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We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.
Keratin gene mutations in disorders of human skin and its appendages

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A B S T R A C T

Keratins, the major structural protein of all epithelia are a diverse group of cytoskeletal scaffolding proteins that form intermediate filament networks, providing structural support to keratinocytes that maintain the integrity of the skin. Expression of keratin genes is usually regulated by differentiation of the epidermal cells within the stratifying squamous epithelium. Amongst the 54 known functional keratin genes in humans, about 22 different genes including, the cornea, hair and hair follicle-specific keratins have been implicated in a wide range of hereditary diseases. The exact phenotype of each disease usually reflects the spatial expression level and the types of mutated keratin genes, the location of the mutations and their consequences at sub-cellular levels as well as other epigenetic and/or environmental factors. The identification of specific pathogenic mutations in keratin disorders formed the basis of our understanding that led to re-classification, improved diagnosis with prognostic implications, prenatal testing and genetic counseling in severe keratin genodermatoses. Molecular defects in cutaneous keratin genes encoding for keratin intermediate filaments (KIFs) causes keratinocytes and tissue-specific fragility, accounting for a large number of genetic disorders in human skin and its appendages. These diseases are characterized by keratinocytes fragility (cytolysis), intra-epidermal blistering, hyperkeratosis, and keratin filament aggregation in severely affected tissues. Examples include epidermolysis bullosa simplex (EBS; K5, K14), keratinopathic ichthyosis (KPI; K1, K2, K10) i.e. epidermolytic ichthyosis (EI; K1, K10) and ichthyosis bullosa of Siemens (IBS; K2), pachyonychia congenita (PC; K6a, K6b, K16, K17), epidermolytic palmo-plantar keratoderma (EPPK; K9, (K11)), monilethrix (K81, K83, K86), ectodermal dysplasia (ED; K85) and steatocystoma multiplex. These keratins also have been identified to have roles in apoptosis, cell proliferation, wound healing, tissue polarity and remodeling. This review summarizes and discusses the clinical, ultrastructural, molecular genetics and biochemical characteristics of a broad spectrum of keratin-related genodermatoses, with special clinical emphasis on EBS, EI and PC. We also highlight current and emerging model tools for prognostic future therapies. Hopefully, disease modeling and in-depth understanding of the molecular pathogenesis of the diseases may lead to the development of novel therapies for several hereditary cutaneous diseases.

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Structure and function of the skin

The human skin is the largest organ of the body, being strategically located at the interfacebetween the interior and exterior, it provides the first line of defense against external insults. Histologically, the skin reveals a built-in synchrony in the outer most epidermis, delineates the existence of a tremendous degree of coordination between tissue-cell layers, and culminates to mediate a multiplicity of functions. From outside-in the skin is composed of a multilayered, non-vascularized, stratified squamous epidermal tissue overlying the thick fibrous connective dermal tissue, which then sits on the hypodermis (Fig. 1a). The multilayered epidermal sheet comprises four (five in palmpoplantar skin) distinct cell layers. The basal layer contains the partially characterized epidermal keratinocyte stem cell population, which divide and are committed to terminal differentiation as they move up, forming the suprabasal and upper layers of the epidermis. The thicker spinous (suprabasal) layers overlay the basal layer, and as cells move up upon commitment to terminal differentiation, they gradually become flattened as they enter the granular layer where they collapse and commence nuclear and organelle degradation and active lipid and protein secretion. This protein and lipid secretion together with cell flattening is critical in maintaining the barrier function of the
Intermediate filaments (IFs) and the keratin IF (KIFs)

The cell cytoskeleton of all multi-cellular organisms consists of three abundant filament systems, which play important roles in the organization and mechanical integrity of tissue cells: the actin microfilaments (MFs; 7–10 nm diameter), intermediate filaments (IFs; 10–12 nm diameter), and interconnected microtubules (MTs; 25 nm diameter). Each filament system is built from a family of proteins with cell-tissue specific regulation of expression, with each protein family being encoded by the corresponding gene family. IFs are by far the most complex of the cytoskeletal proteins (MTs; 25 nm diameter). Each filament system is built from a family of proteins with cell-tissue specific regulation of expression, with each protein family being encoded by the corresponding gene family.

IFs are by far the most complex of the cytoskeletal proteins with at least 60 different IF proteins subcategorized into six broad entities of keratinocytes which form the epidermis. KIFs are encoded by large, well conserved multigene family coding for proteins that form a network of 10–12 nm wide, and represents about three-quarters of all known IF in humans. KIFs build into a dense, three-dimensional transcellular and highly dynamic cytoskeleton network structure spanning within the nucleus and extending to the cell periphery, where they anchor and interact with cell–cell (desmosomes) and cell–matrix (hemidesmosomes) adhesion complexes (Fig. 1b) [3,4]. This organization provides structural stability, flexibility, and ensures the mechanical integrity of the different epithelial cells and tissues. The keratins vary in size between 40–70 kDa and are divided into two groups based on molecular weight: the smaller or low molecular weight acidic type I (40–64 kDa, with PI: 4.7–6.1) and the larger or high molecular weight neutral-basic type II (52–70 kDa, with PI: 5.4–8.4) subgroups of IF proteins [5]. The newly established consensus nomenclature for mammalian keratin genes and proteins is grouped into three categories: (1) epithelial keratins, (2) hair keratins and (3) keratin pseudogenes [6,7]. This nomenclature now includes 28 type I (KRT1, KRT10, KRT12–KRT20, KRT23–KRT28, KRT31–KRT40) genes coding for (K9, K10, K12–K20, K23–K28, K31–K40) and 26 type II (KRT1–KRT8; KRT71–KRT86) genes coding for (K1–K8; K71–K86) keratins. In the human genome, the genes encoding type I and type II keratins are mainly clustered at two different loci on chromosomal regions 17q12–q21 and 12q11–q13, respectively. The epidermal type I keratin genes e.g. KRT1, KRT2 and KRT5, each comprise 9 exons, whereas the genes coding for epidermal Type II keratins e.g. KRT10 and KRT14, each consist of 8 exons (see www.interfil.org and [8]).

Structural and assembly properties of KIFs

Similar to all IFs, keratins share a head-rod-tail structural domain organization, with the basic polypeptide structure consisting of a central α-helical coiled-coil rod domain of about 310 amino acids in size. This central rod domain consists of four helical segments (1A, 1B, 2A and 2B) that are interrupted by three short non-helical flexible linker regions (L1, L12 and L2), and are flanked by variable, non-helical amino-terminal head and carboxy-terminal tail domains (Fig. 2a). The rod domain consists of a repeated sequence of seven amino acid residues (a-b-c-d-e-f-g)n, known as “heptad repeats”. The positions “a” and “d” are known to be occu-

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1 Abbreviations used: KIFs, keratin intermediate filaments; IFs, intermediate filaments; HIP, helix initiation motif; HTP, helix termination peptides; ER, epidermolysis bullosa; BMZ, basement membrane zone; EBS, epidermolysis bullosa simplex; NFJS, Naegeli–Franceschetti–Jadassohn syndrome; DPR, dermatopathia pigmentosa reticularis; DDD, Dowling-Degos disease; PTC, premature termination codon; KPI, keratinopathic ichthyosis; BCIE, bullous congenital ichthyosiform erythroderma; EHRI, epidermal hyperkeratosis; RNEM, ichthyosis hystrix of Currhy-Macklin; SPPK, striate palmo-plantar keratoderma; IBS, ichthyosis bullosa of Siemens (IBS); SEI, superficial epidermolytic ichthyosis; PPK, palmo-plantar keratoderma; PFB, pseudofoliculitis barbae; PC, pachyonychia congenita; IPCRR, International PC Research Registry; ED, ectodermal dysplasia.
The heterodimers align laterally in an overlapping staggered and antiparallel fashion, forming tetramers which then polymerize to elongated chains and form KIFs via lateral stacking [15] (Fig. 2b).

