The Genetics of Skin Fragility

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epidermolysis bullosa, Kindler syndrome, blister, cell adhesion, basement membrane

Abstract

Genetic skin fragility manifests with diminished resistance of the skin and mucous membranes to external mechanical forces and with skin blistering, erosions, and painful wounds as clinical features. Skin fragility disorders, collectively called epidermolysis bullosa, are caused by mutations in 18 distinct genes that encode proteins involved in epidermal integrity and dermal–epidermal adhesion. The genetic spectrum, along with environmental and genetic modifiers, creates a large number of clinical phenotypes, spanning from minor localized lesions to severe generalized blistering, secondary skin cancer, or early demise resulting from extensive loss of the epidermis. Laboratory investigations of skin fragility have greatly augmented our understanding of genotype–phenotype correlations in epidermolysis bullosa and have also advanced skin biology in general. Current translational research concentrates on the development of biologically valid treatments with therapeutic genes, cells, proteins, or small-molecule compounds in preclinical settings or human pilot trials.

INTRODUCTION

EB: epidermolysis bullosa

Skin fragility is defined as the propensity to develop skin blisters and/or erosions after minimal mechanical trauma. In most cases it is genetically determined, but it may also be acquired through various pathomechanisms, such as autoimmunity. In the case of inherited epidermolysis bullosa (EB), the prototype disease of this group, skin fragility and blistering are the major manifestations (47). However, milder fragility may also be associated with other cutaneous features (as in the case of epidermolytic ichthyoses, pachyonychia congenita, or porphyrias) or with more complex diseases (such as acrodermatitis enteropathica, incontinentia pigmenti, or ankyloblepharon–ectodermal defects–cleft lip/palate syndrome) (**Tables 1** and **2**).

Inherited EB is a clinically and genetically heterogeneous group of disorders in which skin blistering is often accompanied by fragility of mucous membranes and involvement of nails, teeth,

Table 1 Epidermolysis bullosa (EB) disorders, causative genes, and affected proteins

Disorder	Subtype	Gene	Protein
EB simplex (EBS)	Acral peeling skin syndrome	*TGM5*	Transglutaminase 5
	Acantholytic EBS	*DSP*	Desmoplakin
		JUP	Plakoglobin
	Skin fragility with hair/nail anomalies or cardiac anomalies	*DSP*	Desmoplakin
		JUP	Plakoglobin
		PKP1	Plakophilin 1
	EBS localized autosomal dominant	*KRT5*, *KRT14*	Keratin 5, keratin 14
	EBS generalized autosomal dominant	*KRT5*, *KRT14*	Keratin 5, keratin 14
	EBS autosomal recessive	*KRT14*	Keratin 14
		DST	Bullous pemphigoid antigen 1
		EXPH5	Exophilin 5
	EBS with mottled pigmentation	*KRT5*	Keratin 5
	EBS with circinate migratory erythema	*KRT5*	Keratin 5
	EBS with muscular dystrophy EBS with pyloric atresia EBS Ogna	*PLEC*	Plectin
Junctional EB (JEB)	JEB severe generalized JEB generalized JEB localized	*LAMA3*, *LAMB3*, *LAMC2*	Laminin-332
	JEB generalized JEB localized JEB late onset	*COL17A1*	Collagen XVII
	JEB with pyloric atresia JEB without pyloric atresia	*ITGB4*, *ITGA6*	$\alpha 6\beta 4$ integrin
	JEB with respiratory and renal involvement	*ITGA3*	$\alpha 3$ integrin subunit
Dystrophic EB (DEB)	DEB autosomal dominant DEB autosomal recessive DEB autosomal recessive severe generalized	*COL7A1*	Collagen VII
Kindler syndrome	—	*FERMT1*	Kindlin-1

Table 2 Non–epidermolysis bullosa disorders with skin fragility and blistering, causative genes, and affected proteins

