

Review Article

Keratins and skin disorders

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Abstract

The association of keratin mutations with genetic skin fragility disorders is now one of the best-established examples of cytoskeleton disorders. It has served as a paradigm for many other diseases and has been highly informative for the study of intermediate filaments and their associated components, in helping to understand the functions of this large family of structural proteins. The keratin diseases have shown unequivocally that, at least in the case of the epidermal keratins, a major function of intermediate filaments is to provide physical resilience for epithelial cells. This review article reflects on the variety of phenotypes arising from mutations in keratins and the reasons for this variation.

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Introduction

The link between keratin intermediate filament proteins and inherited skin fragility disorders was first discovered in the early 1990s [1–3] and proved a turning point in our understanding of intermediate filament function. The growing number of keratin mutations published over the last 10 years as associated causatively with human pathology (more than 400 keratin mutations are now logged on an internet database, www.interfil.org) has begun to draw attention to a number of facts about keratins and keratin disorders which were less well recognized previously.

Firstly, keratin-associated disorders are not as rare as they were thought to be. As more molecular defects are becoming identified, many forms of keratin-associated phenotypes are beginning to be viewed as different points along a single spectrum of mild to severe disease, rather than many separate and unrelated disorders [4]. This is leading towards a coalescence of disease clusters that will have implications for research strategies and funding as well as for health-care budgeting. One can carry out a simple calculation based on a recent estimate of population mutation frequency in the most widely studied keratin disorder, epidermolysis bullosa simplex [5], and extrapolate this to the other keratin diseases where smaller volumes of data exist. This exercise predicts that ‘keratin disorders’ caused by pathogenic keratin mutations in a total of 19 genes so far [6,7], may affect as many as one person in 3000 in the general population.

Similar evolution of molecular pathology has been taking place amongst the collagen diseases [8,9], and

other groups of disorders, such as the limb girdle muscular dystrophies [10], are being reclassified as different types of genes are being identified as causing one sub-form or another. In analysing the molecular mechanisms leading to tissue failure in the keratin disorders, data from one keratin disorder can usually be extrapolated informatively to another [4,6]. In many situations, it will be more useful to consider the keratin disorders as a cluster of hereditary defects involving different members of a large closely-related family of structural genes, rather than a collection of very rare ‘orphan’ diseases.

Another message to emerge from increasing documentation of keratin mutations is that there is an extensive degree of phenotypic variation within the pathology of keratin diseases [4,6]. Firstly, there are profound differences in the phenotypes that result from mutation in different keratin genes, in spite of the close relatedness of these genes. Intermediate filament genes within the same subclass are usually at least 60% identical in sequence [11]. This phenotypic diversity delayed the recognition of the relatedness between different keratin disorders. Secondly, there is well-documented variation in phenotype between different mutations arising in the same gene [12,13], mostly resulting from the position of the mutation within the keratin protein (ie whether in a critical domain for filament assembly or an important protein–protein interaction). Thirdly, and still to be explained, there are also cases of variation in phenotype seen in association with the same mutation in the same keratin gene, such as family members affected to different degrees of severity by a particular keratin mutation [14]. In

other words, modifier genes that can influence a keratin phenotype do appear to exist, but their identity is largely unknown. We will briefly review these levels of variation in keratin disorders affecting the skin.

Phenotypic variation between different keratin genes

Although the keratin genes are all closely related, the effects of mutation in two different keratin genes can be so different as to be not immediately recognizable as related disorders. Keratins are the products of a large gene family, the intermediate filament genes [15]. The family is divided up into six types or subclasses based on the sequence characteristics of the genes and their products, of which keratins make up type I (K9–K20 plus the type I hair keratins) and type II (K1–K8 plus the type II hair keratins) groups [16]. It was recently estimated that there are at least 65 functional intermediate filament genes in the human genome, of which 54 are keratins [17]. Like other intermediate filaments, keratins are characterized by tissue-specific expression patterns which make them useful tools for diagnostic pathology [18–20]. The basic structure of single keratin polypeptides is illustrated in Figure 1, but this overall

structure could apply equally to any other intermediate filament protein. The protein products of this gene family all form α -helical coiled-coil dimers that can rapidly assemble into 10 nm wide filaments without the need for any cofactors or associated proteins other than additional intermediate filament proteins. Most intermediate filaments will assemble as homopolymers, but keratin homodimers are very unstable and heterodimers must be formed to polymerize into filaments. Keratin filaments always consist of equimolar amounts of a type I protein and a type II protein [21], and the keratins are expressed in cells as specific pairs according to the differentiation programme of the cell. Single keratins on their own will not assemble into filaments but are rapidly degraded [22], which helps to maintain the balance between specific type I/type II keratins in a cell.