General features of hereditary cutaneous keratin diseases

In addition to providing structural function, it is evident from human disorders and mouse genetics that the keratin cytoskeleton has more complex roles, which have been associated with additional five broadly defined functions: regulation of apoptosis, cell architecture, stress response, protein synthesis and organelle and vesicle (re)distribution functions [16]. They form complex signaling networks, interacting with various kinases, adaptors and apoptotic proteins to regulate cell growth and cell cycle progression [17,18]. They likely regulate apoptosis via various mechanisms [19], namely by acting as a phosphate “sink” for stress-activated kinases, and preventing the activation of pro-apoptotic substrates [20] or by the regulation of key effectors of the stress-induced metabolic responses through phosphorylation of specific epitopes on keratins [17,21]. Additionally, during physiological wound healing, keratins may regulate various signaling pathways resulting in the regulation of protein synthesis and cell size.

Tremendous progress in our understanding of the etiology and molecular pathogenesis of cutaneous keratins has formed the basis for re-classification, improved diagnosis and pre-implantation genetics with prognostic implications. Moreover, the discovery of specific mutations has facilitated genetic counseling and prenatal testing for severely affected families [22,23].

Heritable keratin-related cutaneous diseases

Several human diseases are caused by defects in genes which encode IF proteins. In the epidermis and associated skin appendages, pathogenic mutations in the coding sequence of keratins and their associated linker proteins have been discerned as the molecular basis of a vast majority of cutaneous disorders. These wide ranges of abnormal genetic skin, appendages and membrane fragility pathologies are commonly termed genodermatoses. These include about 22 different keratin genes, as well as cornea, hair and hair follicle–specific keratins (see the IF database www.interfil.org and [8]). Two decades ago, it was shown for the first time that EB simplex was the first human genetic skin blistering disease shown to be caused by dominant-negative mutations in genes encoding an IF proteins, i.e. the basal epidermal keratins K5 and K14 [24–26]. Since then, our understanding of several other genodermatoses due to defects in keratin genes has considerably advanced. In most conditions, the associated pathology results from fragile keratinocytes expressing the mutated keratin. These diseases commonly termed keratinopathic genodermatoses are individually rare (typically, less than 1:25–50,000 live births), but can be devastating and incapacitating to affected individuals, incurably affecting their quality of life and being occasionally lethal in severe episodes. For most of these disorders, there exists a good correlation between the type of mutated keratin gene, the nature and position of the mutation in the polypeptide, the extent to which the
mutation alters the properties of keratin assembly, and the severity of the clinical phenotype.

Over 90% of pathogenic mutations in keratinopathies are missense mutations with a small number of small in-frame insertion vs deletion mutations and a few intronic splice site defects leading to larger in-frame deletions. At the protein level, the consequences of mutant polypeptides are the expression at normal or near-normal levels with substitutions, deletions or insertion of a different amino acid. The heterodimers of the protein formed from one mutant protein and the wild-type keratin partner then integrate into the keratin network rendering the cytoskeleton susceptible to collapse upon mild or no physical stress [27,28]. Additionally, genetic testing for mutations in KIF and their associated linker proteins, and their site specific expression, reflecting the phenotypic expression pattern delineated various subtypes and variants of each keratin-related disorders. Examples include, epidermolysis bullosa simplex (K5, K14), keratinopathic ichthyosis (K1, K2, K10), palmo-planter keratoderma (K9), pachyonychia congenita (PC) including PC-6a, PC-6b, PC-16 and PC-17 (K6A, K6B, K16 and K17), and monilethrix (K81, K83 and K86), etc. (see www.interfil.org; www.pachyonychia.org and [6,7,12,23]).

Epidermolysis bullosa (EB)

EB is a large and heterogeneous group of genetically defined mechano-bullous skin fragility disorders characterized by fluid filled blistering or erosion of the skin and mucous membrane occurring in response to mild or no physical trauma. About 1 in 20,000 individuals are affected by any of the several EB types recognized currently. Electron microscopy and immunofluorescence antigen mapping have been fundamental in understanding these genodermatoses and revised classification distinguished four major subtypes. Based on the level of skin cleavage within the cutaneous basement membrane zone (BMZ) [29], we now distinguish between: (i) the intra-epidermal EB simplex with cytolysis and fluid filled blister formed intra-epidermally within the basal keratinocytes, usually caused by mutations in either keratin 5 or keratin 14 gene, (ii) the intra-lamina lucida, junctional EB, with the split occurring at the level of the lamina lucida and resulting in blistering with no obvious structural abnormality of tonofilaments, which is caused by defects in laminin-332, collagen XVII, or α6β4 integrin, (iii) the sub-lamina densa, dystrophic EB, with cleavage at the superficial dermis, underneath the lamina densa at the level of the anchoring fibrils (which link the epidermis and dermis), caused by mutations in the gene that encodes collagen VII, often with documented abnormality in tonofilaments, and (iv) the mixed type Kindler syndrome. The intra-epidermal EB is further separated into two subgroups, the basal and suprabasal types, respectively including newly described types such as those due to desmoplakin or plakophilin mutations [29].

To date, all EB subtypes have been characterized at the ultrastructural and molecular levels, with more than 1000 mutations already described in more than 21 genes encoding for structural keratins in the human skin, their appendages and the mucous membranes. Amongst these includes about 193 KRT5 and KRT14, 115 KRT1 and KRT10 and 67 PC mutations (www.interfil.org; www.hgmd.cf.ac.uk and [8]).

Epidermolysis bullosa simplex (EBS) – diseases of K5/K14 mutations

Epidermolysis bullosa simplex (EBS) is a group of rare predominantly autosomal dominant genetic skin diseases affecting approximately 1:25,000–50,000 live births of the population [27,29]. EBS is the first identified and best studied variant of keratin disorders and has become the prototype for understanding disease pathology and genotype–phenotype correlations within a broad spectrum of keratin disorders. In EBS, two major subtypes have been defined: suprabasal and basal EBS [29]. Within the scope of this review, only keratin-related will be described. The suprabasal EBS types such as lethal acantholytic EB (mutations in desmoplakin), plakophilin deficiency (caused by plakophilin-1 mutation) and the basal EBS subtypes; EBS caused by mutations in plectin (EBS with muscular dystrophy and EBS Ogna), mutations in plectin and α6β4 integrin (EBS with pyloric atresia) beyond the scope of this review (for these subtypes see www.interfil.org and [23]). Clinical EBS is usually present at birth and is characterized by intra-epidermal blistering within the basal layer of the epidermis and often with involvement of mucosal epithelia. Blistering which tends to heal without scarring is often associated with mechanical stress, and is usually caused by mutations in keratin K5 or K14. The pathogenic mutations usually occur within regions of the keratin genes that encode “hotspots” in the protein structure, namely the H1 domain of the head region (only for type II keratins), two segments (1A and 2B) of the rod domain, and the central linker region L12 [8]. Upon mild physical trauma, the keratin filament network is easily compromised, resulting in structural failure of the affected keratinocytes and loss of tissue integrity (reviewed in [12,30]). The degree of severity of the clinical phenotype has been directly linked to the position of the pathogenic mutation along the domains of the polypeptide backbone. Recent evidence provides some exceptions to the latter, with milder disease phenotypes resulting from pathogenic mutations in the conserved hotspot region of the KRT genes [31,32]. Based on the clinical severity, recent re-classification distinguishes four major EBS subgroups: (a) the generalized Dowling-Meara EBS (EBS-DM; OMIM 131760), (b) other generalized non-DM EBS (gen non-DM EBS; OMIM 131900), (c) the localized EBS (EBS-Loc; OMIM 131800) and (d) EBS with mottled pigmentation (EBS-MP; OMIM 131960) [29]. In both generalized forms, the most severe Dowling-Meara subtype and the milder generalized non-Dowling-Meara subtype, also previously known as the Koebner form, affected individuals present generalized and pronounced blistering at birth, while the localized EBS is milder with blistering confined to palmar and plantar regions of the body. Nevertheless, other not yet identified genetic or epigenetic modifiers and environmental factors, such as patient lifestyle and climate condition, clearly influence the phenotypic expression as different subtypes of EBS have been associated with the same mutation in several instances [33–35]. However, other additional factors are suggested to exacerbate disease severity [36,37].