Disorder	Clinical features	Gene	Protein
Keratinopathic ichthyoses	Superficial blisters and erosions at birth, ichthyosis	*KRT2*	Keratin 2
		KRT1, *KRT10*	Keratin 1, keratin 10
Pachyonychia congenita	Thick nails, palmoplantar keratoderma, blisters on feet	*KRT6A*, *KRT16*	Keratin 6A, keratin 16
		KRT6B, *KRT17*	Keratin 6B, keratin 17
Ankyloblepharon–ectodermal defects–cleft lip/palate syndrome	Erosions on the scalp, sparse hair, dystrophic nails, hypohidrosis, ankyloblepharon, hypodontia, cleft lip/palate	*TP63*	P63
Hypotrichosis and recurrent skin vesicles	Recurrent skin vesicles, hypotrichosis	*DSC3*	Desmocollin 3
Acrodermatitis enteropathica	Bullous skin lesions, alopecia, diarrhea, failure to thrive	*SLC39A4*	Solute carrier family 39 (zinc transporter) member 4
Incontinentia pigmenti	Blisters and erythema in a linear arrangement, anomalies of eyes and nervous system	*IKBKG*	NF-κB essential modulator (NEMO)
Porphyria cutanea tarda	Skin photosensitivity and blistering with associated features	*UROD*	Uroporphyrinogen decarboxylase
Congenital erythropoetic porphyria	Skin photosensitivity and blistering with associated features	*UROS*	Uroporphyrinogen III synthase
Ehlers–Danlos syndrome types I, II, and VIB	Skin hyperextensibility, bruising and abnormal scarring with associated features	*COL5A1*, *COL5A2*	Collagen type V α1, collagen type V α2
		CHST14	Carbohydrate (*N*-acetylgalactosamine 4-0) sulfotransferase 14
Ehlers–Danlos syndrome type VIIC	Very fragile skin, blue sclerae, joint hypermobility	*ADAMTS2*	ADAM metallopeptidase with thrombospondin type 1 motif

and hair. The spectrum of manifestations related to skin fragility is broad and includes blisters, erosions, wounds (which may become chronic), scars, crusts, milia, skin atrophy, and dyspigmentation (**Figure 1**). Depending on the expression pattern of the defective protein, other organs (such as the muscular system, gastrointestinal tract, heart, kidney, or urogenital tract) may be affected as well. Severe EB subtypes evolve as systemic diseases with secondary multiorgan involvement and failure to thrive, growth retardation, anemia, heart and bone disease, motor impairment, early propensity to skin cancers, and premature death. In contrast, when the physical signs are not classic, mild localized skin fragility may remain unrecognized and the diagnosis may be elusive, or may be unraveled only in the context of aging or associated diseases.

The main adhesive structures that assure the mechanical stability of the skin include the desmosomes, which are responsible for epidermal cell–cell adhesion, and the basement membrane zone (BMZ), which anchors the epidermis to the dermis (**Figure 2**). Both comprise multimolecular suprastructures whose components interact in a highly specific manner. The individual molecules in these structures are targets of pathogenic processes (e.g., genetic mutations and autoantibodies) that lead to skin fragility. Based on the ultrastructural level of skin cleavage, four major EB types are distinguished: EB simplex, junctional EB, dystrophic EB, and Kindler syndrome (38). Eighteen EB-associated genes are currently known, and the vast majority of these encode for structural proteins involved in epidermal and dermal adhesion (11) (**Table 1**).

