroderma) is an autosomal...
dominant disease usually presenting at or soon after birth with erythroderma, widespread skin blistering and erosions. During childhood the phenotype changes, with a diminished tendency to blistering and the formation of an ichthyotic rippled hyperkeratosis of the skin, particularly over flexures. Thickening of the skin is especially marked over palms and soles (palmoplantar keratoderma). Ultrastructural examination reveals keratin filament clumping and cell lysis in suprabasal keratinocytes. Amino acid substitutions have been reported within the conserved helix boundary peptides of keratins K1 and K10 (Rothnagel et al., 1992). The suprabasal pattern of expression of these keratins corresponds to the site of primary epidermal pathology.

A disease which was originally thought to be a milder variant of epidermolytic hyperkeratosis has been defined as a separate entity termed ichthyosis bullosa of Siemens. There is no erythroderma, and although blistering can occur in response to trauma, it is superficial and more often erosions or ‘moulting’ of the skin result. The distribution is predominantly flexural, and other areas of the skin may be normal. Electron microscopy of the epidermis reveals keratin filament clumping, but this is confined to the granular and upper spinous layers of the epidermis, where cytolysis can also occur. Keratin gene mutations underlying this disease were defined within the conserved helix boundary peptides of the differentiation-specific epidermal keratin K2e, which is expressed only in the upper layers of the epidermis (McLean et al., 1994).

**Palmoplantar keratoderma: tissue-specific keratin gene mutations**

Thickening of the skin of the palms and soles with microscopic evidence of epidermal cell lysis is known as epidermolytic palmoplantar keratoderma. This can occur as part of the syndrome of epidermolytic hyperkeratosis, but also occurs alone as a distinct autosomal dominant clinical entity, sometimes referred to as Vorner’s palmoplantar keratoderma. Biopsy of palm or sole skin reveals suprabasal keratin filament clumping and cell lysis, and the underlying mutations occur in the conserved helix boundary peptides of keratin K9 (Reis et al., 1994).

A number of other hereditary palmoplantar keratodermas without epidermolysis are described. Focal non-epidermolytic palmoplantar keratoderma has been shown to result from missense mutations within the helix initiation peptides of keratin K16 (Shamser et al., 1995). Ultrastructurally there is no keratin filament clumping or epidermolysis, but clinical examination of affected family members can reveal orogenital hyperkeratosis and very subtle nail changes. This, together with the genetic mutations, suggests that this condition is a form of pachyonychia congenita (see below).

**Different forms of pachyonychia congenita are caused by mutations in keratins K6a, K16 and K17**

Pachyonychia congenita is an autosomal dominant ectodermal dysplasia. Affected patients suffer from hypertrophic nail dystrophy, nail bed hyperkeratosis, and various additional features allowing distinction between different subtypes (Figure 7).

The Jadassohn–Lewandowsky variant (PC-I) is characterized by focal palmoplantar hyperkeratosis, follicular keratoses and oral leukokeratoses, whereas the Jackson–Lawlor phenotype (PC-II) lacks oral lesions, but includes multiple epidermal follicular cysts (steatocysts), hair abnormalities and natal teeth.

Mutations within the conserved helix initiation peptides of keratins K6a and K16 have been shown to underlie PC-I and of K17 to underlie PC-II (Bowden et al., 1995; McLean et al., 1995). The different patterns of expression of K6a/K16 compared with K17 elegantly correlate with the clinical phenotypes of their respective pachyonychia congenita variants. The identification of K17 mutations in two families suffering from steatocystoma multiplex led the authors to re-evaluate nails of affected patients, and a number of them had subtle changes (Smith et al., 1997). Thus in the same way that focal non-epidermolytic palmoplantar keratoderma may be a variant of PC-I, steatocystoma multiplex may be a variant of PC-II.

To date all mutations described have occurred within the helix boundary peptides of keratins K6a, K16 and K17, but presumably the amino acid substitutions in patients with PC-I or PC-II are more disruptive to keratin filament formation than in focal non-epidermolytic palmoplantar keratoderma and steatocystoma multiplex respectively.

**Mutations in the mucosal keratins K4 and K13 underlie white sponge naevus**

White sponge naevus is an autosomal dominant condition presenting with mucosal hyperkeratosis. White spongy plaques occur most commonly in the mouth, but also in the nose, oesophagus and anogenital mucosa. Electron microscopy of keratin filaments in mucocytes of the epithelium shows a helical structure (Figure 14), which is absent in mutational mosaic epithelium (Stevens et al., 1995).