The generalized EBS-DM subtype is the most severe form being manifested at birth with erythema, widespread blistering, erosions and areas of denuded skin presenting spontaneous clusters of blisters also called “herpetiform” at multiple sites of the body which improve with age (Fig. 3a). Progressive palmo-plantar keratoderma becomes the chief complaint in adulthood. Other hallmark includes callosite formation (Fig. 3b), secondary bacterial infections and sepsis, involvement of mucous membranes, nail dystrophy, healing of lesions without scarring and involvement of the oral mucosa. Inflammation of blisters may be preceded by the formation of transient milia, and healing of the skin with hypo- and hyperpigmentation [27]. Diagnostic criteria include immune epitope antigen mapping and ultrastructural examination of the skin, often present a characteristic pathognomonic electron dense aggregates of K5 and K14 proteins in the cytoplasm of basal keratinocytes harboring the mutation [38].

The pathogenic mutations in EBS-DM are usually missense mutations residing within the highly conserved helix boundary motifs (the HIP of the 1A segment and the HTP of the 2B segment). More than one-third of the reported EBS-DM cases are caused by the KRT14 gene “hot spot” mutation in a highly conserved arginine
residue (Arg125) in the HIP of K14. The likely reason for this genetic hotspot may be owing to the conserved hypermutable CpG dinucleotide known in all type I keratins, which upon mutation leads to the substitution of the arginine codon (CGC) for cysteine (TGC) or histidine (CAC) with unique phenotype.

The gen-non-DM EBS is a more moderate subtype characterized at birth or in early infancy by generalized non-clustered blistering. The clinical presentation in majority of the cases is moderate, without any extra-cutaneous involvement, but with palms, soles and extremities being mostly affected, usually in response to minor trauma and often induced by increased ambient temperature. The disease-associated mutations in the gen non-DM EBS are more centrally located in the rod domain and sometimes more widely distributed along both KRT5 and KRT14 genes, involving the non-helical linker segments (reviewed in [12]).

The EBS-loc, a clinically mild phenotype and frequent form previously known as EBS-Weber Cockayne (EBS-WC), is characterized by late appearing skin blistering restricted to areas of greater friction or trauma such as hands and feet. Children are not affected until they start to crawl or walk and blister formation worsens during warm humid climate, with most common complications presenting as secondary bacterial infections of blister lesions on the extremities. Some affected individuals suffer from focal keratoderma. Mutations in EBS-loc are most frequently distributed in four clusters lying outside of the helix boundaries of K5 or K14, including the non-helical L12 linker motif, or in the amino-terminal homologous domain (H1) of K5, or in the 2B segment of K14 (see www.interfil.org; [8]. However, exceptions do exist and patients with EBS-loc have been identified with mutations in the conserved 1A and 2B helix hotspots [31,32,39]. Ultrastructural abnormalities of the cytoskeleton are far less severe than those seen in EBS-DM and some cases of gen-non-DM EBS. Therefore, based on the site of the pathogenic mutation in keratins expressed by keratinocytes of the basal epidermal layer, it is for the most cases possible to infer a disease phenotype [40,41].

Autosomal recessive epidermolysis bullosa simplex, EBS-AR

Although EBS is mostly inherited in an autosomal dominant mode, approximately 5% of EBS cases are inherited in a recessive mode [42], with about 10 different KRT14 mutations) including nonsense, missense, splice site, deletion and deletion vs insertion mutations identified in (EBS-AR) cases [43]. Yusukawa et al., also reported cases of recessive EBS with compound heterozygous mutations in K5 [9].

Naegeli–Franceschetti–Jadassohn syndrome (NFJS) and dermatopathia pigmentosa reticularis (DPR)

Naegeli–Franceschetti–Jadassohn syndrome (NFJS, OMIM 161000) is a rare autosomal dominant disorder, characterized by palmo-plantar keratoderma, decreased sweating and a reticulate pattern of hyperpigmentation of the skin that occurs with age. Clinically, the disease is mostly manifested by the absence of dermatoglyphs, reticular or motiled hyperpigmentation, hypohidrosis due to diminished sweat gland function, heat discomfort, hair defects, nail dystrophy, enamel (teeth) defects. Also, moderate palmo-plantar hyperkeratosis may co-exist with punctate keratoses showing a linearized pattern [44]. NFJS is usually caused by heterozygous nonsense or frameshift mutations in the KRT14. The disease is allelic to dermatopathia pigmentosa reticularis (DPR) whose dis-

Fig. 3. Clinical features of epidermolysis bullosa simplex (EBS) and epidermolytic ichthyosis (EI). (a) A child’s hand with severe generalized Dowling-Meara form of EBS, characterized by widespread herpetiform blister that heals without scar formation. (b) An Adult with severe EBS-DM whose feet present with painful plantar callosities. (c) Epidermolytic ichthyosis patient showing sharp massive hyperkeratosis of the lower back and (d) a diffuse hyperkeratosis of the hand and flexures with an erythematous background. (Reproduced from [133] with permission from the publisher.)
mutations have been associated to the non-helical head (E1/V1) domain of the KRT14. Mostly, these mutations result in non-sense-mediated mRNA decay (NMD) or in the synthesis of a short unstable peptide, that results in K14 haploinsufficiency, and has been associated with increased susceptibility to tumor necrosis factor-alpha-induced apoptosis [47]. Thus, providing evidence of an inherited keratinopathy resulting from impaired regulation of apoptosis, as well as the link between EBS-MP and DDD, this also infers a potential for defective basal keratins to result in both blistersing and pigmentedary diseases.

