Published in final edited form as:

Exp Cell Res. 2007 June 10; 313(10): 1995–2009. doi:10.1016/j.yexcr.2007.03.029.

DISEASES OF EPIDERMAL KERATINS AND THEIR LINKER PROTEINS

Jouni Uitto¹, Gabriele Richard^{1,*}, and John A. McGrath²

¹ *Department of Dermatology and Cutaneous Biology, Jefferson Medical College, and Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA*

² *Genetic Skin Disease Group, St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, The Guy's, King's College and St. Thomas' School of Medicine, London, UK*

Abstract

Epidermal keratins, a diverse group of structural proteins, form intermediate filament networks responsible for the structural integrity of keratinocytes. The networks extend from the nucleus of the epidermal cells to the plasma membrane where the keratins attach to linker proteins which are part of desmosomal and hemidesmosomal attachment complexes. The expression of specific keratin genes is regulated by differentiation of the epidermal cells within the stratifying squamous epithelium. Progress in molecular characterization of the epidermal keratins and their linker proteins has formed the basis to identify mutations which are associated with distinct cutaneous manifestations in patients with genodermatoses. The precise phenotype of each disease apparently reflects the spatial level of expression of the mutated genes, as well as the types and positions of the mutations and their consequences at mRNA and protein levels. Identification of specific mutations in keratinization disorders has provided the basis for improved diagnosis and subclassification with prognostic implications and has formed the platform for prenatal testing and preimplantation genetic diagnosis. Finally, precise knowledge of the mutations is a prerequisite for development of gene therapy approaches to counteract, and potentially cure, these often devastating and currently intractable diseases.

Keywords

Epidermal keratins; intermediate filaments; epidermal differentiation; hemidesmosomes; desmosomes; genodermatoses; skin fragility; epidermolysis bullosa; epidermolytic hyperkeratosis; ichthyosis

BIOLOGY OF EPIDERMAL KERATINS

Keratins, the major constituents of epithelial cells, represent a family of about 50 proteins that form 10 nm keratin intermediate filaments and are expressed in tissue- and differentiation-specific patterns [1,2]. The intermediate filament network builds into a dense, three-

Address for Correspondence: Jouni Uitto, M.D., Ph.D., Department of Dermatology and Cutaneous Biology, Jefferson Medical College, 233 S. 10th Street, Suite 450 BLSB, Philadelphia, PA 19107, USA, Tel: (215) 503-5785, Fax: (215) 503-5788, E-mail: Jouni.Uitto@jefferson.edu.

*Current Address: GeneDx Inc., 207 Perry Parkway, Gaithersburg, MD 20877

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

dimensional and highly dynamic cytoskeleton spanning between the nucleus and the cell membrane, where they are anchored by interactions with desmosomal and hemidesmosomal linker proteins (Fig. 1). This organization provides both structural stability and flexibility, and ensures the mechanical integrity of different epithelial tissues. Keratins are expressed as obligate heterodimers of acidic (type I) and basic (type II) proteins, which assemble, through a multi-step process, into intermediate filaments (Fig. 2). The genes encoding type I and type II keratins cluster on chromosomal regions 17q12–q21 and 12q11–q13, respectively. Keratin monomers are organized as a central, alpha-helical rod of about 310 amino acids, flanked by variable, non-helical head and tail domains (Fig. 3). The monomers form coiled-coil obligate heterodimers, which polymerize in an overlapping and antiparallel fashion and assemble into intermediate filaments.

In the epidermis, undifferentiated basal keratinocytes express the principal keratins K5 (type II) and K14 (type I), and to a lesser extent K15 (type I), while cells in the upper epidermis switch to the expression of the differentiation-specific keratin pair K1/K10, and in the granular layers also K2e. Other site-specific suprabasal keratins include K9 found predominantly in the palms and soles, and K6, K16 and K17, which are induced by trauma to the skin. Pathogenic mutations in 19 different keratin genes have been discovered so far in a wide range of epithelial fragility disorders affecting skin, mucous membranes, hair, nails, and sebaceous glands [3–5]. In these conditions, the disease pathology in general corresponds to the expression pattern of the defective keratin protein. In addition, a number of genes expressed in desmosomes and hemidesmosomes have been shown to harbor mutations in a manner that the integrity of cells and the stability of cell/cell and cell/matrix interactions are perturbed, resulting in epidermal diseases [6,7].

PATHOLOGY OF EPIDERMAL KERATINS

Epidermolysis Bullosa Simplex – The Disease of *KRT5* and *KRT14* Mutations

Epidermolysis bullosa (EB) is a group of heritable blistering disorders, and the simplex variant of EB (EBS) was the first human keratin disorder to be delineated at molecular level [8,9]. It has become the prototype for understanding disease pathogenesis and genotype-phenotype correlations within this broad group of disorders. EBS is a clinically heterogeneous blistering disorder due to fragility of basal cells of the epidermis [10]. Three major subtypes have been recognized, which all share a common disease pathology involving an abnormal KIF network and cytolysis of basal cells leading to intraepidermal blister formation and skin fragility (Fig. 4). The structural abnormalities in EBS are primarily confined to the basal compartment of the epidermis and result from faulty K5/K14 heterodimers due to mutations in their corresponding genes, *KRT5* and *KRT14*. Mutations in either gene can produce a comparable phenotype reflecting the genetic heterogeneity of EBS. *KRT5* and *KRT14* mutations are usually inherited in an autosomal dominant manner and lead to substitution of conserved amino acid residues, having a deleterious, dominant-negative effect on the assembly of KIF and causes weakening of the cytoskeleton and cell fragility (Fig. 2).

To date, close to 100 distinct pathogenic mutations have been identified in different forms of EBS. They are clustered at eight defined regions (Fig. 3), and their relative positions seem to dictate the severity of disease. The majority of mutations (~60%) occur at two “hot spots” corresponding to the beginning and end of the central rod domain, known as the helix initiation peptide and helix termination peptide, respectively (Fig. 3). Functional *in vivo* and *in vitro* studies have demonstrated that the helix boundaries are zones of overlap between keratin heterodimers and that mutant keratin molecules perturb proper formation of the KIF network of epithelial cells, thus compromising cellular integrity (Fig. 1) [11]. Consequently, these “hot spot” mutations are consistently associated with a severe phenotype. Other mutation sites, such as the homologous domain in the head of type II keratins (H1) or non-helical linker and stutter

motifs, are associated with a milder phenotype. The H1 region has been implicated in filament assembly and contains major phosphorylation sites that are essential for the regulation of KIF dynamics [12], while the linker and stutter regions provide critical flexibility to the otherwise rigid alpha-helical rod [13]. In addition, a few rare autosomal recessive mutations leading to the ‘knock-out’ of keratin 14 in the basal epidermis have also been described in EBS [14–16].

The group of EBS diseases demonstrates considerable clinical variability that can be explained, to a large part, by the types of mutations in the *KRT5* and *KRT14* genes as delineated below. Nevertheless, other not yet identified genetic modifiers and environmental factors, such as life-style or climate, clearly influence the phenotypic expression as different subtypes of EBS have been associated with the same mutation in several instances [17,18].

EBS Dowling-Meara (EBS-DM; OMIM 131760) the most severe subtype manifesting at birth with erythema, widespread blistering, erosions and areas of denuded skin (Fig. 4). While spontaneous development of grouped blisters at any body site slowly improves with age, a progressive palmoplantar keratoderma becomes the chief complaint in adulthood. Other features of EBS-DM include involvement of mucous membranes, secondary skin infections and sepsis, nail dystrophy, transient milia, and healing of the skin with hypo- and hyperpigmentation. Typically, the pathogenic defects comprise heterozygous missense mutations clustering at the highly conserved boundaries of the alpha-helical rod of K5 or K14. A particular arginine codon within the helix initiation peptide in K14 (R125) is the most commonly mutated residue occurring in >30% of EBS-DM cases, probably because it contains a hypermutable CpG dinucleotide. As a result, the arginine codon (CGC) is replaced either by cysteine (TGC) or histidine (CAC) (see Fig. 4). While most keratin gene mutations in EBS are dominant, recessive *KRT14* mutations have been identified in a few families, these mutations predominantly comprise nonsense and splice site mutations leading to premature termination of protein translation and ablation of the affected keratin. While ‘knock-out’ of *KRT14* usually results in severe EBS-DM, mutations in some families have been noted to have a milder phenotype with minimal extracutaneous involvement.

The Köbner subtype of EBS (EBS-K; OMIM 131900) is characterized by milder, generalized blistering of the skin without apparent clustering, often in response to minor trauma and induced by increased ambient temperature. Hands, feet and extremities are most consistently affected, probably because of the greater mechanical trauma at these body sites. *KRT5* or *KRT14* mutations appear more widely distributed across the keratin polypeptides and may lie within and outside the highly conserved helix boundaries as well as in non-helical linker segments (19).