This differentiation-specific expression of keratins is very apparent in skin and related structures, and is the basis for the phenotypic differences seen between the effects of mutating different keratin genes. An overview 'map' of the major keratin expression patterns in the skin epithelia is shown in Figure 2. Keratin genes implicated in human disorders now include nearly all of the keratins expressed as major proteins in any population of keratinocyte-related cells in stratified squamous or complex epithelia, as well as,

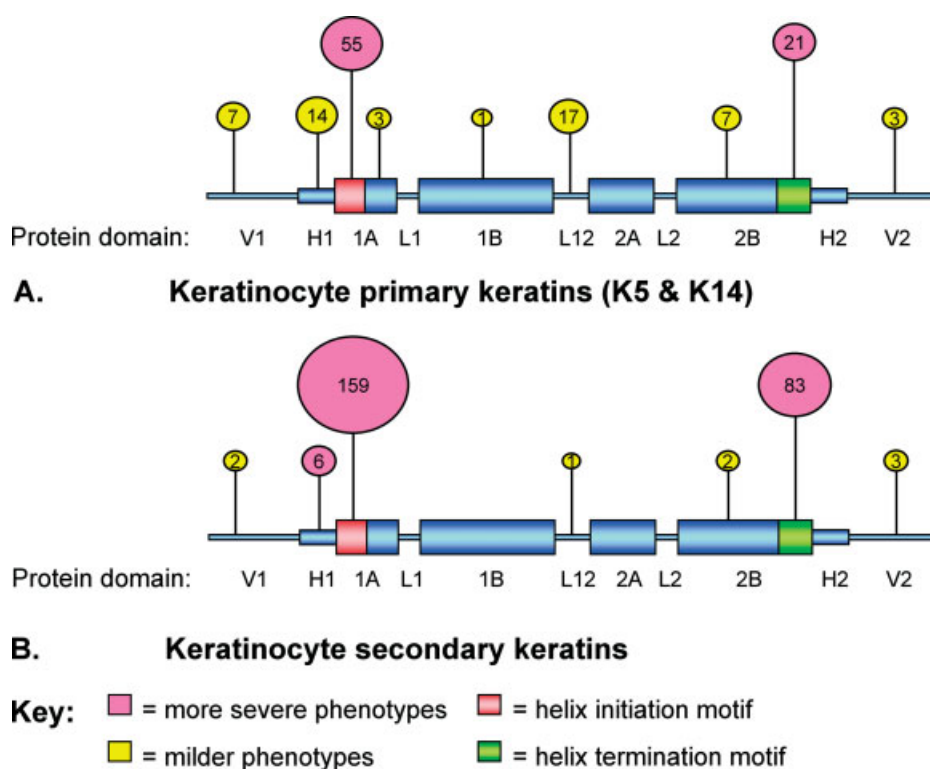


Figure 1. Diagrammatic representation of keratin intermediate filament proteins to show major protein domain structure and the distribution of pathogenic mutations reported to May 2004 in (A) primary keratins (K5 and K14) and (B) secondary or differentiation-specific keratins expressed by keratinocyte-type cells of various stratified squamous epithelia (K1/K10/K9/K2e, K6/K16/K17, K4/K13, K3/K12). Numbers of mutations are indicated (above) for each sub-domain (labelled below the protein structure). Severe mutations (eg leading to Dowling–Meara type EBS for K5/K14) are indicated in red; milder mutations are indicated in yellow. The α -helical rod domains encompass 1A to 2B and the major cluster sites for the most pathogenic mutations are localized in the helix boundary motifs at either end of domains 1A and 2B. For a comparison with similar data from simple epithelial keratins, see Owens and Lane [23]. Data taken from the Intermediate Filament Database, <http://www.interfil.org>