**NON-KERATIN DISORDERS**

Although teratogenic and the most common cause of cerebral dysplasia in the stratum corneum is the desmosome defects caused by mutations in the cornified cell membrane proteins, the role of desmosomes in the formation of normal skin structure is still not fully understood.

Loricrin mutations are associated with the formation of normal squamous epithelial cells and the maintenance of the cornified cell membrane. The desmosomes are thought to play an important role in the differentiation of the cornified cell membrane by facilitating the formation of the desmosome and the intercellular adhesion of the cornified cell membrane.

**Some forms from transglutaminase**

Autosomal dominant severe skin disease with a 'collagenase' phenotype is characterized by severe ichthyotic erythroderma, severe ichthyosis, and a sub-group of mutations of the transglutaminase (TGK) gene. Mutations cause a decrease in the activity of TGK, leading to clinical features of ichthyosis, keratoderma, and cutaneous band-like plaques.

**Loricrin mutation**

Another important role of loricrin is in the maintenance of the cornified cell membrane, and its mutations are associated with the formation of normal squamous epithelial cells and the maintenance of the cornified cell membrane. The desmosomes are thought to play an important role in the differentiation of the cornified cell membrane by facilitating the formation of the desmosome and the intercellular adhesion of the cornified cell membrane.
microscopy of areas of hyperkeratosis reveal keratin filament aggregation within the keratinocytes of the spinous layers reflecting similar pathology to other keratin gene disorders. Mutations have recently been described within the helix initiation peptide of keratins K3 and K14, which are expressed in suprabasal mucosal epithelia (Richard et al, 1995; Rugg et al, 1995).

NON-KERATIN PROTEINS IN DISORDERS OF KERATINIZATION

Although terminal differentiation of stratified squamous epithelia principally involves the organization of keratin filaments, a number of other molecules are involved. Keratin filaments in the stratum corneum are aligned into macrofibres stabilized by disulphide bonds under the influence of filagrin, a protein synthesized in the granular layer. A highly insoluble cornified cell envelope forms within the plasma membrane of the corneocyte from involucrin and loricrin under the action of epidermal transglutaminase.

Lipids derived from lamellar granules also play an important part in the skin barrier. Desquamation of the outer layer of the stratum corneum requires breakdown of intercellular lipids which takes place under the action of steroid sulphatase. It is possible that disruption of these molecules, and many others involved in the process of differentiation, may lead to ichthyotic skin diseases.

Some forms of lamellar ichthyosis result from transglutaminase-1 gene mutations

Autosomal recessive lamellar ichthyosis is a severe skin disorder often presenting at birth with a ‘collodion’ membrane. After this membrane is shed, infants suffer life-long generalized severe ichthyosis with large scales and variable erythroderma. The molecular defects underlying a sub-group of these diseases are disruptive mutations of the keratinocyte transglutaminase gene (TGK, transglutaminase I). These mutations cause reduced expression of TGK and loss of TGK activity (Huber et al, 1995). The resulting clinical phenotype with epidermal thickening and scales attests to the importance of this enzyme in formation of cornified cell envelope and keratinization.

Loricrin mutations cause Vohwinkel's keratoderma

Another inherited disorder which results from a defect of cornified cell envelope formation is Vohwinkel’s keratoderma. Patients with this autosomal dominant condition suffer from hyperkeratosis of the palms and soles described as having a honeycomb appearance. In addition they may develop constricting bands around the digits of the hands and feet, known as pseudoainhum, which can lead to autoamputation. Hyperkeratotic lesions may also occur on the knuckles and dorsum of the hands.

Histology shows a markedly thickened stratum corneum, hypergranulosus and hyperkeratosis with round nuclei retained in the stratum corneum. A heterozygous frameshift mutation has been described in the loricrin gene in one family affected with this disease (Maestrini et al, 1996). The abnormal loricrin protein in these patients’ skin is thought to incorporate less efficiently into the cornified cell envelope and to accumulate in intracellular granules.

Disorders of cutaneous lipid metabolism may result in ichthyosis

X-linked ichthyosis presents with mild to moderate scaling of the skin. Most patients harbour total gene deletions of the steroid sulphatase gene on the X chromosome (Ballabio et al, 1987). Because of the proximity of the steroid sulphatase gene to the genes for Kallman syndrome and for short stature, patients may suffer from these diseases as well as a result of a contiguous gene defect.