EBS Weber-Cockayne (EBS-WC; OMIM 131800) is the most common, relatively mild, localized subtype of EBS, in which blisters are confined to the hands, feet, and areas of friction or trauma. In contrast to the other EBS subtypes, blisters are usually not present at birth but develop later in life in response to mechanical trauma to the skin. Ultrastructural abnormalities of the cytoskeleton are far less severe than those seen in EBS-DM, and may be too subtle to detect by transmission electron microscopy. In this relatively mild form of EBS, pathogenic mutations lie in most cases outside of the helix boundaries, elsewhere in the rod domain of K5 or K14, including the non-helical L12 linker motif or in the amino-terminal homologous domain of K5 (Fig. 3). Dominant point mutations usually result in amino acid substitutions, but a small in-frame deletion in *KRT14* has also been described [15].

EBS with mottled pigmentation (EBS-MP; OMIM 131960) is a rare form of EBS with generalized or acral blistering and small hyper- and hypopigmented spots that form a reticulate pattern. This variant is also associated with thickened, dystrophic nails and punctate

palmoplantar keratoderma. The mottled pigmentation seems unrelated to skin blistering and corresponds with an increased number of melanosomes within basal keratinocytes, dermal macrophages and Schwann cells, as observed by electron microscopy. In most cases, EBS-MP is caused by a recurrent, heterozygous missense mutation in exon 1 of *KRT5* that results in substitution of proline 24 with leucine (p24L) in the non-helical head domain of K5 [20–22]. This mutation has been consistently detected in each of the unrelated families with EBS-MP tested to date. Preliminary investigations of this mutation has revealed that the keratin 5 head domain may directly bind to a cytoplasmic dynein cargo complex transporting melanosomes, thus unraveling the basis for abnormal pigment distribution in this rare disorder [23].

Epidermolytic Hyperkeratosis (EHK; OMIM 113800) – Mutations in the *KRT1* and *KRT10* Genes

EHK, also known as bullous congenital ichthyosiform erythroderma (BCIE), is a relatively rare, autosomal dominant keratinization disorder which presents at birth with generalized erythema, blistering, erosions and peeling of the skin. Later on, hyperkeratotic areas with verrucous scales, mainly involving flexural and intertriginous areas develop, and in the majority of patients palmoplantar hyperkeratosis is present.

The cutaneous pathology in EHK results from the expression of abnormal K1 or K10 proteins that exert a dominant negative effect [24]. Since these two keratins confer structural stability to keratin intermediate filaments in the suprabasal keratinocytes, the blistering is more superficial than in EBS, and there is increased proliferation of suprabasal keratinocytes resulting in ichthyosiform lesions. The mutations primarily involve amino acid residues in the highly conserved helical rod portions of the molecule responsible for intermolecular association of the keratins [25–27]. Thus, the positions of the mutations along the keratin polypeptides and the level of expression of the mutated genes, *KRT1* and *KRT10*, explain the clinical features of this disorder.

Two interesting genetic features are associated with EHK. First, about one half of the mutations are spontaneous, *de novo* mutations, explaining the fact that parents in these cases of this autosomal dominant disease are clinically unaffected [28]. The presence of *de novo* mutations most likely reflects germline mosaicism in one of the parents. Secondly, in many families, one of the parents of a patient with EHK has localized linear epidermal nevi, hamartomas of the epidermis that develop along the lines of Blaschko. These lesions demonstrate pathology similar to EHK and harbor mutations identified in the offspring with full-blown EHK [29, 30]. Thus, these epidermal nevi appear to represent somatic mosaicisms within this disease, the offspring demonstrating full-blown EHK as a result of germ line mosaicism.

Keratin Mutations in Other Genodermatoses – Spectrum of Diseases

In addition to EBS and EHK, two prototypic keratinization disorders resulting from mutations in the *KRT5/KRT14* and *KRT1/KRT10* gene/protein systems, respectively, a number of other genodermatoses have been shown to harbor mutations in the keratin genes. (Table I). Some of these conditions, although clinically clearly distinct from EBS, harbor mutations in the same genes, *KRT5/KRT14*. For example, the Dowling-Degos disease, characterized by progressive and disfiguring hyperpigmentation primarily in the flexural areas, is due to defective *KRT5* expression [31,32]. Similarly, autosomal dominant null mutations in the *KRT14* gene have been shown to cause Naegeli-Franceschetti-Jadassohn syndrome and the allelic pigmentation disorder dermatopathia pigmentosa reticularis, which both share reticulate hyperpigmentation of skin, thickening of the palms and soles as well as signs of ectodermal dysplasia, including abnormal sweating and absence of dermatoglyphics [33]. Phenotypic presentation in these conditions, as opposed to that in the classic forms of EBS, apparently reflects the types and positions of the mutations in the corresponding keratin genes. Similarly, a disease known as

ichthyosis hystrix of the Curth-Macklin type, characterized by severe palmoplantar hyperkeratosis, is due to frameshift mutations in the *KRT1* gene that completely alter the V2 tail domain of this keratin [34]. In addition to these keratinization disorders, other keratin genes can harbor mutations in association of clearly distinct clinical phenotypes. For example, pachyonychia congenita (PC), a heterogeneous group of diseases manifesting with dystrophic nails, thickening and blistering of the palms and soles and associated mucocutaneous and oral lesions, is known to result from mutations in the *KRT6a/KRT16* and *KRT6b/KRT17* gene protein systems in PC type I and type II, respectively [35,36] (Table 1). As another example, ichthyosis bullosa of Siemens demonstrates superficial blistering and shedding of epidermis and harbors mutations in the *KRT2e* gene which is expressed high in the suprabasal layers in the skin [37]. Thus, these phenotypes to a large extent reflect the level of expression of the mutated genes within the stratifying layers of the epidermis, compounded by the types of mutations and their consequences at the mRNA and protein levels.

DISEASES OF HEMIDESMOSOMAL ATTACHMENT COMPLEXES

The Hemidesmosome-Anchoring Filament Complex

The hemidesmosomes, ultrastructurally recognizable attachment complexes, extend from the intracellular compartment of the basal keratinocytes to the lamina lucida in the upper portion of the dermal–epidermal basement membrane (Fig. 1). The intracellular component of hemidesmosomes attaches to the keratin intermediate filament network, while in the extracellular space within the lamina lucida the hemidesmosomes are contiguous with anchoring filaments, thread-like structures which traverse the lamina lucida and tend to concentrate below the hemidesmosomes, hence the designation hemidesmosome-anchoring filament complexes [38]. Early biochemical studies identified five major components of the hemidesmosomes, consisting of polypeptides with molecular masses of approximately 500, 230, 200, 180, and 120 kDa; these were originally designated as HD1–HD5, respectively. HD2 and HD4 have since been shown to correspond to the 230-kDa bullous pemphigoid antigen (BPAG1) and the 180-kDa bullous pemphigoid antigen (BPAG2), respectively. HD3 and HD5 correspond to the $\beta 4$ and $\alpha 6$ integrin subunit polypeptides, respectively, and HD1 corresponds to plectin which is localized to the cytoplasmic side of the hemidesmosomes. The intracellular hemidesmosomal plaque contains the 230-kDa bullous pemphigoid antigen (BPAG1), a non-collagenous protein of the plakin family that serves as an autoantigen in an acquired autoimmune disease, bullous pemphigoid. The 180-kDa bullous pemphigoid antigen (BPAG2), a transmembrane protein, also known as type XVII collagen (COL17A1), together with $\alpha 6\beta 4$ integrin, extends from the intracellular compartment into the extracellular space, thus stabilizing the association of basal keratinocytes to the underlying basement membrane. Consequently, mutations resulting in abnormalities in any one of the protein components of the hemidesmosomes can give rise to different forms of EB.

Mutations in different forms of EB have now been identified in each of the known genes encoding the macromolecular components of the hemidesmosomes, with the exception of BPAG1 [39,40]. Careful examination of the mutation database in these genes reveals genotype-phenotype correlations which reflect the expression of the mutated genes, the types and combinations of the mutations, and their consequences at the mRNA and protein levels.

Within the spectrum of patients with EB, there is a subgroup in which blisters form in and around the hemidesmosomes, and have therefore been referred to as the “hemidesmosomal variants” [41]. Ultrastructural classification initially identified at least three groups of patients with EB whose blisters form at the level of the hemidesmosomes, and whose clinical phenotype is unlike any of the ‘classic’ forms of EB. These three subtypes include epidermolysis bullosa with muscular dystrophy (EB-MD, OMIM 226670), epidermolysis bullosa with pyloric atresia (EB-PA, OMIM 226730), and generalized atrophic benign epidermolysis bullosa (GABEB,

OMIM 226650). Mutations have recently been identified in three hemidesmosome-associated structural protein/gene systems in patients with these rare forms of EB (see below).

The Paradigm of Plectin Disorders

Plectin was initially described as a ~500-kDa component of the hemidesmosome, and was subsequently shown to be an exceptionally large intermediate filament interacting protein [42,43]. Plectin is a plakin family member with similarities to both desmoplakin and bullous pemphigoid antigen 1, it is highly conserved between rat and human, and it has a wide tissue distribution, including the central nervous system and muscle. Plectin has a remarkable number of versatile binding affinities for other proteins, including keratins 5 and 14, vimentin, glial fibrillary acidic protein, the neurofilament protein triplet, and lamin B, suggesting that it can tether one filamentous network to another [44]. The binding of keratin intermediate filament to nucleus may involve binding of plectin to nesprin-3, an outer nuclear membrane protein (45). Although plectin is expressed in nearly all cell types, its precise cytoplasmic localization is cell-type specific, and it can appear diffusely throughout the cell as a cytomatrix component or in a restricted distribution as a focal adhesion protein.