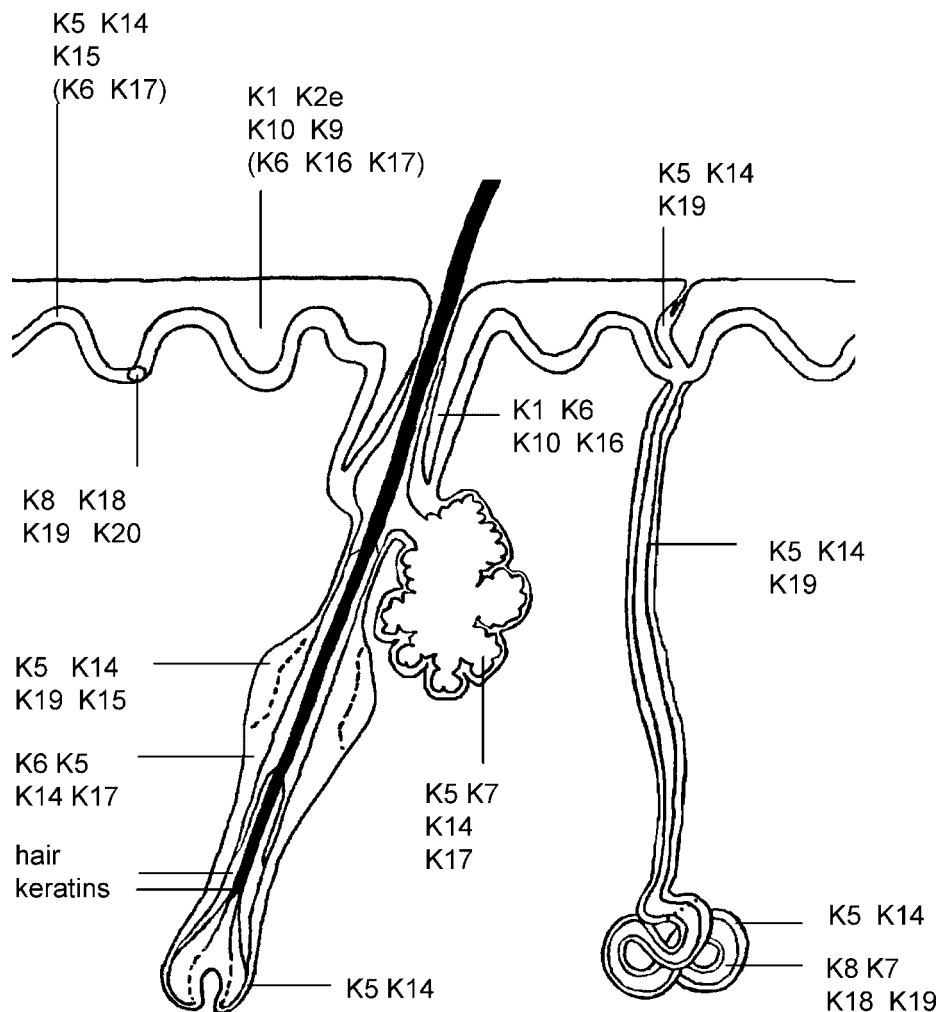


Figure 2. Summary of the major consensus patterns of keratin expression in epidermis and epidermal appendages. This map is not exhaustive as new keratins are still emerging from the human genome

to a lesser extent, simple epithelial keratins K8 and K18 (associated with liver, gut, and pancreas disorders — see Owens and Lane [23]). In this review article we will review the disorders known to be caused by mutations in keratinocyte keratins associated with various different subpopulations of cells in the skin and other external barrier layers.

K5/K14 and epidermolysis bullosa simplex

Keratinocytes are the predominant cell type in the stratified squamous keratinizing epithelium of the epidermis. They begin their progressive differentiation in the basal layer attached to the basal lamina of extracellular matrix by hemidesmosomes, and to each other by desmosomes, into which the keratin filaments are linked. The basal keratinocytes are undifferentiated and still capable of proliferation, and this cell compartment includes some of the stem cells of the epidermis as well as transit amplifying cells. These cells all express K5 (type II) and K14 (type I) as their major, primary keratins. In addition, stable basal cells also express K15 [24,25] and at some locations and in certain circumstances, K17 and K19 may be expressed [26–28].

Mutations in K5 or K14 cause epidermolysis bullosa simplex, in which the basal cells are fragile and may fracture if the epidermis is subjected to even quite mild physical trauma such as rubbing or scratching (Figure 3); this intraepidermal cytolysis of the basal keratinocyte cells leads to fluid-filled blisters. There are different distributions and degrees of severity of skin blistering, traditionally regarded as being clinically distinct, that come under the heading of epidermolysis bullosa simplex (EBS). All three forms can show associated palmoplantar keratoderma, with the epidermis and stratum corneum becoming greatly thickened. Dowling–Meara EBS is the most severe form, diagnosed by the appearance of clustered herpetiform blisters from birth at friction sites anywhere on the body, and can be life-threatening in neonates (Figure 3). This form is also associated with the appearance of electron-dense aggregates [29] of K5/K14 keratin protein [30] in the cytoplasm of some of the basal keratinocytes by electron microscopy; the aggregates may or may not predispose those particular cells to rupture. These aggregates used to be the definitive diagnostic criterion for Dowling–Meara EBS [29].



Figure 3. Examples of the clinical phenotypes arising from mutations in different keratins expressed in specific sub-compartments of the stratified squamous epithelia. (A) An infant with the severe Dowling–Meara variant of epidermolysis bullosa simplex. This patient carries heterozygous missense mutation R125C in the K14 gene, the most common ‘hotspot’ defect in this disorder. (B) A patient with the milder, site-restricted Weber–Cockayne form of epidermolysis bullosa simplex, showing blisters on the soles of the feet. (C) The feet of a patient with bullous congenital ichthyosiform erythroderma, showing severe epidermolytic hyperkeratosis. This patient carries a mutation in the K1 gene. (D) This young child suffers from pachyonychia congenita type I, in this instance due to a missense mutation in the K16 gene. The palmoplantar keratoderma in this condition is focal — occurring mainly on the pressure points. (E) Epidermolytic palmoplantar keratoderma is a form of epidermolytic hyperkeratosis restricted to the palms and soles, where the causative gene, K9, is exclusively expressed. This patient has a 3 base-pair insertion mutation leading to an additional amino acid in the highly conserved helix termination motif of the K9 polypeptide. (F) The main feature of pachyonychia congenita types I and 2 is hypertrophic nail dystrophy, seen here in an infant with PC-I caused by a K16 mutation. Figures courtesy of Sue Morley, Peter Steijlen, Colin Munro, and Irene Leigh