Sjögren-Larsson syndrome is an autosomal recessive severe generalized ichthyosis presenting at or soon after birth. The underlying molecular defect is a deficiency of fatty aldehyde dehydrogenase, and deleterious mutations have been found in this gene. The mechanism of the disease is unclear, but probably relates to deranged lipid metabolism in the stratum corneum. The disease is important as children also suffer from severe mental retardation and spasticity, presumably due to fatty acid accumulation in the central nervous system (De Laurenzi et al, 1996).

CONCLUSION

The basis of inherited disorders of keratinization is now much clearer following recent advances in molecular genetics of these disorders (Table 1). As yet treatment of most of these disorders is limited, but it is to be hoped that new therapeutic strategies can now be developed. Corrective gene therapy is the obvious avenue of future research, but additional disease modifying treatments could also be devised on the basis of an improved understanding of the molecular pathology.
I am very grateful for the help and advice of David Paige and Anthony Quinn in the preparation of this manuscript. Figure 2 is reproduced courtesy of Dr J McMillan and Figure 4 courtesy of Professor RAI Eady.


**KEY POINTS**

- Keratinization is a process of terminal differentiation of cornified squamous epithelium. This involves keratin filaments together with cornified envelope proteins, lipids and enzymes.
- Inherited disorders of keratinization cause abnormal scaling of the skin, with various associated features.
- Mutations within critical regions of keratin genes result in filament aggregation and epithelial cell collapse. Phenotypes in inherited keratin gene disorders depend on the pattern of expression of the keratin subtype affected.
- Mutations in genes involved in cornified cell envelope formation and cutaneous lipid metabolism result in ichthyotic skin disorders.
- Genotype-phenotype correlation in these disorders gives fresh insight into the normal function of epithelial proteins.

**TABLE 1. Disorders of keratinization**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression</th>
<th>Disease(s)</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>K5/K14</td>
<td>Basal keratinocytes, stratified epithelia</td>
<td>EB simplex</td>
<td>Generalized blistering</td>
</tr>
<tr>
<td>K1/K10</td>
<td>Suprabasal keratinocytes, stratified epithelia</td>
<td>Epidermolysis hyperkeratosis</td>
<td>Generalized blistering and erythroderma, generalized ichthyosis</td>
</tr>
<tr>
<td>K9</td>
<td>Palm and sole</td>
<td>Epidermolysis PPK</td>
<td>Thickening, blistering of palms and soles</td>
</tr>
<tr>
<td>K2e</td>
<td>High epidermis; cornified epithelia</td>
<td>Ichthyosis bullosa of Siemens</td>
<td>Peeling of skin, limited ichthyosis</td>
</tr>
<tr>
<td>K6a, K16</td>
<td>Suprabasal; palm, sole, appendages, mucous</td>
<td>Pachyonychia, non-epidermolysis PPK</td>
<td>Thickening of palms, soles, l follicular hair and oral keratoses</td>
</tr>
<tr>
<td>K17</td>
<td>Suprabasal; palm, sole, appendages</td>
<td>Pachyonychia II, stafatoicysta multiplex</td>
<td>Thickening of palm, sole; epidermal cysts, stand-on-end hair, nail keratoses</td>
</tr>
<tr>
<td>Loricin</td>
<td>Granular layer epidermis</td>
<td>Vohwinkel's keratodera</td>
<td>Thickening of palms, soles; pseudoainhum; hyperkeratoses</td>
</tr>
<tr>
<td>TG-1</td>
<td>Granular layer epidermis</td>
<td>Lamellar ichthyosis</td>
<td>Generalized ichthyosis</td>
</tr>
<tr>
<td>SS</td>
<td>Probably stratum corneum</td>
<td>X-linked ichthyosis</td>
<td>Generalized ichthyosis</td>
</tr>
<tr>
<td>FAD</td>
<td>Probably stratum corneum</td>
<td>Sjögren-Larsson syndrome</td>
<td>Ichthyosis, mental retardation, spasticity</td>
</tr>
</tbody>
</table>

References:
EB = epidermolysis bullosa; FAD = fatty aldehyde dehydrogenase; PPK = palmar plantar keratodema; SS = sterile sulphatase; TG-1 = transglutaminase 1

**WHAT IS**

- The term pl ionizing irr n the treatn

**WHAT IS**

- Photochem tosensitzin; usually UV therapeutic
- Photo(ch effects, to : cell replicat cutaneous a

**WAVELN PHOTO(C**

- The wavele apy compr: netic radiati light (400-4000 nm) of clinical use, whereas U tion with p known as p