EB with muscular dystrophy—The typical presentation of EB-MD, a rare variant of EB, involves neonatal blistering and late-onset muscular dystrophy with nail and tooth abnormalities, and severe mucocutaneous involvement, including laryngeal webs and urethral strictures, has been reported [46]. Initial examination of biochemical and synthetic properties of cultured keratinocytes from patients with EB-MD using antibodies against plectin showed that this protein was completely absent in the cells of several patients with EB-MD, and subsequently, mutations in the plectin gene (PLEC1) could be demonstrated in a number of families [46–49]. While EB-MD is a rare autosomal recessive disorder, most of the mutations reported thus far result in premature termination codons on both alleles of plectin, either by nonsense, insertion/deletion or splicing mutations, and the phenotype in these patients is remarkably consistent manifesting with neonatal blistering and progressive muscle weakness from the first or second decade of life on. In some patients, however, small in-frame deletions or insertions have been disclosed with significantly milder phenotype and the onset of the muscle weakness on the third or fourth decade of life [49,50]. Thus, identification of plectin mutations in patients with EB-MD has provided insights into the phenotype-genotype correlations in this disorder as well as the relationship between the skin, the musculoskeletal and the nervous systems.

The Onga variant of EB is caused by a plectin missense mutation—While several mutations in the plectin gene have been identified, primarily in recessively inherited EB-MD, a different type of mutation in plectin leads to a rare, autosomal dominant form of EB, known as the Onga variant (OMIM 131950). The EB Onga phenotype results from a specific missense mutation (R2110W) within the rod domain of plectin originally identified in a Norwegian kindred [51]. It has since been shown that EB Onga is not restricted to a single Norwegian kindred as previously believed, but a German family with this phenotype was also found to carry the identical mutation. Clinically, the EB Onga patients demonstrate hemorrhagic blistering of the skin, but unlike patients with EB-MD, there is no muscle phenotype, and muscle biopsies from several EB Onga patients revealed normal staining patterns with antibodies against plectin. Clearly, the genotype-phenotype relationships among plectin mutations in the different forms of EB are unusually complex and still emerging.

EB with pyloric atresia—Another rare subtype of EB, characterized by blistering of the skin and congenital pyloric atresia (EB-PA), was previously classified as a form of junctional EB, and its phenotypes can range from moderate to early postnatal demise. Mutations in the genes encoding the two polypeptides of the $\alpha 6 \beta 4$ integrin receptor (*ITGA6* and *ITGB4*) were

initially identified in EB-PA [7,41,52]. Furthermore, more recently plectin gene mutations have also been identified in the severe forms of EB-PA, attesting to further genetic heterogeneity in this EB variant [53,54]. A survey of the reported mutations in these genes indicates that premature termination codons are associated predominantly with the lethal EB-PA variants, whereas missense mutations are more prevalent in non-lethal forms. However, the consequences of the missense mutations are position dependent, and substitutions of highly conserved amino acids within the individual integrin receptors may have lethal consequences. The phenotypic manifestation of pyloric atresia from mutations in the *ITGB4* and *ITGA6* genes suggest a tissue-specific role for this integrin both in the skin and the gastrointestinal tract.

Mutations in the Type XVII Collagen Gene (BPAG2) in Generalized Atrophic Benign EB

Underscoring the genetic heterogeneity of EB is the demonstration of mutations in the *COL17A1* gene in a subset of patients known as generalized atrophic benign EB (GABEB). Clinically, these patients demonstrate a moderate blistering tendency, associated with a characteristic constellation of extracutaneous involvement, including dystrophy of the fingernails, focal scarring alopecia of the scalp, loss of eyelashes, dental anomalies, and patchy macular hyperpigmentation [41]. The genetic lesion in the original GABEB family, described in a geographically limited area in Austria, is a premature termination codon mutation transmitted through many branches of the large inbred family, and subsequently, many different types of mutations in *COL17A1* have been observed, including missense, nonsense, splice-site and insertions/deletions, resulting in premature termination codons [7,41,55–57]. While there is some variation in phenotypic severity, GABEB is in most cases non-lethal, and there does not appear to be a strict genotype-phenotype correlation with respect to *COL17A1* mutations. Recently, a dominantly inherited glycine substitution mutation in *COL17A1* was identified in a GABEB family, in which the proband had inherited a premature termination codon on the second allele [56]. Interestingly, all heterozygous carriers of the glycine substitution alone had markedly abnormal dentition and enamel pitting. Clearly, the glycine substitution mutation in *COL17A1* also impacts upon the basement membrane of the developing tooth, a previously undisclosed function for this transmembrane collagen.

In summary, the hemidesmosomal variants of EB reflect mutations from different genes and the specific clinical constellations result from the combination of different types of mutations within the mutated genes (Table 2). Whereas the mechanistic consequences of these mutations have been explored at the mRNA and protein levels, the functional role of these components in HD assembly is also becoming increasingly clear through recent biochemical studies of protein–protein interactions. For example, an interaction between the 180-kDa bullous pemphigoid antigen and $\alpha 6$ integrin has been described, the disruption of HD assembly by a tailless $\beta 4$ integrin subunit was reported, and roles for the integrin receptors in mediating signal transduction continue to emerge [38]. Whereas these components clearly represent functionally interdependent structural macromolecules in the dermal–epidermal adhesion zone, recent studies suggest a functionally dynamic and interactive role of some of these proteins in cell–matrix communication. Finally, it is noteworthy that despite an extensive survey of the genes known to be involved in EB, a number of families have proven negative for mutations, suggesting additional, as yet undisclosed, candidate genes and uncharacterized protein components for this heterogeneous group of disorders.

BIOLOGY AND PATHOLOGY OF DESMOSOMES

Desmosomes are important cell–cell adhesion junctions found predominantly in epithelia and the heart, but also in the meninges and cortex of lymph nodes [58]. In addition to providing attachment points for intermediate filaments, these intercellular bridges provide strength and rigidity to cells and contribute to aspects of tissue signaling, differentiation, proliferation and development. Functionally, desmosomes consist of three families of proteins, (a) the armadillo

proteins (plakoglobin and certain plakophilins), (b) cadherins (four desmogleins and three desmocollins), and (c) plakins (mainly desmoplakin but in some desmosomes also including envoplakin, periplakin, plectin, bullous pemphigoid antigen 1, corneodesmosin and microtubule actin cross-linking factor) (see Fig. 1). Each desmosome is made up of a complex network of these components, the precise composition of which may vary in a site- and differentiation-specific manner. Clues to the important function of the desmosomal proteins in epithelial cell biology have emerged from a variety of studies including naturally occurring and engineered mutations, animal models and other *in vitro* experiments. Notably, over the last decade or so, human gene mutations have been reported in eight components of desmosomes. These discoveries have often highlighted novel or unusual phenotypes, including abnormal skin barrier function, alterations in epidermal differentiation, and developmental anomalies of various ectodermal appendages, especially hair, as well as a range of extracutaneous pathologies. The human diseases thus provide intriguing insight into the biological complexities of cell-cell contact and intercellular communication [59,60].

Plakophilin 1 Mutations in Ectodermal Dysplasia – Skin Fragility Syndrome

The first human mutations in a desmosome component were identified in the plakophilin 1 gene, *PKP1*. Specifically, McGrath *et al.* [61] described a child with a combination of skin fragility (erosions, fissures, scale-crust, keratoderma) and abnormalities of ectodermal development (small size, scanty hair, reduced sweating, astigmatism), who was a compound heterozygote for loss-of-function mutations on both alleles of the *PKP1* gene, p.Q304X and c.1132ins28; these mutations occur within the second and fourth *arm*-repeats of the protein [62,63]. Clinically, the phenotype was termed ectodermal dysplasia-skin fragility syndrome (OMIM604536) and eight other clinically similar individuals with different loss-of-function mutations in *PKP1* have been reported subsequently [64,65]

Clues to the desmosomal pathology in these cases came from skin biopsy analysis which showed widening of spaces between keratinocytes throughout most of the epidermis (apart from the basal layer) with ultrastructural evidence of a reduced number of small, poorly-formed desmosomes (Figure 5A). In addition to cell-cell separation, the keratinocyte intermediate filament network was clearly abnormal, showing condensed keratin filaments that were retracted in a perinuclear distribution and which lacked insertion into the cell membrane. Close inspection showed that the intercellular keratinocyte weakness involved “pinching off” of hypoplastic desmosomes with the plane of fragility occurring within the intracellular desmosomal plaque (Figure 5B).

A candidate gene approach, based on reduced or absent expression of desmosomal proteins using immunofluorescence microscopy, was then used to identify the primary abnormalities in *PKP1*. Subsequently, cell biologic studies on keratinocytes lacking *PKP1* have demonstrated its importance in the formation of desmosomal plaques and in stabilizing desmosomal proteins [66]. Plakophilin 1 has been shown to bind to desmoglein 1, desmocollin 1, desmoplakin, keratin (at least *in vitro*) and actin [67]. The redistribution of keratin filaments away from the cell periphery in patient’s keratinocytes lacking *PKP1* provides some support for an *in vivo* interaction between *PKP1* and keratin filaments, but further assessment in these cells showed that the major desmosomal plaque protein desmoplakin (DSP) was also redistributed to the cytoplasm, illustrating the bond between *PKP1* and desmoplakin *in vivo* and demonstrating that the major role of *PKP1* is in stabilizing desmosomes in the spinous layers of the epidermis.