Milder forms of EBS are classified as Köbner (generalized blistering) and Weber–Cockayne EBS (targeting hands and feet). The variations in clinical severity of the different forms of EBS generally correlate well with the position in the protein at which the mutation occurs (Figure 1 and see below), as observed previously [13,31]. Mutations in K5 or K14 have been identified in about three-quarters of all the patients diagnosed clinically as having EBS, resulting in 129 separate cases of autosomal dominant mutations currently catalogued in the www.interfil.org database, 62 of which are in K5 and 67 in K14. No mutations in

any other keratins have been found to lead to basal cell blistering in the epidermis, although partially similar phenotypes can be caused by mutations in keratin-associated proteins such as the desmosomal protein desmoplakin [32] or the hemidesmosome component plectin [33,34].

K1/K10 and bullous congenital ichthyosiform erythroderma

The switch from undifferentiated basal keratinocyte to a keratinocyte committed to differentiation involves

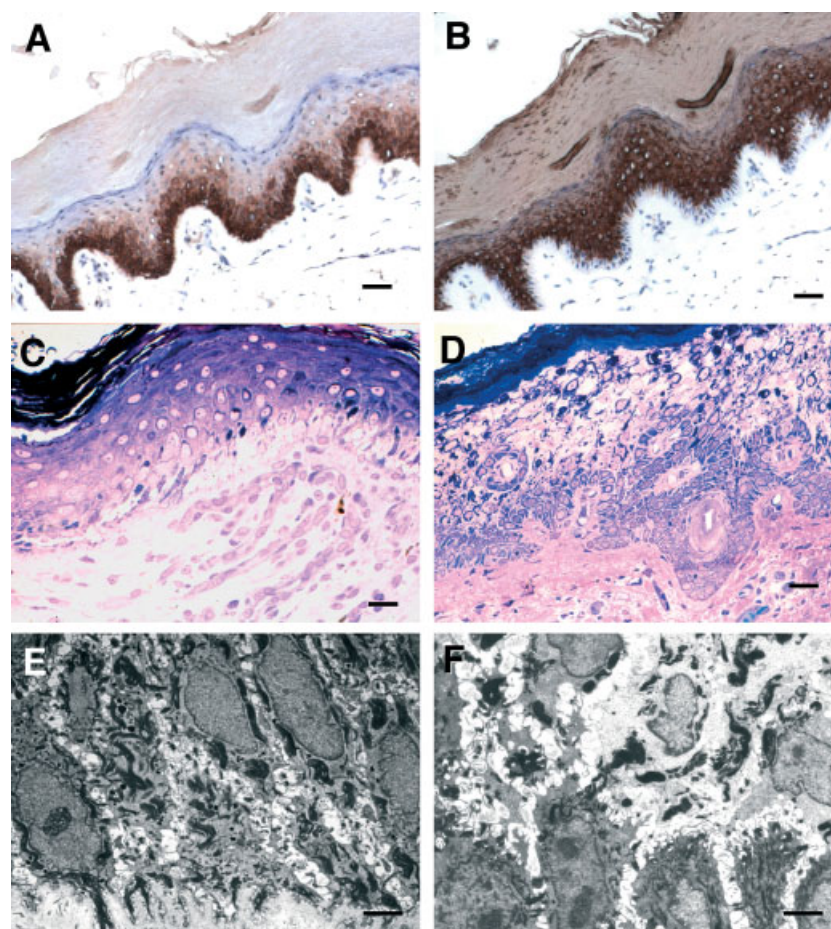


Figure 4. Histological effects of mutation in the major epidermal keratins. Expression of K5 (and K14, not shown) in the basal cells of epidermis (A) is correlated with the cytolysis seen in basal keratinocytes only in patients with epidermolysis bullosa simplex (C). By electron microscopy, basal cell filaments can be seen to be clumped and irregularly distributed in these cells (E). In contrast, K10 (and K1, not shown) is expressed in suprabasal cells (B) and mutations in this keratin lead to fragility of the suprabasal cell layers (D): filaments are clumped and cells are disrupted in the suprabasal layers whilst the basal cells remain intact (F). Primary antibodies used are AE14 to K5 (panel A) and LHPI to K10 (panel B). Scale bars = 50 μm (A, B), 20 μm (C, D), and 0.25 μm (E, F). Panels C–F courtesy of Robin Eady

a conformational change in integrin extracellular matrix receptors and a change in keratin synthesis programme, accompanying reduced adhesion to the basal lamina. The order of these events is unproven but it seems possible that the change is triggered by mechanical pressure in the basal layer of cells. The nature of the change in keratin synthesis depends on the body site, but in interfollicular epidermis the induction of synthesis of first K1 and subsequently K10 is seen as synthesis of K5 and K14 is shut down. K1 and K10 are the major secondary differentiation-specific keratins of interfollicular epidermis and are expressed by suprabasal epidermis and any other stratified squamous epithelia that becomes orthokeratinized. Expression of K1/K10 appears to inhibit cell proliferation [35] and cells moving up into the suprabasal layers become post-mitotic, and progressively more terminally differentiated as they continue their journey up towards the epidermal surface.