### Table 1

<table>
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<th>Human cutaneous keratin disorder</th>
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<td>K14</td>
<td>Basal keratinocytes of the epidermis</td>
<td>Localized, generalized and/or herpetiform blistering, PPK</td>
</tr>
<tr>
<td>Localized EBS (EBS-Loc)</td>
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<tr>
<td>Generalized non-Dowling-meera EBS (gen-non-DM-EBS)</td>
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<tr>
<td>EBS Dowling Meara (EBS-DM)</td>
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<tr>
<td>Epidermolysis bullosa simplex (EBS-AR)</td>
<td>AR</td>
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<td>EBS with migratory circinate erythema (EBS-CE)</td>
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<td>Generalized blistering, circinate erythrodema, brown hyperpigmentation, reduced KIF in basal keratinocytes</td>
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<td>Dowling-Degos disease (DDD)</td>
<td></td>
<td>K5</td>
<td>&quot;</td>
<td></td>
<td>Hyperkeratotic papules, reticulate, progressive and disfiguring hyperpigmentation, primarily in the flexural areas</td>
</tr>
<tr>
<td>Naegeli–Franceschetti–Jadassohn syndrome (NFJS)/dermatopathia pigmentosa reticularis (DPR)</td>
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<td>K14</td>
<td>&quot;</td>
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<td></td>
</tr>
<tr>
<td>Epidermolytic ichthyosis (EI)</td>
<td>AD</td>
<td>K1</td>
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<td>Suprabasal keratinocytes of the epidermis</td>
<td>Erythroderma, blister formation, development of hyperkeratosis, PPK</td>
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<td>AD</td>
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<td></td>
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<td>Epidermolytic ichthyosis (EI) Ichthyosis hystrix Curth-Macklin (HCM)</td>
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<td>K1</td>
<td>K10</td>
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<td>Erythroderma, blister formation, hyperkeratosis</td>
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<td>K1</td>
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<td>Upper suprabasal keratinocytes of palmoplantar skin</td>
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<td>Upper suprabasal keratinocytes of palmoplantar skin</td>
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<td>Diffuse PPK, sharp demarcation with erythematous borders, SEHK/suprabasal KIF clumping</td>
</tr>
<tr>
<td>Pachyonychia congenita (PCGa, PC16)</td>
<td></td>
<td>K6a</td>
<td>K16</td>
<td>Suprabasal epidermis of palm, soles and epidermal appendages e.g. fingernails, toenails</td>
<td>Thickening of toenails and fingernails, plantar keratoderma, plantar pain, palmar keratoderma, pilosebaceous cysts, follicular hyperkeratosis, oral leukokeratosis, hyperhidrosis</td>
</tr>
<tr>
<td>Pachyonychia congenita (PCGb, PC17)</td>
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<td>K6b</td>
<td>K17</td>
<td></td>
<td>Thickenning of toenails and fingernails, plantar keratoderma, plantar pain, palmar keratoderma, pilosebaceous cysts, follicular hyperkeratosis, oral leukokeratosis, hyperhidrosis</td>
</tr>
<tr>
<td>Focal non-epidermolytic PPK (FNEPPK)</td>
<td></td>
<td>K16</td>
<td>&quot;</td>
<td>Suprabasal layers of palmoplantar epidermis (palms and soles)</td>
<td>Focal PPK with oral, genital, and/or follicular lesions</td>
</tr>
<tr>
<td>Monilethrix</td>
<td>AR, co-dominance</td>
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<td>K752</td>
<td>Hair shaft (clumps of the structural proteins)</td>
<td>Hair fragility and total or patchy alopecia, hair shaft deformation</td>
</tr>
<tr>
<td>Pseudofolliculitis barbare (PFB)</td>
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<td>K85</td>
<td>&quot;</td>
<td>Hair follicle root sheath</td>
<td>Hair follicles ingrowth, follicular infections, affects mainly black individuals, inflammatory papules and pustules</td>
</tr>
<tr>
<td>Ectodermal dysplasia of hair and nail type</td>
<td>AD/AR</td>
<td>K85</td>
<td>&quot;</td>
<td>Hair shaft</td>
<td>Hypotrichosis and nail dystrophy, associated with keratoderma and ichthyosis, hair scalp fragility</td>
</tr>
</tbody>
</table>
**Dowling-Degos disease (DDD) – disease of K5**

Dowling-Degos disease (DDD) is a rare genodermatosis inherited in an autosomal dominant manner with varying degree of penetrance and is caused by mutation in the KRT5 [48,49]. The disease is manifested during adulthood often in the 40s, with clinical features including small rounded reticulate freckle looking hyperpigmented macules often in the flexural areas including the armpits, neck, under mammary folds and in the groins or the gluteal folds. Other involved areas may include the scalp, trunk and arms [49]. Major histopathological characteristics include acanthosis and papillomatosis, with a digitiform (fingerlike) pattern of dermal papillae, epidermal keratin cyts and dilatation of keratinized hair follicles. It present with diffuse deposits of melanin in the basal epidermal layer in addition to diverse amount of dermal papillary melanophages and superficial perivascular infiltration of lymphocytes. In some cases genital involvement has been reported often with progressive and symmetric pigmentation [49,50]. The inconsistency in arriving at a consensus in classifying hereditary pigmentary dermatoses, has made Dowling-Degos disease to be defined as a clinicopathological entity with several subtypes including the Galli–Galili disease, an acantholytic form and the reticulate acropigmentation of Kitamura, the acral form of the dermatosis [48,49,51]. Molecular genetic analysis has identified deletion, duplication, and nonsense mutations resulting in premature termination codon (PTC) in DDD with K5 null mutations [52,53]. However, the dominant inheritance pattern in DDD is often due to K5 haploinsufficiency instead of the dominant-negative K5 mutations. In the acantholytic variant of DDD, the Galili–Galili disease [48], Sprecher et al. identified a disruptive KRT5 initiation codon mutation that results in either the synthesis of a K5N-deletion mutant or haploinsufficiency [54] (see Table 1., and reviews [12,23,27] for other forms of EBs). Conclusively, identification of novel mutations, genotype–phenotype correlations, disease modifiers and in-depth molecular pathogenesis in EBs will allow improved understanding of the disease pathogenesis and futuristic development of treatment as well as better patient management.

**Keratinopathic ichthyosis (KPI) – diseases of K1/K2/K10 mutations**

Keratinopathic ichthyosis (KPI), represent a family of ichthyoses occurring due to mutations in keratins referred to as superficial keratin keratodermas which include epidermolytic ichthyosis (EI; K1/K10 mutations) and superficial epidermolytic ichthyosis (SEI; K2 mutations (ichthyosis bullosa of Siemens) [55].

**Epidermolytic ichthyosis (EI) – disorders of K1 and K10 mutations**

Epidermolytic ichthyosis (EI; OMM 113800) is a form of congenital ichthyosis with a prevalence of 1 in 200,000–300,000 people [56]. EI, also previously known as bullous congenital ichthyosiform erythroderma (BCIE) or epidermolytic hyperkeratosis (EHK), is a relatively rare congenital skin fragility disease which is predominantly autosomal dominantly inherited [55]. EI is caused by mutations in either of the genes coding for epidermal suprabasal keratins, K1 and K10 expressed in keratinocytes of the suprabasal layers of the epidermis.

These disorders are characterized at birth with generalized erythroderma (redness of the skin) (Fig. 3c), with severe blistering accompanying widespread hyperkeratosis or thickening of the uppermost layer of the skin, erosions and peeling of the skin upon mild trauma (Fig. 3d). Hallmarks include superficial ulcerations that develop on the flexural regions of the skin. As a result of epithelial barrier disruption, neovascularization with EI are at risk of developing electrolyte imbalances, severe infection and sepsis. EI is often associated with rapid healing of denuded areas with recurrent episodes of blisters on a background of erythroderma that may persist throughout life. Later on in adulthood, blistering becomes infrequent; hyperkeratotic plaques with verrucous scales, mainly involving flexural and intertriginous areas develop, but can also appear on the scalp, neck and infragluteal folds. In most of the patients, palmo-plantar hyperkeratosis is present and bacterial colonization of the macerated scales causes a distinct foul odor [57–61]. As mentioned, the cutaneous pathology in EI results from the expression of abnormal K1 or K10 proteins [62], but contrary to the deep blisters found in EBS, blistering is more superficial EI. In E1 disease, there is increased proliferation of suprabasal keratinocytes that results in the formation of ichthyosiform lesions as opposed to herpetiform lesions seen in EBS-DM. Ultrastructurally, basal keratinocytes appear normal, but irregularly shaped pathognomonic KIFs aggregates are identified in suprabasal keratinocytes, appearing as dense peri-nuclear shells as the primary event, with secondary suprabasal cytolyis, blistering and hyperkeratosis [42].