Another plakophilin family member, *PKP2*, is also associated with inherited disease but involving the heart rather than the skin. Specifically, heterozygous mutations in *PKP2* represent the major cause of familial arrhythmogenic ventricular cardiomyopathy, an autosomal dominant condition characterized by ventricular arrhythmias, syncope and sudden death, usually precipitated by exertion [68]

Desmoplakin Mutations – A Spectrum of Skin, Hair and Heart Phenotypes

Evidence for a primary inherited abnormality of DSP emerged from a linkage study that mapped an autosomal dominant pedigree with striate palmoplantar keratoderma (Figure 6A) to 6p24, a locus containing the *DSP* gene [69]. Microscopy of palmar skin showed massive hyperkeratosis along with dysadhesion between suprabasal keratinocytes and abnormal cell-cell and cell membrane-cytoskeletal adhesion (Figure 6B and C) and screening of genomic DNA identified a heterozygous nonsense mutation, p.Q331X, in the *DSP* gene [69]. Thus, the keratoderma in this family was due to DSP haploinsufficiency, demonstrating that 50 percent of normal DSP levels are sufficient for normal epidermal functioning in nonpalmoplantar skin but insufficient for withstanding trauma to the palms and soles. Sixteen further human mutations in the *DSP* gene have subsequently been reported [70,71].

The phenotypes resulting from DSP mutations are protean, reflecting either autosomal dominant or autosomal recessive inheritance, and resulting in clinical abnormalities that may affect skin, hair or heart [71]. Some of the mutations provide a graphic illustration of the profound biological consequences of abnormal DSP. For example, the first recessive pathogenic mutation found in the *DSP* gene was a homozygous frameshift mutation, c.7901delG, identified in affected members of three Ecuadorean families [72] with Carvajal syndrome (OMIM605676), which comprises palmoplantar keratoderma, dilated left ventricular cardiomyopathy and woolly hair. This mutation produces a stop codon leading to the formation of a truncated protein lacking the carboxyl terminal tail domain. Clinically, affected patients suffer from heart failure in their teens, and their skin abnormalities are usually confined to areas prone to mechanical stress, such as the palms and soles. In contrast, the almost complete ablation of the desmoplakin tail may produce an even more severe phenotype consisting of progressive widespread sloughing of the epidermis, generalized alopecia, absence of nails and the presence of neonatal teeth [73]. This condition, termed “lethal acantholytic epidermolysis bullosa” (OMIM609638), has been described in a single neonate who was a compound heterozygote for the *DSP* gene mutations, p.R1934X and c.6370delTT.

Plakoglobin Gene Mutation Causes Naxos Disease

Only one pathogenic mutation in the gene encoding the other major desmosomal plaque protein, plakoglobin (JUP), has been reported [74]. A homozygous 2-base pair deletion, c.2157delTG, was shown to be the cause of an autosomal recessive disorder involving heart, skin and hair abnormalities, known as Naxos disease (OMIM601214) which partially resembles the phenotype in plakoglobin-deficient transgenic mice [75]. The clinical features of Naxos disease comprise cardiomyopathy, nonepidermolytic diffuse palmoplantar keratoderma and irregular woolly hair. The disorder is named after the Mediterranean island Naxos on which approximately 1 in 1000 of the population is affected [76]. All affected individuals have the same mutation, c.2157delTG, which occurs close to the carboxyl terminus and alters the last five aminoacids within the 13th arm-repeat, truncating the protein by 56 residues.

Desmoglein 1 Mutations in Palmo-Plantar Keratoderma

In addition to inherited gene abnormalities in desmosomal plaque components, mutations have also been reported in the extracellular part of certain cadherins. Mutations in the desmoglein 1 gene (*DSG1*) in an inherited skin disorder were first identified in a three-generation Dutch family with striate palmoplantar keratoderma (i.e. similar phenotype to the cases reported by Armstrong *et al.*, [69] with DSP haploinsufficiency) who had a heterozygous acceptor splice site mutation, IVS2-1G>A, in the *DSG1* gene [77]. This mutation occurs within the prosequence of DSG1 but results in skipping of exons 2–4 and generates a peptide that is only 25 amino acids in length. This suggests that the disease in this family is due to haploinsufficiency of DSG1. Eight further mutations within the extracellular part of DSG1

were subsequently reported [78–81]. Clinically, affected individuals usually have features of keratoderma on their palms and soles (typically striate but sometimes focal); rarely are there any other cutaneous abnormalities although thickening/scaling on trauma-prone sites such as the knees, ankles or finger knuckles and mild nail dystrophy have also been documented [81]. Mutations in the *DSG1* gene also affect the number and size of desmosomes as well as expression of other skin proteins [82]. Notably, in palmar skin, DSG1 haploinsufficiency leads to a reduced number of smaller desmosomes in suprabasal layers. By confocal microscopy, there is reduced expression of keratins K5, K14 and K10 as well as upregulation of K16; involucrin labeling is also disrupted [82].

Desmoglein 4 Mutations Cause Hair Abnormalities

Mutations in the desmoglein 4 gene, *DSG4*, are the cause of localized autosomal recessive hypotrichosis (OMIM607903) [83]. Clinically, affected individuals have hypotrichosis involving the scalp, chest, arms and legs, but there is sparing of the axillary and pubic hair and eyelashes. On the scalp, small papules are present due to the inability of the hair to extrude through the skin [83]. The mutation first identified, EX5-8del, deletes all of the second extracellular domain as well as part of the first and third extracellular domains. A homozygous missense mutation, p.A129S, has also been reported in an Iraqi subject with localized autosomal recessive hypotrichosis [84]. This amino acid substitution is located within a highly conserved motif (RAL) corresponding to the HAV region of classical cadherins, a domain that is important in cell-cell adhesion. In 2006, further missense, splice site, frameshift and nonsense mutations in the *DSG4* gene were reported [85–87]. However, the phenotype in these cases was described as autosomal recessive monilethrix. Although the beaded hair phenotype of monilethrix typically presents as an autosomal dominant disorder (OMIM158000) due to heterozygous mutations in the hair keratin genes, *KRTHB1*, *KRTHB3* and *KRTHB6*, recessive forms of the disease also exist and these appear to result from mutations on both alleles of *DSG4*. Most of the mutations are located within the extracellular part of DSG4. Although localized autosomal recessive hypotrichosis and recessive monilethrix are listed as distinct entities, considerable phenotypic overlap exists and the nature of the mutations in the extracellular part of DSG4 also shows similar gene pathologies.

CLINICAL IMPLICATIONS OF MOLECULAR DIAGNOSTICS IN EPIDERMAL DISORDERS

Collectively, the identification of a large number of naturally occurring human mutations in genes encoding protein components of the keratin intermediate filaments and their linker protein complexes has disclosed a spectrum of inherited diseases, many with rare and unusual phenotypes. Assessing the consequences of these mutations on the integrity and function of hemidesmosomes, desmosomes and the intermediate filaments has also provided valuable insight into biologically significant protein-protein interactions and other fundamental elements of epithelial structural biology. The conclusions drawn from these observations on phenotype-genotype correlations in humans are often verified by development of transgenic animals as models which recapitulate the features noted in patients [88,89]. However, in spite of the extensive molecular characterization of the keratins and keratin-associated diseases over the last two decades, several mysteries still persist. For example, mutations in *KTR5* or *KRT14* genes have failed to be detected in up to ~30% of cases of EBS using conventional mutation screening and sequencing protocols. It is plausible that a large number of these families harbor mutations in keratin linker protein genes, such as the plectin gene [45], but some mutations may also reside in the keratin genes in the regions that are not routinely analyzed. Similarly, in approximately 25% of patients with desmosomal skin fragility disorders search for mutations in the known candidate genes has not been successful, suggesting that there may be additional, less characterized genes that may participate in cell-cell interactions.

Thus, elucidation of the molecular pathology in such cases has the potential to provide further insight into the major function of epithelial keratins and associated anchoring and signaling proteins in epidermal physiology and its perturbations in diseases.

The progress in basic molecular biology of keratins and in understanding the underlying molecular mechanisms in a number of keratin disorders have major implications for clinical diagnosis of these skin fragility syndromes. Specifically, identification of distinct mutations has allowed development of refined, molecular-based classification with prognostic implications, and the knowledge of the precise mutations has provided the basis for prenatal testing and preimplantation genetic diagnosis in the most severe cases [90–93]. Finally, knowledge of the precise genetic lesions in the candidate genes is a prerequisite for development of gene therapy approaches to counteract, and potentially cure, these often devastating and currently intractable genodermatoses [94].

Acknowledgements

The authors thank Carol Kelly for assistance. Dr. Irwin McLean kindly provided the immunofluorescence illustration shown in Figure 1.