BMZ: basement membrane zone

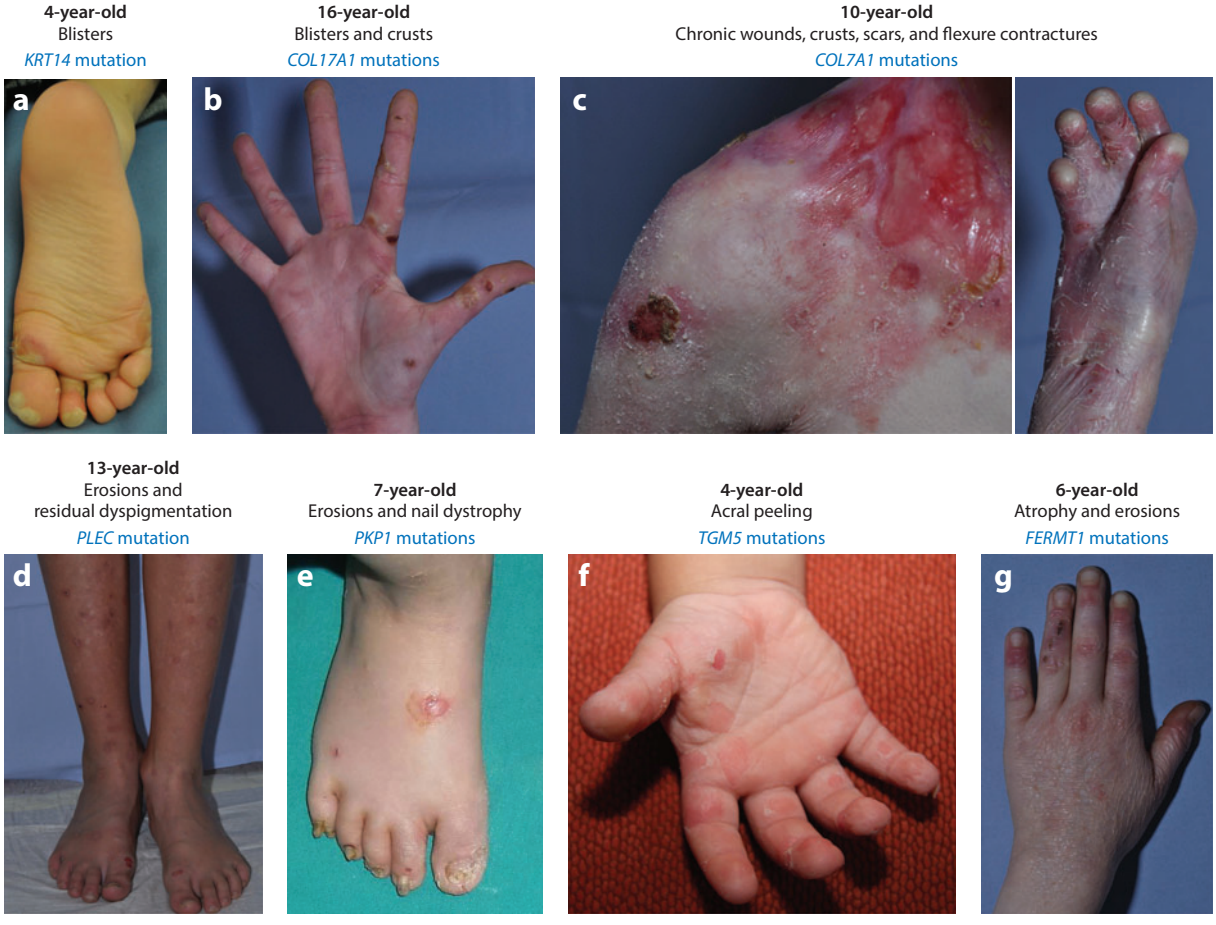

Figure 1

The spectrum of clinical manifestations related to skin fragility. (*a*) Blisters on the foot of a 4-year-old boy with localized epidermolysis bullosa (EB) simplex resulting from a *KRT14* missense mutation. (*b*) Blisters and crusts on the hand of a 16-year-old girl with junctional EB resulting from *COL17A1* mutations. (*c*) Chronic wounds, crusts, and scars (*left*) and flexure contractures of the hand (*right*) in a 10-year-old girl with severe generalized dystrophic EB resulting from *COL7A1* mutations. (*d*) Erosions and residual dyspigmentation on the lower legs of a 13-year-old boy with dominant EB simplex (Ogna) resulting from a *PLEC* mutation. (*e*) Erosions and nail dystrophy on the foot of a 7-year-old boy with *PKP1* mutations (courtesy of Dr. Ebtesam M. Abdalla, Department of Human Genetics, Alexandria University, Egypt). (*f*) Pronounced acral peeling on the hand of a 4-year-old boy with *TGM5* mutations. (*g*) Pronounced "cigarette-paper-like" atrophy and erosions on the hand of a 6-year-old girl with *FERMT1* mutations (Kindler syndrome).