Mutations in keratins K1 and K10 are associated with bullous congenital ichthyosiform erythroderma (BCIE), also sometimes referred to as EH or EHK (epidermolytic hyperkeratosis, the principal clinical

feature of this disorder). In this disorder, blisters and reddened skin are often seen at or soon after birth, but with passing time the blisters give way to increasingly thickened ichthyotic skin. Instead of the basal cell layer being fragmented as in EBS, the basal layer remains intact but the suprabasal cells become fragmented easily (see Figure 4). The epidermis becomes hyperproliferative, probably in response to cytokines trickling from the chronic partial wound stimulus of suprabasal cell fragmentation. The stratum corneum becomes unusually thick, producing the clinical phenotype of ichthyosis. The keratin mutations in K1 and K10 which lead to these disorders are found predominantly in the helix boundary motifs of either keratin [36–38], in the H1 region of K1 [39], and occasionally in the L12 linker region [40].

There are currently 35 reported mutations in K1 and 47 in K10, as registered in the intermediate filament database (www.interfil.org). Because there is so much similarity between the sequence features and the morphology of all intermediate filament proteins, extrapolations can be made between the molecular consequences of these clinically diverse disorders.

The mutation cluster sites emerging for K1 and K10 are mostly those which in EBS would indicate a severe mutation, whereas relatively few mutations are emerging in the 'mild' hotspot sites. The most likely reason for this is that K5 and K14 proteins persist in the suprabasal layers even though new protein synthesis has stopped when cells lose contact with the basal lamina. The stable proteins K5 and K14 probably provide a scaffold for the assembly of K1 and K10 [41,42]. This gives an extra level of structural reinforcement in these cells, such that 'mild' mutations may not produce any phenotype and the only mutations to be pathologically visible are those in the 'severe' mutation cluster sites.

K2e and ichthyosis bullosa of Siemens

Suprabasal keratinocytes can express other secondary keratins in various body sites. Late in differentiation of the interfollicular epidermis, K2e is expressed in suprabasal keratinocytes [43], and mutations in this keratin have been associated with another form of epidermal blistering and superficial skin thickening known as ichthyosis bullosa of Siemens (IBS) [44–46]. Similarly to K1/K10, mutations in K2e have been mostly reported to lie within the rod ends, with the majority in helix 2B. None have been found so far in the H1 or L12 domains. There have been 26 mutations reported in K2e so far (www.interfil.org) and some of these mutations have also been found to give rise to a BCIE-like phenotype [46,47].

K9 and epidermolytic palmoplantar keratoderma

Keratin 9 is a type I keratin expressed in suprabasal cells in the epidermis of palm and sole [48], where it may contribute a specific reinforcing effect to withstand the greater stress of these skin regions [27]. K9 was thus a strong candidate gene for palmoplantar-specific keratoderma, and is indeed the source of a number of mutations causing this disorder [49–51]. A total of 38 mutations have been reported in K9 in association with epidermolytic palmoplantar keratoderma, ie thickening of the palm and sole epidermis with a degree of cytolysis. As with the other keratodermas, it may be the cytolysis that results in proliferation signals to the epidermis in an aberrant wound response.

K6, K16, and K17 in pachyonychia congenita

Stress response keratins K6, K16, and K17 are rapidly induced on injury or inflammation, and are also constitutive components of the epithelium in several epidermal appendages such as hair follicle and nail [16,52,53]. Mutations in the stress response keratins give rise to pachyonychia congenita, characterized by grossly thickened nails [54]. Type 1 pachyonychia congenita (Jackson–Lawler type, or PC-1) patients have thickened nails plus white plaques in the mouth and other orogenital epithelia, frequent follicular hyperkeratosis, and pronounced keratoderma on the hands

and feet [55]. This disorder is linked to mutations in K16 (12 reported) and K6a (13 reported in the database) [54–56]. Pachyonychia congenita type 2 (Jadassohn–Lewandowsky form) is linked to K17 (23 reported mutations) and also to one of the K6 genes, K6b (one report) [54,57]. PC-2-affected individuals have thick nails without prominent oral leukoplakia but can also have pili torti (twisted hairs), pilosebaceous cysts, and a high incidence of natal teeth, ie teeth erupted or exposed prematurely at birth [55]. These two different phenotypes are closely correlated with the cell and tissue types in which these keratins are expressed constitutively: whilst K16 is a major secondary keratin in orogenital epithelia and in palmoplantar epidermis [27], K17 is only a minor component of these tissues in the fully developed epidermis but is significantly expressed in the deep hair follicle where the hair shaft is being formed [52]. It is likely that the cellular weakness resulting from K17 mutations leads to breakdown of the outer root sheath epithelial cells and loss of the constraints of the deep hair follicle during anagen, which normally has the effect of helping to mould the shape of the forming hair shaft. There is another condition known as steatocystoma multiplex, in which cysts are formed in association with the hair follicle. This disorder was previously regarded as an unrelated clinical entity, but it is now known to be caused by mutations in the same K6b and (or) K17 genes as PC-2 [14,58]. The follicular keratoses associated with mutations in K6a represent a milder, more superficial form of cysts than the steatocystoma multiplex phenotype.