References

1. Coulombe PA, Omary MB. Hard” and “soft” principles defining the structures, function and regulation of keratin intermediate filaments. *Curr Opin Cell Biol* 2002;14:110–122. [PubMed: 11792552]
2. Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, Maltais L, Omary MB, Parry DA, Rogers MA, Wright MW. New consensus nomenclature for mammalian keratins. *J Cell Biol* 2006;174:169–174. [PubMed: 16831889]
3. Lane EB, McLean WH. Keratins and skin disorders. *J Pathol* 2004;204:355–366. [PubMed: 15495218]
4. Corden LD, McLean WH. Human keratin diseases: Hereditary fragility of specific epithelial tissues. *Exp Dermatol* 1996;5:297–307. [PubMed: 9028791]
5. Smith F. The molecular genetics of keratin disorders. *Am J Clin Dermatol* 2003;4:347–364. [PubMed: 12688839]
6. Kottke MD, Delva E, Kowalczyk AP. The desmosome: Cell science lessons from human diseases. *J Cell Sci* 2006;119:797–806. [PubMed: 16495480]
7. Varki R, Sadowski S, Pfendner E, Uitto J. Epidermolysis bullosa. I Molecular genetics of the junctional and hemidesmosomal variants. *J Med Genet* 2006;43:641–652. [PubMed: 16473856]
8. Ishida-Yamamoto A, McGrath JA, Chapman SJ, Leigh IM, Lane EB, Eady RA. Epidermolysis bullosa simplex (Dowling-Meara type) is a genetic disease characterized by an abnormal keratin-filament network involving keratins K5 and K14. *J Invest Dermatol* 1991;97:959–968. [PubMed: 1721080]
9. Chan YM, Yu QC, Fine JD, Fuchs E. The genetic basis of Weber-Cockayne epidermolysis bullosa simplex. *Proc Natl Acad Sci USA* 1993;90:7414–7418. [PubMed: 7688477]
10. Horn HM, Tidman MJ. The clinical spectrum of epidermolysis bullosa simplex. *Br J Dermatol* 2000;142:468–472. [PubMed: 10735952]
11. Hermann H, Wedig T, Porter RM, Lane EB, Aebi U. Characterization of early assembly intermediates of recombinant human keratins. *J Struct Biol* 2002;137:82–96. [PubMed: 12064936]
12. Omary MB, Ku NO, Tao GZ, Toivola DM, Liao J. Heads and tails” of intermediate filament phosphorylation: multiple sites and functional insights. *Trends Biochem Sci* 2006;31:3834–94.
13. Steiner PM, Marekov LN, Fraser RD, Parry DA. Keratin intermediate filament structure. Crosslinking studies yield quantitative information on molecular dimensions and mechanism of assembly. *J Mol Biol* 1993;230:4364–52.
14. Rugg EL, McLean WH, Lane EB, Pitera R, McMillan JR, Dopping-Hepenstal PJ, Navsaria HA, Leigh IM, Eady RA. A functional “knockout” of human keratin 14. *Genes Dev* 1994;8:2563–2573. [PubMed: 7525407]

15. Chan Y, Anton-Lamprecht I, Yu QC, Jackel A, Zabel B, Ernst JP, Fuchs E. A human keratin 14 “knockout”: The absence of K14 leads to severe epidermolysis bullosa simplex and a function for an intermediate filament protein. *Genes Dev* 1994;8:2574–2587. [PubMed: 7525408]
16. Corden LD, Mellerio JE, Gratian MJ, Eady RA, Harper JI, Lacour M, Magee G, Lane EB, McGrath JA, McLean WH. Homozygous nonsense mutation in helix 2 of K14 causes severe recessive epidermolysis bullosa simplex. *Hum Mutat* 1998;11:279–285. [PubMed: 9554744]
17. Rugg EL, Horn HM, Smith FJ, Wilson NJ, Hill AJ, Magee GJ, Shemanko CS, Baty DU, Tidman MJ, Lane EB. Epidermolysis bullosa simplex in Scotland caused by a spectrum of keratin mutations. *J Invest Dermatol* 2007;127:574–80. [PubMed: 17039244]
18. Covello SP, Smith FJ, Sillevius Smitt JH, Paller AS, Munro CS, Jonkman MF, Uitto J, McLean WH. Keratin 17 mutations cause either steatocystoma multiplex or pachyonychia congenita type 2. *Br J Dermatol* 1998;139:4754–80.
19. Chen MA, Bonifas JM, Matsumura K, Blumenfeld A, Epstein EH Jr. A novel three-nucleotide deletion in the helix 2B region of keratin 14 in epidermolysis bullosa simplex: Delta E375. *Hum Mol Genet* 1993;2:1971–1972. [PubMed: 7506606]
20. Uttam J, Hutton E, Coulombe PA, Anton-Lamprecht I, Yu QC, Gedde-Dahl T Jr, Fine JD, Fuchs E. The genetic basis of epidermolysis bullosa simplex with mottled pigmentation. *Proc Natl Acad Sci USA* 1996;93:9079–9084. [PubMed: 8799157]
21. Irvine AD, McKenna KE, Jenkinson H, Hughes AE. A mutation in the V1 domain of keratin 5 causes epidermolysis bullosa simplex with mottled pigmentation. *J Invest Dermatol* 1997;108:809–810. [PubMed: 9129237]
22. Shurman D, Losi-Sasaki J, Grimwood R, Kivirikko S, Tichy E, Uitto J, Richard G. Epidermolysis bullosa simplex with mottled pigmentation: Mutation analysis in the first reported Hispanic pedigree with the largest single generation of affected individuals to date. *Eur J Dermatol* 2006;16:132–135. [PubMed: 16581562]
23. Irvine AD, Rugg EL, Lane EB, Hoare S, Peret C, Hughes AE, Heagerty AH. Molecular confirmation of the unique phenotype of epidermolysis bullosa simplex with mottled pigmentation. *Br J Dermatol* 2001;144:40–45. [PubMed: 11167681]
24. Ishida-Yamamoto A, McGrath JA, Judge MR, Leigh IM, Lane EB, Eady RA. Selective involvement of keratins K1 and K10 in the cytoskeletal abnormality of epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma). *J Invest Dermatol* 1992;99:19–26. [PubMed: 1376754]
25. McLean WH, Morley SM, Higgins C, Bowden PE, White M, Leigh IM, Lane EB. Novel and recurrent mutations in keratin 10 causing bullous congenital ichthyosiform erythroderma. *Exp Dermatol* 1999;8:120–123. [PubMed: 10232402]
26. Sybert VP, Francis JS, Corden LD, Smith LT, Weaver M, Stephens K, McLean WH. Cyclic ichthyosis with epidermolytic hyperkeratosis: A phenotype conferred by mutations in the 2B domain of keratin K1. *Am J Hum Genet* 1999;64:732–738. [PubMed: 10053007]
27. Tal O, Bergman R, Alcalay J, Indelman M, Sprecher E. Epidermolytic hyperkeratosis type PS-1 caused by aberrant splicing of KRT1. *Clin Exp Dermatol* 2005;30:64–67. [PubMed: 15663507]
28. Cohen PR, Ulmer R, Theriault A, Leigh IM, Duvic M. Epidermolytic acanthomas: Clinical characteristics and immunohistochemical features. *Am J Dermatopathol* 1997;19:232–241. [PubMed: 9185908]
29. Ishida-Yamamoto A, Takahashi H, Iizuka H. Immunoelectron microscopy links molecules and morphology in the studies of keratinization. *Eur J Dermatol* 2000;10:429–435. [PubMed: 10980462]
30. Paller AS, Syder AJ, Chan YM, Yu QC, Hutton E, Tadini G, Fuchs E. Genetic and clinical mosaicism in a type of epidermal nevus. *N Engl J Med* 1994;331:1408–1415. [PubMed: 7526210]
31. Betz RC, Planko L, Eigelshoven S, Hanneken S, Pasternack SM, Bussow H, Bogaert KV, Wenzel J, Braun-Falco M, Rutten A, Rogers MA, Ruzicka T, Nothen MM, Magin TM, Kruse R. Loss-of-function mutations in the keratin 5 gene lead to Dowling-Degos disease. *Am J Hum Genet* 2006;78:510–519. [PubMed: 16465624]
32. Liao H, Zhao Y, Baty DU, McGrath JA, Mellerio JE, McLean WH. A heterozygous frameshift mutation in the V1 domain of keratin 5 in a family with Dowling-Degos disease. *J Invest Dermatol*. 2006e-published (August 17)