Similar to EBS, the majority of disease-causing mutations in EI are missense mutations that usually occur within the highly conserved sequence of the alpha-helical rod and the non-helical H1 domains of K1 and K10. Also, the milder variants of the disease have been associated with mutations located outside the helix boundary motifs or in the L12 linker region [63]. Analogous to EBS disorders, the positions of the mutations along the keratin polypeptides, the degree of disruption and the level of expression of the mutated KRT1 and KRT10 genes usually explain the clinical features of EI as well [64–66].

In EI, rare dinucleotide alterations in KRT10 [64,67] including spontaneous de novo point, deletion, deletion vs insertion and splice site mutations in KRT1 and KRT10 have been associated with half of the reported cases [66,68]. It is well established that the nature of the mutations can predict the disease phenotype and is known that while KRT1 mutations are associated with palmo-plantar keratoderma, KRT10 mutations are associated with the non-palmoplantar variants [56]. Such assertion of association appears to be unique for KRT1 mutations; but exceptions do exist where KRT10 mutations have been identified in patients with severe EI and palmo-planar keratoderma [68,69]. As in severe EBS, a genetic “hot spot” that affects a highly conserved arginine residue (p.Arg156) exist in EI. Also, similar to milder forms of EBS, a missense HTP mutation p.Ile479Thr in K1 in some cases have been associated with mild ichthyotic phenotype [65] as well as only epidermolytic palmo-plantar keratoderma in others [70].

Based on genotype–phenotype correlation studies, a more complex link is known to exist where the genetic background can modulate the disease phenotype. A proof of principle is the report illustrating that the conserved 156 codon mutations in KRT10, usually associated with severe phenotype as well lead to milder EI phenotype [71]. This is interestingly similar to EBS disorder where different nucleotide changes at the same site on the polypeptide results in different disease phenotypes, examples are the following KRT5 mutations K5_p.Iso183 [32,72] and the K5_p.Val186 mutations [73,74].

Even though EI is usually an autosomal dominant trait, several recent reports have delineated recessive inheritance pattern, involving either donor splice site, nonsense or termination codon mutations [75–77]. Characteristic ultrastructural features include, sparse, homogenous and amorphous KIFs network associated with keratin aggregates, and nonsense KRT10 mutation leading to a loss of K10 expression [78]. In other cases, homoygous nonsense KRT10 mutations have been reported. The heterozygous phenotypes were non-phenotypic carriers, demonstrating that a normal K10 allele is sufficient to maintain a normal KIF network in the absence of mechanical stress [77].
The nevoid variant of EI, also termed epidermal nevus of the epidermolytic hyperkeratotic type exists and is characterized by ichthyosiform lesions that are usually distributed along the Blaschko lines and that alternate with normal skin. The nevoid distribution of EI is due to the mosaicism of K1/K10 mutations. Heterozygous missense mutations in KRT10 were discovered in skin lesions, which were absent in normal skin and led to the postulation that during embryogenesis mosaicism occurred as a result of postzygotic spontaneous mutations in KRT1 and KRT10 [79,80]. Children with full-blown EI, born by EI patients with the nevoid variant, usually have underlying gonadal and cutaneous mosaicism [12]. Analogously, mosaicism has been reported in EBS and in palmoplantar verrucous nevus [12].

The annular variant of EI also termed cyclic ichthyosis with epidermolytic hyperkeratosis (EHK) is also a rare disease that has been reported in only seven families so far. At birth, the individuals show classical EI erythroderma, superficial erosions but with improvement of symptoms in early infancy. Characteristic clinical features include flares of polycyclic psoriasisiform plaques that persist for several weeks to several months with only benign localized disease in adulthood. Except for palmoplantar hyperkeratosis, the skin between the flares usually looks normal. Definitely, the identification of KRT1 and KRT10 mutations suggested a subtype of EI [65,67,81,82].

**Ichthyosis hystrix of Curth-Macklin (IHCN) – disorder due to mutations in the V2 domain of K1**

Ichthyosis hystrix (IHCN) is a rare distinct type of autosomal dominant skin disorder characterized by extensive hyperkeratosis, with or without palmoplantar keratoderma, which results from heterozygous frameshift mutation in KRT1 [83]. Except for the absence of epidermolyis, histological features are similar to those of epidermolytic hyperkeratosis or other cornification disorders like erythrokeratodermia variabilis. Ultrastructural features include perinuclear vacuolization, binucleated cells and suprabasal non-aggregated shell-like tonofilaments. Here, the KRT1 mutations most affect the V2 domain and dramatically alter it biochemical properties, but does not interfere with KIF formation. Instead, the mutation is postulated to relate to intracellular mis-orientation of loricrin, responsible for the organization of the cornified cell envelope [83,84]. Beside, a heterozygous frameshift mutation was identified in the V2 domain in close proximity to the IHCN mutation in KRT1, which results in striate palmoplantar keratoderma (SPPK), a heterogenic disorder associated with pathogenic mutations in KRT1, which is occasionally associated with mutations in desmosomal proteins, desmoglein 1 and desmoplakin. Consequently, this frameshift mutation lead to partial loss of the glycine loop motif in the V2 domain of KRT1 [85], and therefore suggests a distinct pathogenic pathways in the tail domain of KRT1.

**Ichthyosis bullosa of Siemens (IBS) – disorders linked to K2 mutations**

Ichthyosis bullosa of Siemens (IBS, OMIM 146800), a more superficial epidermolytic ichthyosis (SEI), is an autosomal dominant keratinization disorder, with similar but milder clinical features compared to EI. Clinical characterized include, the absence of congenital erythroderma and hyperkeratosis and is mostly localized to flexural areas. The blistering is more superficial and has been called molting (Mauersung), and the granular degeneration is restricted to the uppermost spinous and granular layers with characteristic desquamation that result in denuded skin areas [86–88]. Aggregates of keratin filament bundles and superficial cytolysis often correspond to the epidermal tissue layer of K2 expression. The majority of pathogenic mutations affect the HTM of K2 although mutations have been reported in the 1A and 2B domain with the possibility of a Methyl-CpG deamination mutation hot spot, K2.p.Glu487Lys. It is often clinically very difficult to clearly distinguish mild EI from severe IBS, inferring that molecular genetic analysis is a definitive predictive diagnosis. However, IBS patients presenting with severe skin phenotypes previously clinically misdiagnosed as EI were upon molecular genetic analysis correctly diagnosed [89,90]. In the other hand, a heterozygous missense KRT1 mutation, p.Glu478Asp was reported in a family with mild EI presenting with clinical and histological features similar to IBS [90].