K3/K12 in Meesmann epithelial corneal dystrophy

Keratins K3 and K12 are expressed uniquely in the anterior corneal epithelium and mutations in these keratins give rise to a condition known as Meesmann epithelial corneal dystrophy [59–61]. This condition is also characterized by a cell fragility phenotype. Small cysts, which are detectable in the clinic with a slit lamp, form within the corneal stratified epithelium where intraepithelial cytolysis has taken place. To date, there have been 12 mutations in K12 reported but only one in K3 (www.interfil.org). Again these mutations are found in the helix boundary motifs of the keratin protein, and as with the other disorders involving secondary or differentiation-specific keratins, the mutations recorded to date are dominant ones.

K4/13 in white sponge naevus

Although white sponge naevus (WSN) is not strictly a disorder of skin, the genes responsible are expressed by oral keratinocytes (following the mucosal path of differentiation). WSN is a benign condition affecting buccal mucosa and other orogenital epithelia, producing plaques of loosened white epithelium. Histologically, the resemblance of these lesions to those of PC-1 and BCIE is very clear: suprabasal cells fragment and

the thickened epithelium appears white. This disorder was discovered to be caused by dominant mutations in K4 and K13, the pair of secondary keratins that are characteristic of this type of mucosal, parakeratinized stratified squamous epithelium [62–64]. These and other mutations identified to date in WSN all lie in the helix boundary motifs of either of these two keratins, with three mutations reported for K4 and five mutations reported for K13 (see www.interfil.org for details).

Disorders caused by hair keratins

Keratins K1–K20 are all expressed in the ‘soft’ epithelial sheet tissues of the body that line and delimit not only the exterior surface, but also internal ducts and glands. There are a further 20 or so keratins that have now been identified within the cells of ‘hard’ keratin tissues of hair, nail, filiform papillae of the tongue, and possibly Hassall’s corpuscle of the thymus [16,65,66]. If the main consequence of keratin mutations is to render the cells expressing them fragile and vulnerable to trauma-induced cytolysis, then it might be expected that such cell breakdown would be less likely to be apparent in these hard tissues where the keratin intermediate filaments are usually embedded in a matrix of cross-linked, oxidized specialized proteins of highly cornified structures. However, even here there are now known to be examples of keratin mutations that lead to structural tissue defects. Monilethrix is a condition in which the hair shaft is both fragile and develops with a periodic beaded appearance. Mutations have been identified in monilethrix families in the helix initiation motif of two type II hair keratin genes, hHb6 [67,68] and hHb1 [69]. The current mutation count stands at 33 for hHb6 and seven for hHb1 (www.interfil.org).

Another condition has recently been identified as being associated with a sequence variation in a hair follicle keratin [7]. This is pseudofolliculitis barbae, a shaving-induced condition in which hairs do not grow straight out but curl under the skin, giving rise to an inflammatory reaction to the ingrown hair. There is a strong association of this condition with a potentially disruptive polymorphism in the helix initiation motif of keratin K6hf, a K6 gene that is expressed in hair follicles. It appears that this sequence variant predisposes some individuals to this reaction.

Simple epithelial keratins

In the skin and skin appendages, simple epithelial keratins are expressed in very few locations. K8 and K18 are found in sweat gland secretory cells; K7 is found in sebaceous gland and sweat gland and has been reported in some cells in the hair follicle; and K19 is found in sweat gland secretory and duct cells and in the bulge region of the outer root sheath of the hair follicle [28,70]. All the four keratins have been reported to be expressed in Merkel cells [71],

but K20 is not otherwise found in skin. No mutation in any of these keratins has yet been proven to directly and singly cause any human pathology, either in skin or elsewhere, although there is evidence for an association with defects in the liver, pancreas, and intestinal epithelium (see review article by Owens and Lane [23]).

Phenotypic variation from mutations within one keratin gene

Phenotypic variation can also be seen between cases of keratin disorders caused by different mutations within the same keratin gene, even between members of the same family. These clinical variants are largely dependent on the position of the mutation and, to a lesser extent, the type of mutation, within the keratin protein domain structure (Figure 1). Therefore, the longer-term clinical outcome is predictable to some extent where the mutation is known.

Diverse phenotypes of K5/K14 mutations

The original template for genotype–phenotype correlation in keratin disorders has come from studies of the autosomal dominant forms of epidermolysis bullosa simplex (EBS). The vast majority of mutations in Dowling–Meara EBS patients are missense or small in-frame deletions occurring in the helix boundary motifs of keratins K5 and K14 [2,3], as shown in Figure 1. These are the two conserved sequence motifs that mark either end of the α -helical rod domain. The rod domain is essentially the construction unit of the polymeric filament and the sequences at the rod ends are critical for correct subunit alignment and docking during filament assembly [72]. There is a particularly prominent mutation hotspot in the codon for the arginine amino acid at position 125 in K14, resulting from an intrinsically unstable CpG dinucleotide in the DNA. This arginine/CpG dinucleotide is conserved in many type I keratins and the analogous mutation hotspot appears in many of the keratin diseases and accounts for a significant proportion of all pathological keratin mutations.