33. Lugassy J, Itin P, Ishida-Yamamoto A, Holland K, Huson S, Geiger D, Indelman M, Bercovich D, Uitto J, Bergman R, McGrath J, Richard G, Sprecher E. Naegeli-Franceschetti-Jadassohn syndrome and dermatopathia pigmentosa reticularis: Two allelic ectodermal dysplasias caused by *KRT14* dominant mutations. *Am J Hum Genet* 2006;79:724–730. [PubMed: 16960809]
34. Richardson ES, Lee JB, Hyde PH, Richard G. a novel mutation and large size polymorphism affecting the V2 domain of keratin 1 in an African-American family with severe, diffuse palmoplantar keratinoderma of the ichthyosis hystrix Curth-Macklin type. *J Invest Dermatol* 2006;126:79–84. [PubMed: 16417221]
35. Smith FJ, Liao H, Cassidy AJ, Stewart A, Hamill KJ, Wood P, Joval I, Steensel MA, Bjorck E, Callif-Daley F, Pals G, Collins P, Leachman SA, Munro CS, McLean WH. The genetic basis of pachyonychia congenita. *J Invest Dermatol Symp Proc* 2005;10:21–31.
36. McLean WH, Smith FJ, Cassidy AJ. Insights into genotype-phenotype correlation in pachyonychia congenita from the human intermediate filament mutation database. *J Invest Dermatol Sym Proc* 2005;10:31–36.
37. Akiyama M, Tsuji-Abe Y, Yanagihara M, Nakajima K, Kodama H, Yaosaka M, Abe M, Sawamura D, Shimizu H. Ichthyosis bullosa of Siemens: Its correct diagnosis facilitated by molecular genetic testing. *Br J Dermatol* 2005;152:1353–1356. [PubMed: 15949009]
38. Litjens SH, de Pereda JM, Sonnenberg A. Current insights into the formation and breakdown of hemidesmosomes. *Trends Cell Biol* 2006;16:376–383. [PubMed: 16757171]
39. Pulkkinen L, Uitto J. Mutation analysis and molecular genetics of epidermolysis bullosa. *Matrix Biol* 1999;18:29–42. [PubMed: 10367729]
40. Uitto J, Richard G. Progress in epidermolysis bullosa: Genetic classification and clinical implications. *Am J Med Genet C Semin Med Genet* 2004;131C:61–74. [PubMed: 15468152]
41. Pulkkinen L, Uitto J. Hemidesmosomal variants of epidermolysis bullosa. Mutations in the $\alpha 6\beta 4$ integrin and the 180-kD bullous pemphigoid antigen/type XVII collagen genes. *Exp Dermatol* 1998;7:46–64. [PubMed: 9583744]
42. Andra K, Lassmann H, Bittner R, Shorny S, Fassler R, Propst F, Wiche G. Targeted inactivation of plectin reveals essential function in maintaining the integrity of skin, muscle, and heart cytoarchitecture. *Genes Dev* 1997;11:3143–3156. [PubMed: 9389647]
43. Wiche G. Role of plectin in cytoskeleton organization and dynamics. *J Cell Sci* 1998;111:2477–2486. [PubMed: 9701547]
44. McMillan JR, Akiyama M, Rouan F, Mellerio JE, Lane B, Leigh IM, Owaribe K, Wiche G, Fujii N, Uitto J, Eady RAJ, Shimizu H. Plectin defects in epidermolysis bullosa simplex with muscular dystrophy. *Muscle and Nerve* 2007;35:24–35. [PubMed: 16967486]
45. Wilhelmsen K, Litjens SH, Kuikman I, Tshimbalanga N, Janssen H, van den Bout I, Raymond K, Sonnenberg A. Nesprin-3, a novel outer nuclear membrane protein, associates with the cytoskeletal linker protein plectin. *J Cell Biol* 2005;171:799–810. [PubMed: 16330710]
46. Pfendner E, Rouan F, Uitto J. Progress in epidermolysis bullosa: The phenotypic spectrum of plectin mutations. *Exp Dermatol* 2005;14:241–249. [PubMed: 15810881]
47. McLean WHI, Pulkkinen L, Smith FJD, Rugg EL, Lane EB, Bullrich F, Burgeson RE, Amano S, Hudson DL, Owaribe K, McGrath JA, McMillan JR, Eady RAJ, Leigh IM, Christiano AM, Uitto J. Loss of plectin causes epidermolysis bullosa with muscular dystrophy: cDNA cloning and genomic organisation. *Genes Dev* 1996;10:1724–1735. [PubMed: 8698233]
48. Smith FJD, Eady RAJ, Leigh IM, McMillan FRY, Rugg EL, Kelsell DP, Bryant SP, Spurr NK, Geddes JF, Kirschig G, Milana G, de Bono AG, Owaribe K, Wiche G, Pulkkinen L, Uitto J, McLean WHI, Lane EB. Plectin deficiency results in muscular dystrophy with epidermolysis bullosa. *Nature Genet* 1996;13:450–457. [PubMed: 8696340]
49. Pulkkinen L, Smith FJD, Shimizu H, Murata S, Yaoita H, Hachisuka H, Nishikawa T, McLean WHI, Uitto J. Homozygous deletion mutations in the plectin gene (*PLEC-1*) in patients with epidermolysis bullosa simplex associated with late-onset muscular dystrophy. *Human Mol Genet* 1996;5:1539–1546. [PubMed: 8894687]
50. Shimizu H, Takizawa Y, Pulkkinen L, Murata S, Kawai M, Hachisuka H, Udonon M, Uitto J, Nishikawa T. Epidermolysis bullosa simplex associated with muscular dystrophy: Phenotype-

- genotype correlations and review of the literature. *J Am Acad Dermatol* 1999;41:950–956. [PubMed: 10570379]
51. Koss-Harnes D, Hoyheim B, Anton-Lamprecht I, Gjesti A, Jorgensen RS, Jahnsen FL, Olaisen B, Wiche G, Gedde-Dahl T Jr. A site-specific plectin mutation causes dominant epidermolysis bullosa simplex Ogna: Two identical de novo mutations. *J Invest Dermatol* 2002;118:87–93. [PubMed: 11851880]
 52. Pulkkinen L, Kimonis VE, Xu Y, Spanou EN, McLean WHI, Uitto J. Homozygous $\alpha 6$ integrin mMutation in junctional epidermolysis bullosa with congenital duodenal atresia. *Hum Molec Genet* 1997;6:669–674. [PubMed: 9158140]
 53. Pfendner E, Uitto J. Plectin gene mutations can cause epidermolysis bullosa with pyloric atresia. *J Invest Dermatol* 2005;124:111–115. [PubMed: 15654962]
 54. Nakamura H, Sawamura D, Goto M, Nakamura H, McMillan JR, Park S, Kono S, Hasegawa S, Paku S, Nakamura T, Ogiso Y, Shimizu H. Epidermolysis bullosa simplex associated with pyloric atresia in a novel clinical subtype caused by mutations in the plectin gene (PLEC1). *J Mol Diagn* 2005;7:28–35. [PubMed: 15681471]
 55. McGrath JA, Gatalica B, Christiano AM, Li K, Owaribe K, McMillan JR, Eady RAJ, Uitto J. Mutations in the gene encoding the 180-kD bullous pemphigoid antigen (BPAG2), a hemidesmosomal transmembrane collagen (type XVII), in generalized atrophic benign epidermolysis bullosa. *Nature Genet* 1995;11:83–86. [PubMed: 7550320]
 56. McGrath JA, Gatalica B, Li K, Dunnill MG, McMillan JR, Christiano AM, Eady RA, Uitto J. Compound heterozygosity for a dominant glycine substitution and a recessive internal duplication mutation in the type XVII collagen gene results in junctional epidermolysis bullosa and abnormal dentition. *Am J Pathol* 1996;148:1787–1796. [PubMed: 8669466]
 57. Darling TN, McGrath JA, Yee C, Gatalica B, Hametner R, Bauer JW, Pohla-Gubo G, Christiano AM, Uitto J, Hintner H, Yancey KB. Premature termination codons are present on both alleles of the bullous pemphigoid antigen 2/type XVII collagen gene in five Austrian families with generalized atrophic benign epidermolysis bullosa. *J Invest Dermatol* 1997;108:463–468. [PubMed: 9077475]
 58. Green KJ, Gaudry CA. Are desmosomes more than tethers for intermediate filaments? *Nat. Rev Mol Cell Biol* 2000;1:208–216.
 59. Kottke MD, Delva E, Kowalczyk AP. The desmosome: cell science lessons from human diseases. *J Cell Sci* 2006;119:597–806.
 60. Dusek RL, Godsel LM, Green KJ. Discriminating roles of desmosomal cadherins: beyond desmosomal adhesion. *J Dermatol Sci.* 2007(e-pub; in press)
 61. McGrath JA, McMillan JR, Shemanko CS, Runswick SK, Leigh IM, Lane EB, Garrod DR, Eady RA. Mutations in the plakophilin 1 gene result in ectodermal dysplasia/skin fragility syndrome. *Nat Genet* 1997;17:240–244. [PubMed: 9326952]
 62. Heid HW, Schmidt A, Zimbelmann R, Schafer S, Winter-Simanowski S, Stumpp S, Keith M, Figge U, Schnolzer M, Franke WW. Cell type-specific desmosomal plaque proteins of the plakoglobin family: plakophilin 1 (band 6 protein). *Differentiation* 1994;58:113–131. [PubMed: 7890138]
 63. Choi HJ, Weis WI. Structure of the armadillo repeat domains of plakophilin 1. *J Mol Biol* 2005;346:367–376. [PubMed: 15663951]
 64. Wessagovit V, McGrath JA. Clinical and molecular significance of splice site mutations in the plakophilin 1 gene in patients with ectodermal dysplasia-skin fragility syndrome. *Acta Derm Venereol* 2005;85:386–388. [PubMed: 16159727]
 65. Ersoy-Evans S, Erkin G, Fassihi H, Chan I, Paller AS, Sürücü S, McGrath JA. Ectodermal dysplasia--skin fragility syndrome resulting from a new homozygous mutation, 888delC, in the desmosomal protein plakophilin 1. *J Am Acad Dermatol* 2006;55:157–161. [PubMed: 16781314]
 66. South AP, Wan H, Stone MG, Dopping-Hepenstal PJ, Purkis PE, Marshall JF, Leigh IM, Eady RA, Hart IR, McGrath JA. Lack of plakophilin 1 increases keratinocyte migration and reduces desmosome stability. *J Cell Sci* 2003;116:3303–3314. [PubMed: 12840072]
 67. Hatzfeld M. Plakophilins: Multifunctional proteins or just regulators of desmosomal adhesion? *Biochim. Biophys Acta* 2007;1773:69–77.
 68. van Tintelin JP, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld AC, Wilde AA, van der Smagt J, Boven LG, Mannens MM, van Langen IM, Hofstra RM, Otterspoor LC, Doevendans PA, Rodriguez

