Keratin and the skin: past, present and future

Keratin was originally a general term used to describe the waterproof layer of dead cells on the surface of the skin—the stratum corneum, or hair and nails. With increased understanding of the cytoskeleton, the word keratin(s) now more commonly refers to a group of proteins which play a crucial role as scaffolding filaments within epithelial cells. This group of former back-stage proteins have been thrust into the spotlight recently because of their structural significance. Abnormalities of keratins are the cause of several genodermatoses, and these conditions are a contemporary focus for gene therapy research.

Keratins thus exist in tissues as pairs, in specific sites or in patterns characteristic of particular epithelia. This specificity suggests a highly-controlled, defined role for these keratin pairs, with some keratins found in the moist environment of the buccal mucosa (K4 and K13) or cornea (K3 and K12) whilst others are found on the dry surface of the skin (K1 and K10). Alteration in keratin expression occurs during differentiation and can be changed during disease or repair, when for example K6 and K16 appear. Yet more keratins, specific to hair and wool are still being defined.

For many years it was suspected that keratins may be involved in the pathogenesis of the inherited skin fragility syndrome, epidermolysis bullosa simplex (EBS). In this condition, which develops soon after birth, the skin blisters easily with mild trauma. By light microscopy, the blister appears as a split at the

![Figure 1](https://example.com/figure1.png)

**Figure 1.** The keratin filament network in cultured keratinocytes (immunostained with anti-keratin monoclonal antibody LP34). Bar, 20 μm.
level of basal keratinocytes, and electron micrographs show clumping of intermediate filaments in those cells. Later, immuno-electron microscopy confirmed the presence of the basal keratins K5 and K14 in these filament aggregates. Based on data ranging from transgenic animal models, cultured epithelial cells, linkage analysis and sequencing data from families with EBS, it was established that this disease is caused by single point mutations in the genes coding for either K5 or K14, the keratin pair found in basal keratinocytes. These mutations have a dominant negative effect: a single point mutation in one allele of one of the genes for the keratin pair K5 or K14 produces the disease, which is then inherited in an autosomal dominant fashion. The fact that mutations in keratins resulted in skin fragility firmly established the role of the intermediate filaments as important structural proteins (until then this was surmised but not proven). More work is now being done to understand the functional implications of these keratin mutations at the cellular level.

Numerous different mutations in K5 or K14 have now been documented in patients with EBS. Many of the mutations occur at ‘hotspot’ positions in the keratin proteins, especially at each end of the helical rod domain, where the protein sequence is evolutionally highly conserved and probably critical for filament assembly. Mostly the position of the mutation correlates with the clinical severity of the disease. The clustering of mutations has enabled more rapid screening of families for mutations (including ante-natal screening) and provides an insight into how the keratin filaments assemble within the cells.

The search has continued for more families with inherited disorders of skin, hair or nails, involving clinicians collaborating with scientists to find mutations in other keratins expressed in the skin. Careful clinical examination, combined with histology, including electron microscopy, linkage analysis and gene sequencing formed the backbone of this work. Diseases currently attributable to mutations in keratin genes include epidermolysis bullosa simplex (K5/K14), bullous congenital ichthyosiform erythroderma (K1/K10), ichthyosis bullosa of Siemens (K2e), pachyonychia congenita Type I (K6/K16), pachyonychia congenita Type II (K17), white sponge naevus (oral lesion) (K4, K13), and epidermolytic palmoplantar keratoderma (K9) (see reviews). In about 25% of patients with an EBS phenotype, many of them sporadic cases, no keratin mutations have been found, either at the hot spots or along the length of the gene (fortunately keratin genes are relatively short, at around 1 kilobase). Attention therefore turned to the structural proteins in the desmosomal and hemidesmosomal junctions into which keratin filaments loop. These junctions attach the epithelial cells to each other or to the basement membrane, respectively. In a sub-type of EBS associated with muscular dystrophy, mutations were traced to the protein plectin, present in desmosomes in both skin and muscle. A variant of dystrophic EB (with some phenotypic resemblance to EBS, but where the split in the skin occurs below the basement membrane of the epidermis) associated with pyloric atresia, has now been shown to be due to mutations in the genes of the integrin α6β4, which is also part of the hemidesmosome (reviewed in reference 16).

Much work has also been done over this period investigating other inherited skin diseases not associated with keratin abnormalities. The more severe forms of EB, when blisters form below the basal lamina are due to mutations in laminin 5 (junctional EB) and collagen VII (dystrophic EB). Some congenital ichthyoses are due to mutations in the transglutaminase enzymes, while mutations in other differentiation specific proteins such as loricrin have been found in Erythrokeratoderma and Vohwinkels’ syndrome. The search continues for a candidate protein in Darier’s disease, which also links to chromosome 12, near the keratin locus.

For the affected families in whom mutations have been found, recognition and understanding of their disease has brought relief and also hope. Ante-natal diagnosis is already possible and the prospect of gene therapy looms in the future.

The skin offers several advantages for gene therapy. It is readily accessible, the results are easily visible and regional treatment is a possibility. Keratinocytes can be grown in culture, providing in vitro systems to test therapeutic approaches. Disadvantages include the difficulty of targeting stem cells in the epidermis, since these cells are not easily distinguished from other basal cells. Another disadvantage with many of the keratin disorders is the dominant negative effect of the mutations. It would be necessary to knock out the damaged gene completely and remove all the disruptive protein to correct the cell fragility, since as little as 5% of mutant protein can be disruptive. Interestingly, a few families with recessive EBS have a complete absence of K14, creating an effective functional ‘knock out’ model. This could be an ideal scenario for gene therapy, since all that is required is to reintroduce a normal gene: unfortunately this form of the disease is very rare. More needs to be understood about the control of keratin gene expression. Factors which affect upstream promoters of the gene may be a more suitable target to manipulate. Much work is being done towards gene therapy, but it remains in the future.

Thus, through the combination of clinical resource and scientific expertise, the causes of several inherited skin diseases have been established. Much has
also been achieved in the past decade towards a better understanding of the normal processes of differentiation and the organization of the epidermis, which may help in understanding other more common dermatoses. The search continues for causes of other genodermatoses, including hair disorders.

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References