The two milder forms of EBS are caused by mutations outside the helix boundary peptides. One is the Köbner form, which is characterized by blisters occurring anywhere on the body but not necessarily in clusters. The other is the milder Weber–Cockayne type of EBS, in which blisters are generally restricted to the hands and feet. ‘Mild’ mutation clusters associated with these forms are recognizable in the second half of the 1A domain, the L12 domain, and central 2B domain of K5 and K14 [73,74] (summarized in Figure 1). The H1 sub-domain, which is absent from type I keratins, is an additional mutation hotspot for these milder phenotypes. There is also a recurrent mutation in the V1 domain of K5 that has consistently been associated with the EBS-mottled pigmentation

phenotype — another mild clinical variant [75,76]. In our experience, clinical severity prediction is more difficult for mutations occurring at the borders between these hotspots, such as mutations in the middle of the 1A domain, as one might expect.

All these cluster sites must reflect places in the protein molecules where sequence drift is not tolerated because this part of the protein is essential for correct function, although the consequences for function are milder and more subtle for the Weber–Cockayne mutations than for the Dowling–Meara ones (see Figure 2). The absence of mutations in other regions presumably means that mutations in these other places are not pathogenic and are therefore clinically silent.

Diverse phenotypes of K1 mutations

Genotype–phenotype correlation has been slower to emerge for keratins other than K5 and K14; however, certain trends are now evident, particularly for K1, where a wide range of overlapping phenotypes is well established. Mutations in K1 and its partner K10 were originally shown to cause bullous congenital ichthyosiform erythroderma (BCIE) [36–39]. This disorder is characterized clinically by blistering and erythroderma in infancy and severe generalized epidermolytic hyperkeratosis in adulthood (Figure 3). Recently, a number of patients have emerged with K1 mutations where epidermolytic hyperkeratosis is almost exclusively limited to palms and soles, a phenotype known as ‘K1 keratoderma’ [77,78]. Some of these mutations are larger in-frame deletions affecting the helix boundary motifs and are therefore different from the classic missense mutations in the same domains commonly reported in BCIE. Similarly, frameshift mutations in the V2 domain of K1 have been found in striate keratoderma [79] and in a phenotype resembling ichthyosis hystrix of Curth–Macklin [80]. In other cases, however, the genotypic difference is less obvious. For example, substitutions of amino acid I479 in K1 have variously been associated with BCIE-like, milder ichthyosis-like phenotypes [81] and K1 keratoderma [82]. Thus, phenotype prediction is less clear-cut with K1 mutations compared with EBS. In terms of K10 mutations, relatively few milder mutations have been reported to date. It has been suggested that since K1 is the probable partner of both K9 and K10 in palmoplantar epidermis and the partner of K10 elsewhere in the skin, K1 mutations are more likely to result in palmoplantar keratoderma phenotypes.

Diversity of phenotypes with K16 and K17 mutations

Outside of the major epidermal keratins, K5/K14 and K1/K10, less is known about variations in phenotype, possibly since these groups of keratins are limited to smaller cell populations. One recent emerging trend comes from pachyonychia congenita (PC) and involves later onset of this keratinizing disorder,

described as PC-tarda [83] in association with less disruptive mutations. The first example was a patient with PC-1 in whom nail dystrophy did not appear at birth (as is normally the case) but began in the second decade of life. The mutation in this case was located in the central 2B domain of K16, a region that in the K14 molecule is associated with mild EBS phenotypes [84]. The second example involves a family with late-onset PC-2, where the causative mutation was in the second half of the 1A domain of K17, again a region associated with mild EBS [85].

Two trends are therefore emerging in relation to genotype–phenotype prediction in the keratin disorders. Firstly, less disruptive mutations may lead to site-restricted variants of a given disease such as EBS-WC, EBS-K or K1 keratoderma. Secondly, milder mutations may lead to later onset of clinical symptoms, as appears to be the case in PC-tarda. Some keratin disorders are clearly subject to hormonal influences, notably PC-2, where pilosebaceous cysts occur only after puberty [55], presumably when the sebaceous glands become active. There are also reports, albeit still anecdotal, of changes in the clinical manifestation of keratin disorders during pregnancy. There may be other more subtle hormonal or longer-term developmental effects that influence the overall clinical presentation arising from a given keratin defect. Understanding these modifying factors may provide useful insights into therapy design for this group of genetic diseases.