**Palmo-planter keratoderma (PPK) – disorders of palmoplantar K9 mutations**

Palmo-planter keratodermas (PPK), a group of heterogeneous keratinopathic genodermatoses characterized by hyperkeratotic skin confined to the palms and soles, usually clinically grouped into three distinct patterns: diffuse, focalize and punctate. Based on the identification of the underlying genetic defects in these disorders, a revised classification now exist as a result of the previous difficulties in classifying based on phenotypic and morphological criteria.

Epidermolytic PPK (EPPK, OMIM 144200), an autosomal dominant genodermatosis that develops within the first months after birth, typically manifests as diffuse hyperkeratosis strictly confined to the palms and soles presenting sharp demarcations with erythematous borders. Ultrastructurally, studies revealed aggregates of keratins in the palmoplantar epidermis, with varying degree of hyperkeratosis between families and within individuals in a family. Other hallmarks include flexural finger joints knuckle pad-like keratoses and nails clubbing [91]. Another disease form, the Unna-Thost form, which is often clinically identical to Vörner disease, can be distinguished histologically and ultrastructurally by the absence of EHK and suprabasal KIF clumping, respectively [65]. A spectrum of pathogenic substitutions including missense or small in-frame insertion vs deletion mutations of KRT 9, have been reported in several families with EPPK, with the beginning of the 1A rod domain and the end of the 2B rod domain of the KRT 9 as mutation hotspots [92–96]. In addition, mutations have been identified in the variable V1 and V2 domains, which are responsible for the greatest sequence variation in different keratins, and some have been associated with non-epidermolytic palmo-planter keratoderma [87]. Another rare PPK ultrastructurally characterized by of the presence of “tonotubules”, first described in a large German family, called PPK with “tonotubular” keratin, has been described in five families [97]. Molecular analysis revealed two missense mutations closed to the 1B domain of K1 [98,99]. As these tubular structures have not been described in other keratin disorders, they suggest a different role for the 1B domain in the formation of KIF.

**Keratin defects in skin appendages (hair and nail) and other genodermatoses**

**Monilethrix – disease of the hair K81/K83/K86 mutations**

Monilethrix is a rare predominantly autosomal dominant disorder that affects the hair with characteristic shaft anomalies, featuring uniform elliptical nodes with intermittent constrictions resulting in hair fragility and diffuse or patchy alopecia. Inheritance is usually accompanied by a high penetrance and variable expressivity with a few reports of autosomal recessive and sporadic cases [100]. Clinical features include a small area of hair dystrophy to total alopecia, hair shaft deformation with characteristic moniliform appearance caused by the nodes and internodal thinning of the shaft, and abnormalities of cuticle, cortex and keratiniz-
ing zones of hair follicles. The internodes are characterized by wrinkled cortical cells leading to hair fragility, and lack of medulla [101]. Invagination of the cuticle cells into the cortex, degeneration, cytoplasmic vacuolation and abnormal tonofibrils are seen in cortical cells which are particularly affected in the hair matrix [102]. In addition to the clinical signs of short, sparse, fragile, non-growing hair, patients may present with keratosis pilaris, koilonychia with rare, systemic problems such as mental and physical retardation, syndactyly, teeth and nail dystrophies [103,104]. Improvements during adolescence and pregnancy have been documented, suggesting a hormonal influence. Light microscopy is usually diagnostic with the hair showing a typical moniliform (beaded) structure. Ultrastructural findings show cortical defects and aggregates of hair shaft structural proteins [7105] suggesting trichocyte keratins as the prime disease candidates. Pathogenic mutations have been delineated in genes that code for the basic hair keratins K81, K83 and K86 in humans, often presenting normal lanugo hair in neonates [104]. Mutations in the HTM of KRT86 (previously denoted KRT8B6), in K86 (Hb6; 90%) and in HTM of KRT81 (Hb1; 100%) in K81 have been identified [106]. Keratin K83 (Hb3) is co-expressed with K81 (Hb1) and K86 (Hb6) in the mid-cortex and shares an almost identical rod domain sequence, but only one mutation in the 2B region (p.Glu407Lys) has been identified to date in a monilethrix family with co-dominance [107,108]. Multiple cases with autosomal recessive inheritance and mutations in desmoglein 4 (DSG4), a cadherin subfamily member expressed in the hair cortex and upper cuticle, have been reported [109–111]. Mutated DSG4 is now associated with localized autosomal recessive hypotrichosis, and with the great variability in the moniliform trait, this suggests a clinical overlap between monilethrix and localized autosomal recessive hypotrichosis.

Pseudofolliculitis barbae – disease due to predisposition to K75 polymorphism

Pseudofolliculitis barbae (PFB), is a common hair disorder with chronic, irritating, and potentially disfiguring condition that usually develops after shaving hair from predilection sites (beard area), leading to hair follicles ingrowth and follicular infections [112]. Incidence rate of the disorder is difficult, but some studies showed that it can affect up to 1 out of every 5 caucasian individuals and often occurs much more commonly in black persons due to a genetic predisposition to curled hair. Clinical features include the appearance of inflammatory papules and pustules. Molecular analysis in severely affected males and asymptomatic females revealed a single-nucleotide polymorphism that results in a disruptive p.Ala161Thr substitution in the 1A alpha-helical segment of

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Fig. 4. Clinical features of pachyonychia congenita (PC) affecting the plantar epidermis and the nails with varying severity and keratin gene mutations. (a) Patient (PC-17) presenting with mild plantar keratoderma due to K17 (K17_p.92Asn>Ser) mutation. (b) Patient (PC-16) with severe and painful plantar keratoderma due to a K16 (K16_p.124Leu>Pro) mutation. (c) Patient (PC-16) with mild to severe hypertrophic nail dystrophy with a K16 (K16_p.132Leu>Pro) mutation. (d) Patient (PC-6a) with severe hypertrophic nail dystrophy involving the nail beds and having a K6a (K6a_p.171 Asn>Lys) mutation.
This has been suggested to be partially responsible for the phenotypic expression and represents an additional genetic risk factor for PFZ [113].