- LM, van Gelder IC, Hauer RN. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation* 2006;113:1650–1658. [PubMed: 16567567]
69. Armstrong DK, McKenna KE, Purkis PE, Green KJ, Eady RA, Leigh IM, Hughes AE. Haploinsufficiency of desmoplakin causes a striate subtype of palmoplantar keratoderma. *Hum Mol Genet* 1999;8:143–148. [PubMed: 9887343]
 70. Lai-Cheong JE, Wessagowit V, McGrath JA. Molecular abnormalities of the desmosomal protein desmoplakin in human disease. *Clin Exp Dermatol* 2005;30:261–266. [PubMed: 15807686]
 71. Lai-Cheong JE, Arita K, McGrath JA. Genetic diseases of junctions. *J Invest Dermatol*. 2007;in press
 72. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, Whittock N, Leigh IM, Stevens HP, Kelsell DP. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum Mol Genet* 2000;9:2761–2766. [PubMed: 11063735]
 73. Jonkman MF, Pasmooij AM, Pasmans SG, van den Berg MP, Ter Horst HJ, Timmer A, Pas HH. Loss of desmoplakin tail causes lethal acantholytic epidermolysis bullosa. *Am J Hum Genet* 2005;77:653–660. [PubMed: 16175511]
 74. McKoy G, Protonotarios N, Crosby A, Tsatsopoulou A, Anastasakis A, Coonar A, Norman M, Baboonian C, Jeffery S, McKenna WJ. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet* 2000;355:2119–2124. [PubMed: 10902626]
 75. Bierkamp C, McLaughlin KJ, Schwarz H, Huber O, Kemler R. Embryonic heart and skin defects in mice lacking plakoglobin. *Dev Biol* 1996;180:780–785. [PubMed: 8954745]
 76. Antoniadis L, Tsatsopoulou A, Anastasakis A, Syrris P, Asimaki A, Panagiotarios D, Zambartas C, Stefanadis C, McKenna WJ, Protonotarios N. Arrhythmogenic right ventricular cardiomyopathy caused by deletions in plakophilin-2 and plakoglobin (Naxos disease) in families from Greece and Cyprus: genotype-phenotype relations, diagnostic features and prognosis. *Eur Heart J* 2006;25:2208–2216. [PubMed: 16893920]
 77. Rickman L, Simrak D, Stevens HP, Hunt DM, King IA, Bryant SP. N-terminal deletion in a desmosomal cadherin causes the autosomal dominant skin disease striate palmoplantar keratoderma. *Hum Mol Genet* 1999;8:971–976. [PubMed: 10332028]
 78. Hunt DM, Rickman L, Whittock NV, Eady RA, Simrak D, Dopping-Hepenstal PJ, Stevens HP, Armstrong DK, Hennies HC, Kuster W, Hughes AE, Arnemann J, Leigh IM, McGrath JA, Kelsell DP, Buxton RS. Spectrum of dominant mutations in the desmosomal cadherin desmoglein 1, causing the skin disease striate palmoplantar keratoderma. *Eur J Hum Genet* 2001;9:197–203. [PubMed: 11313759]
 79. Kljuic A, Gilead L, Martinez-Mir A, Frank J, Christiano AM, Zlotogorski A. A nonsense mutation in the desmoglein 1 gene underlies striate keratoderma. *Exp Dermatol* 2003;12:523–527. [PubMed: 12930313]
 80. Keren H, Bergman R, Mizrahi M, Kashi Y, Sprecher E. Diffuse nonepidermolytic palmoplantar keratoderma caused by a recurrent nonsense mutation in DSG1. *Arch Dermatol* 2005;141:625–628. [PubMed: 15897387]
 81. Milingou M, Wood P, Masouye I, McLean WH, Borradori L. Focal palmoplantar keratoderma caused by an autosomal dominant inherited mutation in the desmoglein 1 gene. *Dermatology* 2006;212:117–122. [PubMed: 16484817]
 82. Wan H, Dopping-Hepenstal PJ, Gratian MJ, Stone MG, Zhu G, Purkis PE, South AP, Keane F, Armstrong DK, Buxton RS, McGrath JA, Eady RA. Striate palmoplantar keratoderma arising from desmoplakin and desmoglein 1 mutations is associated with contrasting perturbations of desmosomes and the keratin filament network. *Br J Dermatol* 2004;150:878–891. [PubMed: 15149499]
 83. Kljuic A, Bazzi H, Sundberg JP, Martinez-Mir A, O'Shaughnessy R, Mahoney MG, Levy M, Montagutelli X, Ahmad W, Aita VM, Godon D, Uitto J, Whiting D, Ott J, Fischer S, Gilliam TC, Jahoda CA, Morris RJ, Panteleyev AA, Nguyen VT, Christiano AM. Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotrichosis and acquired pemphigus vulgaris. *Cell* 2003;113:249–260. [PubMed: 12705872]

84. Messenger AG, Bazzi H, Parslew R, Shapiro L, Christiano AM. A missense mutation in the cadherin interaction site of the desmoglein 4 gene underlies localized autosomal recessive hypotrichosis. *J Invest Dermatol* 2005;125:1077–1079. [PubMed: 16297213]
85. Schaffer JV, Bazzi H, Vitebsky A, Witkiewicz A, Kovich OI, Kamino H, Shapiro LS, Amin SP, Orlov SJ, Christiano AM. Mutations in the desmoglein 4 gene underlie localized autosomal recessive hypotrichosis with monilethrix hairs and congenital scalp erosions. *J Invest Dermatol* 2006;126:1286–1291. [PubMed: 16543896]
86. Shimomura Y, Sakamoto F, Kariya N, Matsunaga K, Ito M. Mutations in the desmoglein 4 gene are associated with monilethrix-like congenital hypotrichosis. *J Invest Dermatol* 2006;126:1281–1285. [PubMed: 16439973]
87. Zlotogorski A, Marek D, Horev L, Abu A, Ben-Amitai D, Gerad L, Ingber A, Frydman M, Reznik-Wolf H, Vardy DA, Pras E. An autosomal recessive form of monilethrix is caused by mutations in DSG4: clinical overlap with localized autosomal recessive hypotrichosis. *J Invest Dermatol* 2006;126:1292–1296. [PubMed: 16575393]
88. Arin MJ, Roop DR. Disease model: Heritable skin blistering. *Trends Mol Med* 2001;7:422–424. [PubMed: 11530338]
89. Vijayaraj P, Sohl G, Magin TM. Keratin transgenic and knockout mice: functional analysis and validation of disease-causing mutations. *Methods Mol Biol* 2006;360:203–252. [PubMed: 17172732]
90. Smith FJD, McKusick VA, Nielsen K, Pfendner E, Uitto J, McLean WHI. Cloning of multiple keratin 16 genes facilitates prenatal diagnosis of pachyonychia congenita type 1. *Prenat Diagn* 1999;19:941–946. [PubMed: 10521820]
91. Pfendner E, Nakano A, Pulkkinen L, Christiano AM, Uitto J. Prenatal diagnosis for epidermolysis bullosa: A study of 144 consecutive pregnancies at risk. *Prenat Diagn* 2003;23:447–456. [PubMed: 12813757]
92. Fassihi H, Grace J, Lashwood A, Whittock NV, Braude PR, Pickering SJ, McGrath JA. Preimplantation genetic diagnosis of skin fragility-ectodermal dysplasia syndrome. *Br J Dermatol* 2006;154:546–550. [PubMed: 16445790]
93. Uitto J, Pfendner E, Jackson LG. Probing the fetal genome: Progress in non-invasive prenatal diagnosis, Probing the fetal genome: Progress in non-invasive prenatal diagnosis. *Trends Mol Med* 2003;9:339–343. [PubMed: 12928035]
94. Mavilio F, Pellegrini G, Ferrari S, Di Nunzio F, Di Iorio E, Recchia A, Maruggi G, Ferrari G, Provasi E, Bonini C, Capurro S, Conti A, Magnoni C, Giannetti A, De Luca M. Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells. *Nat Med* 2006;12:1397–1402. [PubMed: 17115047]

Figure 1.