Milder phenotypic variants have not yet been described for a number of keratin disorders, eg ichthyosis bullosa of Siemens or white sponge naevus. It is quite possible that milder forms of these diseases, which are already site-restricted and fairly benign, may not produce any clear pathology. This certainly appears to be the case in families affected by Meesmann epithelial corneal dystrophy, where a number of completely asymptomatic individuals have been shown to carry ‘strongly pathogenic’ mutations (as predicted from their position in the keratin molecule) in the K12 gene [59,86]. Many, if not all, of these symptom-free patients have visible microcysts by slit-lamp examination but for some unknown reason these do not cause discomfort or visual impairment in certain individuals. It is therefore quite likely that less disruptive mutations in K3 or K12 might go unnoticed and therefore be indistinguishable from polymorphisms on the basis of clinical phenotype alone.

Variation between cases with the same mutation

There is further evidence for the existence of genetic modifying factors influencing the phenotypes in keratin disorders in cases where phenotypes of quite different severity result from the same mutation arising in two different families, or even in the same family. One well-documented example was a report of one kindred

with the classic PC-2 phenotype and another unrelated family with steatocystoma multiplex but no nail dystrophy, both of whom had the same mutation, R94C, in the helix initiation motif of K17 [14]. The phenotypes were consistent within each of the two families, implying a genetic background effect.

In another example, radically different phenotypes arose from essentially the same frameshift mutation in the V2 domain of K1: relatively mild striate keratoderma in one family [79] and extremely severe ichthyosis hystrix in another [80]. In this instance, the two families were of different ethnicity, again pointing to an effect at the population level. These examples, taken with the strain-dependent phenotypes observed in transgenic mouse models of keratin diseases [87,88], point to the existence of a variety of genetic modifying factors that attenuate or exacerbate keratin disease phenotypes.

Recently, there has been a report of a second modifying mutation within a keratin gene [89]. In this study, a family presented with several members affected by autosomal dominant EBS, some of whom were mildly affected, others quite severely affected. All the affected persons carried a mutation in the 1A domain of K5, E170K. The more severely affected individuals also carried a second mutation on the other K5 allele, E418K in the centre of the 2B domain. People in the family carrying only the latter mutation had no skin blistering phenotype, although the E418K mutation was shown to produce a low level of keratin aggregation when expressed in cultured cells. Neither of these mutations was detected in 100 ethnically matched chromosomes and so were not common sequence variants in the population. Thus, the E418K mutation acts as a polymorphism in the heterozygous state but is able to exacerbate a mild phenotype when in the compound heterozygous state [89]. It is not known what the effect of a homozygous E418K mutation would be in humans but it is quite possible that this might produce mild recessive EBS, similar to other reports in the literature [90]. This study points the way towards understanding at least some of the factors involved in generating phenotypic variation within a kindred with a given mutation. Very often, genetic testing laboratories do not fully screen genes but halt the screening process as soon as a mutation is detected and confirmed. This is a particularly common practice in keratin gene screening since analysis generally starts with the most prevalent mutation hotspots (Figure 1), and only when these are negative are the other exons or other keratin gene screened. Thus, important modifying factors within a keratin pair can easily go unnoticed.

In addition to other keratin genes, a whole range of keratin-associated proteins are good candidates for genetic modifying factors. For example, loss-of-expression mutations in plectin, a cytoskeletal cross-linker protein found in hemidesmosomes, cause a mild form of EBS with muscle disease [33,34]. Dominant plectin mutations have recently been implicated

in EBS in the absence of a muscle phenotype [91]. It is therefore possible that polymorphisms or recessive mutations in this gene might modify an EBS phenotype resulting from a keratin mutation. Similarly, keratins are linked to desmosomes by a variety of proteins, including desmoplakin, plakoglobin, and plakophilin. Mutations in all of these genes give rise to skin fragility phenotypes [32,92,93] and so a recessive allele of any of these genes might be expected to modify the phenotype of EBS and, indeed, all other keratin disorders since desmosomes are prominent structural components of all epithelia. A candidate gene approach in suitable families where mild and severe phenotypes can be readily distinguished is probably the only way that phenotypic modifying factors can be identified in humans, since it is rare that large enough pedigrees with such variant phenotypes for a genetic linkage approach are identified (we have not succeeded in identifying one yet). However, mouse models of keratin disorders where strain-dependent phenotypes are present are very suitable for genetic linkage mapping and so these types of studies may eventually lead back to modifiers in humans. The search for these factors, especially the unknown and less predictable ones, is worthwhile since it may pave the way to novel gene-based or pharmacological therapies.

Summary

The keratin gene defects affecting skin and associated structures illustrate the ability of molecular genetics to shed light on the *in vivo* functions of a sub-cellular system — in this case, the intermediate filament cytoskeleton. This polymeric protein network had been well studied biochemically and in cultured cells, but it was a major event in the field when the first human disease associations and transgenic mouse models revealed, in a highly conclusive manner, the critical structural role that keratin filaments play in maintaining epithelial cell integrity in a range of high-stress tissues. The challenge now is to determine the tissue-specific functions of the individual keratin expression pairs: presumably all keratins are not equal, and we need to identify their important functional differences. Only then can we properly address the next major challenge in the application of this understanding to human disease — ie how one can influence keratin gene expression therapeutically, by either gene-based or pharmacological strategies, and perhaps treat some of these incurable and distressing genetic conditions.

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