Pachyonychia congenita (PC) – disorder of plantar and nail K6/K16/ K17 mutations

Pachyonychia congenita (PC; OMIM 167200 and 167210), is a rare autosomal dominant genodermatosis chiefly affecting tissues of the ectodermal origin such as the palmoplantar epidermis, nail bed, mucosae and the pilaosebaceous unit. The fundamental clinical features are painful palmoplantar (preponderantly plantarkeratoderma) (Fig. 4a and b); hypertrophic nail dystrophy (Fig. 4c and d); oral leukokeratosis; and a variety of cysts arising from hyperkeratosis of pilaosebaceous apparatus. Occasionally, plantar epidermal blistering with varying degree of involvement of the skin (including cysts and follicular hyperkeratosis), larynx, oral mucosa, teeth and tongue [114,115]; Wilson2, Eliason3, PC is often inherited in an autosomal dominant manner and is caused by pathogenic mutations in at least four keratin (KRT) genes (KRT6A, KRT6B, KRT16, and KRT17) that encode the keratins K6a, K6b, K16, and K17, respectively. Previously, based on minor phenotypic differences, classification distinguished two PC subtypes (PC-1, or Jadasoohn-Lewandowski subtype; and PC-2, or the Jackson-Lawler subtype), chiefly based on the presence or absence of pilosebaceous cysts [114,115]. This classification was based on clinical, but non-genetically analyzed cases, but with the advent of data from studies analyzing hundreds of patients in the International PC Research Registry (IPCRR), presenting intriguing phenotypic overlap between PC subtypes, the limitations of this previous classification has become clear. For instance regardless of the genotype, many PC patients have some form of epidermal cysts (Wilson1). Based on the IPCRR data, a novel consensus classification based on the mutated gene and their clinical subtype has been adopted [116]. This new consensus classification categorizes PC as PC-6a, PC-6b, PC-16 and PC-17, where for instance PC-6a indicates a patient with a mutation in the gene encoding K6a etc. For complete dataset on this novel consensus nomenclature see Wilson1 and Eliason2. Even though the clinical feature that resulted in naming of the condition is hypertrophic nail dystrophy, focal plantar keratoderma associated with severe debilitating pain is the major problem encountered by PC patients, with serious negative impact on quality of life (Eliason2). Similar to other keratin genodermatoses discussed above, most pathogenic PC-related keratin mutations are heterozygous missense mutations or small insertion/deletion mutations mostly inherited as an autosomal dominant trait, for literature see www.interfil.org and [8]. These mutations renders cytoskeletal fragility causing cytolysis and hyperkeratosis of the basal/suprabasal layers of palmoplantar skin, as well as epidermal appendages and oral mucosa, predominantly expressing K6a, K6b, K16 and K17 [28]. The severity of the clinical features of PC can vary quite widely both among and within families and based on recently updated information from 271 genetically analyzed PC patient samples in the International PC Research Registry (IPCRR) showing differential involvement of the various PC-associated keratin and their respective tissue involved (Table 2, IPCRR data on file November 2010).

The reason for this variability is not known, but is likely a combination of genetic/epigenetic and environmental factors including


Table 2

Summary of the percentage contribution of the different ectodermal tissues in PC disease severity, including the percentage of the different PC-related keratin genes K6a, K6b, K16 and K17 mostly expressed in suprabasal layers of palmoplantar skin, as well as epidermal appendages and oral mucosa. Additional unpublished data from International PC Research Registry (IPCRR) shows that in 487 genetically tested patients more than 90% of the disease-causing PC mutations are associated with the four “classic” PC keratins namely: about 40% are K6a, 8% are K6b, 27% are K16 and 16% are K17 while 10% of patients diagnosed with PC have other non-PC mutations (such as Connexin, PPK, etc.) or mutations yet to be discovered.

Table 2

<table>
<thead>
<tr>
<th>IPCRR data November 2010, N = 271</th>
<th>Pachyonychia congenita (PC) genes involved (%)</th>
<th>Total N = 271</th>
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<tr>
<td>K6a N = 125</td>
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<tr>
<td>K16 N = 79</td>
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</tr>
<tr>
<td>K6b N = 20</td>
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<td>K17 N = 47</td>
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<td>Larynx – hoarsiness</td>
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</tr>
<tr>
<td>Hyperhydrosis</td>
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Ectodermal dysplasia of the hair and nail type keratins

Ectodermal dysplasia (ED) is a congenital syndrome characterized by abnormal development of tissues of ectodermal origin such as skin and its appendages (the hair, nails, teeth and sweat glands). There exist about 200 known ectodermal dysplasias with about 30 genetically tested and characterized at the molecular level. The clinical characteristics and the molecular basis of ED are poorly understood. Most of the hair and nail type ectodermal dysplasia (HNED; OMIM 602032), the congenital disorder characterized by hypotrichosis and nail dystrophy, can show either an autosomal dominant [129] or autosomal recessive [130,131] pattern of inheritance. They are mostly associated with abnormalities, such as keratoderma or ichthyosis, skeletal anomalies, cardiac irregularities, mental or psychomotor retardation and some hair scalp fragility.
Ultrastructural examination shows inconsistent thickness of the hair shaft, without periodic variation in hair diameter as observed in monilethrix hair [102]. A homozygous mutation in the type II hair keratin gene KRT85, has been identified in a consanguineous Pakistani family with autosomal recessive PHNED [132] [132]. In two other consanguineous Pakistani families with PHNED two distinct homozygous mutations in the KRT85 gene were disclosed in both families with an autosomal recessive inheritance [133]. However, only five families have been reported with two distinct KRT85 mutations so far, making genotype-phenotype correlations for KRT85 mutations more difficult to ascertain. In a cohort, some patients exhibited severe hair and nail phenotypes, suggesting a more disruptive potential for the p.Arg78His mutation compared to the mutation p.Pro483ArgfsX18 mutation in the K85 protein. The K85 is expressed in the lowermost matrix, precortex, cortex, and cuticle of the hair shaft (follicle) [134], as well as in the basal compartment and in the lower keratogenous zone of the apical and ventral nail matrix [135]. It has been suggested that genetic dysfunction in K85 can disrupt KIF formation resulting in abnormal desmosomal assembly in hair and nails [133]. Therefore, the absence of the protein during hair and nail formation can explain the severe hair and nail phenotype [7].

Animal and in vitro models of keratin-related cutaneous diseases

The molecular characterization and assessment of therapeutic outcomes for inherited cutaneous disorders requires faithful preclinical models such as animal models and in vitro proliferating and bio-engineering models of the affected skin. A significant increase in the knowledge that governs the biology and pathophysiology of keratinopathic genodermatoses has benefited tremendously from the development and use of knockout and transgenic mice models [136–138], and more recently in vitro models of such disorders.

Animal models of keratin diseases have been useful for understanding the function of keratins, to identify and delineate molecular defects in human keratinopathic-disorders, and to develop potential therapies. Several native pathogenic mutations with blistering phenotypes have been identified in other animals, such as dogs, horses and sheep [139], most of which have provided in-depth understanding of the role of animal models in disease pathogenesis. Three main groups of animals exist: animals in which a specific keratin genes have been deleted [140–142]; animals expressing keratins into which mutations have been introduced [138,143]); animals in which the expression pattern of keratin has been altered either by over-expression of normal keratin [144] or by the use of promoters to direct keratin expression to a different tissue or group of cells [145]. Most human keratin disorders are dominant disorders resulting from missense mutations, Whereas most transgenic mice are keratin knockouts with recessive phenotypes and thus their phenotype may not be directly comparable to those of humans. To date, a plethora of evidence providing intriguingly new insight in the complexity of the effects mediated by signaling pathways, which result in phenotype modification in diseases are still emerging. A prototype example is the cell-mediated inflammation in keratin 5-deficient mice [36]. Genetically engineered mouse models have been generated for the major EBS, EI and PC and reviewed in [146].
In vitro models of inherited keratin disorders provide useful systems with which to elucidate the cellular, morphological, biochemical and biophysical mechanisms in the disease pathogenesis, and represent an initial step towards the development of novel therapeutic strategies. Since introducing mutant keratins into keratinocytes lead to positive keratin-aggregates formation, often with negligible correlation between in vitro and donor phenotype severity, alternative strategies created finite and permanent cell lines from donor patients with known pathogenic keratin mutations. Primary patient-derived keratin-defective keratinocytes have been used as model for studying the impact of stress-induced keratin aggregation.

However, primary keratinocytes exhibited finite replicative life-spans in culture and developed heterogeneous behavior with increasing passage numbers, a limitation that makes them suboptimal for long-term reproducible and more extensive functional evaluations, especially when testing new treatment rationales. Complementing such knowledge with biochemical, biophysical and pharmacological assays using patient-derived cells culture models will rapidly enhance development of therapy.