Schematic representation of the structure of desmosomes and hemidesmosomes, attachment complexes at the cell-cell and cell-matrix interface, respectively. The keratin intermediate filament network is visualized by immunofluorescence on the upper right corner. In basal keratinocytes of the epidermis, keratins 5 and 14 form the network which attaches to desmoplakin in desmosomes and to plectin in hemidesmosomes. Critical protein-protein interactions of the desmosomal and hemidesmosomal components are required for physiologic integrity of the basal layer of epidermis and its attachment to the underlying matrix or to adjacent keratinocytes, and genetic or immunologic perturbations in the hemidesmosome and/or desmosome network structures result in skin fragility.

Figure 2.

Schematic representation of the physiology of keratin intermediate filament (KIF) assembly (left panel) and mechanisms of genetic perturbations that can lead to KIF pathology (right panel). Under physiologic conditions, the keratin mRNAs are translated on the ribosomes of epithelial cells to synthesize type I and II keratins (i). In the cytoplasm, type I and type II keratin polypeptide heterodimerize (ii) and align in parallel and registry (ii). Pairs of heterodimers align in an anti-parallel, overlapping fashion to form tetramers (iv), which polymerize to elongated chains (v) and form the KIFs through lateral packing (vi). The presence of a mutation in a keratin gene can lead to different pathological processes (right panel). As an example in autosomal recessive disorders, a mutation that introduces a premature termination codon or affects mRNA splicing can result in the synthesis of a truncated polypeptide (ii). Concomitant nonsense-mediated mRNA decay or enhanced degradation of the truncated polypeptides can result in absence of the mutant protein and lack of formation of the corresponding heterodimers

and KIF, resulting in haploinsufficiency. Alternatively, the persistence of truncated altered keratin polypeptides can prevent heterodimer formation and KIF assembly. In autosomal dominant keratin disorders, most mutations result in non-conservative amino acid replacement at sites of high sequence conservation (*iii*). The mutant keratin polypeptides then interfere in a dominant negative manner with head-to-tail interactions and proper alignment of heterodimers (*iv*), as well as a elongation and lateral packing during KIF assembly (*v*), thus producing a severe phenotype. Mutations with a less severe phenotype often reside outside the helix boundaries and may have a more subtle effect on KIF assembly or may affect keratin phosphorylation or interactions with other proteins. (Modified from Uitto *et al.*, Epidermolysis Bullosa; in Principles of Molecular Medicine (Runge, M.S. and Patterson, C., Eds.), Humana Press, Totowa, NJ, Second Edition, pp. 1024–1034, 2006).

Figure 3.

Schematic representation of the structure of a keratin protein depicting the structural domains and common mutation sites in epidermolysis bullosa simplex (EBS). The non-helical head domain consists of E1, V1 and H1 (only in type II keratins). The α -helical rod domain is composed of four segments, 1A, 1B, 2A, and 2B (with stutter-S), which are interrupted by non-helical linker domains, L1, L12, and L2. The H2 (only in type II keratins), V2 and E2 segments form the non-helical tail domain. The highly conserved helix initiation and termination motifs, the regions containing the mutational hotspots, are striped. Common sites of dominant mutations and their corresponding EBS phenotypes are depicted above the diagram; the locations of recessive mutations are shown below. The relative height of the bars reflects the relative frequency of the mutations. Abbreviations: EBS-MP, EBS with mottled pigmentation; EBS-WC, Weber-Cockayne subtype of EBS; EBS-DM, Dowling-Meara subtype of EBS; EBS-K, K6b subtype of EBS; EBS-MCE, EBS with migratory circinate erythema; DDD, Dowling-Degos Disease; NFJS/DPR, Naegeli-Franceschetti-Jadassohn syndrome/ Dermatopathia pigmentosa reticularis. FS/Stop: frameshift and nonsense mutations; Ins/Dup: insertions and duplications; Del: deletions.

Figure 4.

Clinicopathological features of EBS-DM. (A) Characteristic blisters in an annular (herpetiform) distribution on the thigh of a 9-year old girl who has the heterozygous mutation p.R125C in the *KRT14* gene. Several of the blisters are blood-filled and appear blue-black in color whereas the skin inflammation secondary to keratinocyte cytolysis is pinkish-red. (B) Light microscopy reveals cleavage (+) close to the dermal-epidermal junction as well as aggregated filaments within basal keratinocytes (Huber stain; bar = 25 μ m). (C) Transmission electron microscopy from the edge of the blistered skin reveals keratin filament clumping (*) and basal cell cytolysis (+) (bar = 2 μ m). (D) In contrast, in normal human skin no aggregation of keratin filaments or cytolysis is seen (bar = 0.5 μ m)

Figure 5.

Structural pathology in a skin biopsy from a patient with ectodermal dysplasia-skin fragility syndrome resulting from loss-of-function mutations in the *PKP1* gene. (A) Transmission electron microscopy of normal keratinocytes showing intercellular spaces but desmosomal intercellular connections and keratin filament networks that extend to the edges of the cells (bar = 2 μ m). (B) Transmission electron microscopy of skin harboring *PKP1* mutations reveals widening of spaces between adjacent keratinocytes (double arrows) and a condensed appearance of the keratin filament network which is retracted around cell nuclei (single arrows) (bar = 2 μ m); (C) At higher magnification, cell-cell separation is occurring because of fragility within the desmosomal plaque and small poorly formed desmosomes are being pinched off

(dotted circle). Failure of keratin filaments to connect to desmosomes is also evident (bar = 0.1 μm).

Figure 6.

Clinical and structural abnormalities resulting from DSP haploinsufficiency. (A) Heterozygosity for a nonsense mutation in the *DSP* gene results in striate palmpoplantar keratoderma; linear thickening of the skin can be seen on the fingers; (B) Biopsy from the hyperkeratotic palm reveals massive thickening of the stratum corneum (Richardson's stain; bar = 25 μ m); (C) At higher magnification, keratin filaments in suprabasal layers (region depicted by the double arrow) appear more condensed compared to the cytoskeleton in basal keratinocytes and there is widening of intercellular spaces (Richardson's stain; bar = 10 μ m); (D) In contrast, light microscopy of normal palm skin shows no keratin filament aggregation in suprabasal layers (Richardson's stain; bar = 10 μ m).

Table 1

Epidermal Diseases with Keratin Mutations

Disease	Mutated keratin genes	Histopathologic/clinical features
Epidermolysis bullosa simplex	<i>KRT5/KRT14</i>	Cytolysis of basal keratinocytes; relatively mild blistering tendency as a result of trauma shortly after birth
Epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma)	<i>KRT1/KRT10</i>	Keratinocyte fragility in upper epidermis; erythematous blistering at birth, later development of hyperkeratosis
Linear epidermal nevus	<i>KRT1/KRT10</i>	Epidermal hamartomas representing somatic mosaicism of epidermolytic hyperkeratosis
Diffuse epidermolytic PPK (Vörner syndrome)	<i>KRT1/KRT9</i>	Yellowish thickening of the palms and soles on erythematous base; occasional blistering
Diffuse non-epidermolytic PPK (Unna-Thost disease)	<i>KRT1</i>	Well-demarcated yellow thickening of the entire palms and soles
Ichthyosis bullosa of Siemens	<i>KRT2e</i>	Superficial blistering and shedding of epidermis; later development of dark brown hyperkeratosis
Pachyonychia congenita type I (Jadassohn-Lewandowsky syndrome)	<i>KRT6/KRT16</i>	Dystrophic nails at birth; thickening and blistering of palms and soles; patchy leukokeratosis of the mucous membranes
Pachyonychia congenita type II (Jackson-Lawler syndrome)	<i>KRT6b/KRT17</i>	Nail changes as in PC type I; in addition, multiple steatomas and epidermal cysts; appearance of natal teeth, no oral lesions.
Ichthyosis hystrix (Curth-Macklin syndrome)	<i>KRT1</i>	Severe epidermal hyperkeratosis; failure of keratin IF binding, and retraction of cytoskeleton from nucleus; mutation affects the Y2 tail domain of KRT1
Naegeli-Franceschetti-Jadassohn syndrome (allelic to dermatopathia pigmentosa reticularis)	<i>KRT14</i>	Absence of dermatoglyphics; reticulate hyperpigmentation of skin; thickening of palms and soles; abnormal sweating
Dowling-Degos disease	<i>KRT5</i>	Progressive, disfiguring hyperpigmentation in a reticulate pattern on the flexural areas

Table 2

Hemidesmosomal Variants of Epidermolysis Bullosa

Disease*	Inheritance ⁺	Mutated gene	Characteristic consequences of mutation	Clinical features
EB-MD	AR	<i>PLEC1</i>	PTC	Mild blistering at birth; later-onset progressive muscular dystrophy
EB-PA	AR	<i>PLEC1, ITGB4/ITGA6</i>	PTC	Congenital pyloric atresia; skin blistering of varying severity at birth
EBS-Ogna	AD	<i>PLEC1</i>	Missense	Rare condition; life-long hemorrhagic blistering, no muscular dystrophy
GABEB	AR	<i>COL17A1</i>	PTC, missense	Life-long blistering tendency; scarring alopecia; tooth and nail abnormalities

* EB, epidermolysis bullosa; EB-MD, EB with muscular dystrophy; EB-PA, EB with pyloric atresia; EBS-Ogna, EB simplex Ogna variant; GABEB, generalized atrophic benign EB.

⁺ AD, autosomal dominant; AR, autosomal recessive