Established immortalized keratin-mutant keratinocyte cell lines are invaluable tools required at early stage for devising therapeutic strategies, and the phenotypic rescue of inherited skin disorders using genetically manipulated cultured cells have shown proofs of principle [147–149]. To date, several immortalized patient-derived keratin defective cell lines have been established as long-term reproducible cellular models of EBS, EI and PC [150–154]. Future challenges remain to rapidly make use of such established models to further explore the detailed pathomechanisms, test new treatment options and develop tools with which to curb current treatment limitations for these disorders.

**Effects of keratin mutations and physical stress on cytoskeleton resilience in keratin-defective cells**

Analysis of primary and immortalized keratin-mutant keratinocytes demonstrated some common features as well as certain divergence related to the phenotypic variation seen in the patients in vivo. These changes could presumably be due to an increased physiological stress burden on the severely mutant cells, incurred by the requirements of handling mutated keratins [72,153,155]. It has also been observed that when keratin-defective cells are grown in conditions favoring desmosomal connections (serum containing medium) as well as at increased confluency; they are more resistant to the effect of physical stress. This emphasizes the necessity of uniformity in cellular confluency when performing such experiments. Cells grown in culture have been extremely useful for the establishment of different functional assays such as heat, mechanical and osmotic shock, which explore the effects of stress-induced KIF remodeling. Basal/suprabasal keratinocyte expressing keratin mutations revealed pathologic keratin positive aggregates in both EBS and EI disease models in vitro (Fig. 5a and b) [151,154–158]. Moreover, the effect of stress on mutant keratin aggregation in both basal and suprabasal keratin defective cell lines were shown to be similar to those of their respective primary cells [152]. These data infer that immortalization did not seem to interfere with the intrinsic properties of the patient-derived primary keratinocytes, and thus point out the importance of the data provided by experiments on these cell lines.

**In vitro tissue engineering of keratin defective epidermis**

Since keratin diseases are tissue fragility disorders, which may not be adequately reproduced in monolayer cultures, attempt to mimic the disease phenotype in reconstructed epidermis and other dermal equivalent substrates in vitro, has been made prior to testing the effects of new drugs on animal models and clinical development. These cells were able to differentiate and reconstitute a 3-D engineered epidermal tissue in vitro on cell culture inserts or de-epidermalized dermis. It was shown that cells from patients with severe disease recapitulated the histological and phenotypic alterations reminiscent of the in vivo donor phenotype [152,159]. (Fig. 5c and d), a phenomenon consistent with a previous report in an in vitro recessive dog model of EI [152,160,161]. Recently, organotypic epidermal model was successfully used to evaluate the effectiveness of “self-delivery” siRNA. The end point was the reduction of pre-existing fluorescent reporter gene expression compared to the minor effect in controls conditions. Also, a marked reduction of mutant keratin mRNA expression compared to the wild-type was observed [162]. Additionally, a recent approach applied a combination of in vitro bio-engineered skin and engraftment onto immuno-compromized mice (the skin-humanized mouse model) [163–165], which has been developed and is amenable for genetic intervention to clinically treat PC [166]. Thus, these models represent useful tools for future development of clinical remedies for keratin-related skin disorders.

**Application of animal and in vitro models**

The development of new bio-engineered skin systems in combination with establishing a robust humanized skin approach, provide an optimized translation of human skin physiology and pathophysiology. Thus, the blend of regenerating human skin in immunodeficient mice following bio-engineered skin (in vitro) grafting appears a perfect correlation between animal, in vitro and in vivo fidelity in translational medicine [163,164]. Such combination of organotypic epidermis and skin-engrafted mouse models may generate large numbers of keratin-defective skin-engrafted mice [163–165], potentially enabling the test of novel gene-based or pharmacological therapy for these untreatable skin-disorders, and recent report already showed proof of principle [162,167].

**Problems related to treatment of keratin genodermatoses and prospects for emerging therapies**

Currently no curative therapy for keratin-related cutaneous disorders exists, and some may argue that current therapies are tedious, only moderately effective and involve significant risks of side-effects. However, therapy development for some keratinopathies has improved considerably over the years and this is becoming obvious for EBS, KPI and more recently, PC. Unfortunately, it was thought that most of the hurdles lie in the lack of in-depth understanding of the detailed molecular signature of the defect. This is not the case now as significant knowledge is available, yet most of the treatment outcomes are still below expectation. There is an obvious need for more research on the disease mechanisms and new therapies for these discomfiting genodermatoses, aiming for best restoration of near normal to normal functioning of the skin and its appendages. The severe forms of keratinization disorders represent a serious handicap, requiring life-long, management, therapy and affecting the patient’s quality of life. Associated complications, such as heat intolerance owing to chronic skin infections mainly in EI patients for instance, add to the incapacitation of the patients and often require constant medical attention. Therefore, medical and ethical considerations, concomitant with funding agencies should support the development of experimental approaches to treatments. These may include cutaneous gene therapy, natural products, small molecule-based pharmacological and drug-based discovery therapy approaches to overcome treatment limitation thus helping this group of patients.
It is clear that, many different approaches must be tried. For example, in the case of dominant negative gene mutations such as in EBS, KPI (EI and SEI) and PC discussed above, it should at least be possible to silence a mutated keratin allele by applying RNAi technology. It was hoped that some encouraging in vitro results will lead to clinical trials [168], which have now been proven viable for phase 1b clinical trial of PC [166,169–171]. The combination of in vitro and animal model in a new bio-engineering technique is fruitful for therapies of a skin disorder [166]. In the case of recessive disorders where a protein is missing, the principle of ex vivo gene transfer using cultured and re-transplanted keratinocytes to the patient might be possible [172]. Therefore, although systemic gene therapy exploiting viral vectors is risky, it probably represents one of the many ways forward. This idea is supported by the more recent application of the spliceosome-mediated RNA trans-splicing (SmART) technology, to specifically replace exons 1–7 of the KRT14 in an EBS-DM patient cell line, which suggested a promising tool for treatment of dominant genetic disorders [173]. As the molecular dissection of these genodermatoses is being unraveled since the last two decades, it certainly will be exciting to follow the progress in this field over the next few years.

Concluding remarks

Our understanding of how various keratin genes are involved in causing inherited disorders of the skin and its appendages have increased tremendously over the last two decades, revealing a spectrum of diverse pathogenic mechanisms of abnormal structural keratin proteins. It is hoped that this new knowledge together with refined in vitro and animal models will lead to many novel therapeutic avenues for specific subtypes of keratinopathic genodermatoses. These approaches may perhaps include gene, natural products and drug discovery therapies for severely affected patients. Indeed, keratinopathies can be very disabling, requiring laborious treatment several times a day, and can as well be relatively mild that requires only occasionally application of emollients or prevention of exacerbating conditions (heat, moisture or harnessed by other modifier factors, e.g. cytokines, epigenetic/ge-netic and environmental, etc.). From both a diagnostic and therapeutic point of view, the many different subtypes of genodermatoses represent a challenge for a caring physician, who must learn to understand the underlying pathology as well as to scientists whose relentless efforts will soon benefit the general patient public. However, scientist and clinicians have and are collaborating enormously to handle these hurldles. It is likely that the choice of the best treatment for each individual will increasingly become simplified as more highly geared technological approaches and products are developed. Increased knowledge regarding the regulatory functions of keratins and in-depth molecular dissection of the pathologies may provide potentially new targets for the development of novel therapeutic strategies to counteract these incapacitating and intractable keratinopathic genodermatoses. These choices will largely depend on advantageous exploitation of the possible biophysics of the skin in normal and pathological situation.

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