THE EVOLUTION AND PRESENT STATE OF THE FIELD OF GENETIC SKIN FRAGILITY

The medical and scientific history of skin fragility can be regarded in four phases: clinical description, biochemical characterization, molecular genetic analysis, and use of high-throughput genomic analysis to extend genotype–phenotype correlations (11). Blistering, as the main sign of skin fragility, was the origin of the term epidermolysis bullosa, which was coined by Koebner (69) more than 120 years ago. The genetic background and molecular pathomechanisms of the major types were uncovered approximately a century later (**Figure 3**). In the first half of the

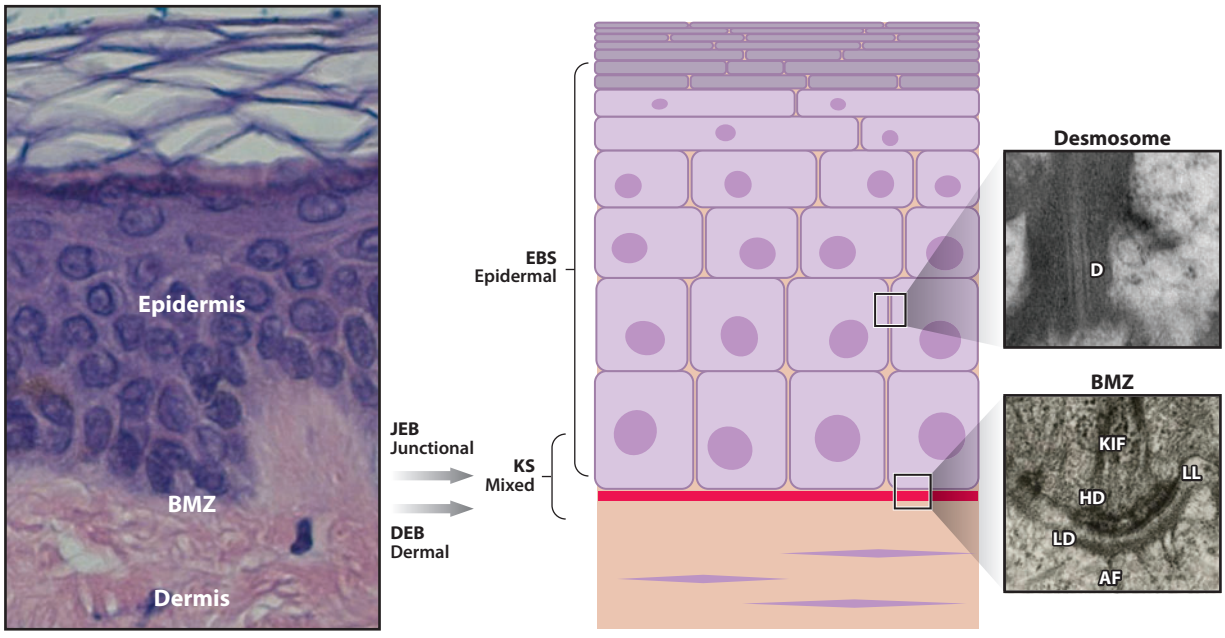

Figure 2

Structural basis of cutaneous adhesion and levels of skin cleavage in epidermolysis bullosa (EB). The left panel shows hematoxylin and eosin (H&E) staining of a skin section; the right panel shows a corresponding schematic representation. The levels of skin cleavage in the main EB types are indicated below each name. In EB simplex (EBS), blistering occurs within the epidermis in either the basal or the suprabasal layer. Junctional EB (JEB) and dystrophic EB (DEB) are characterized by subepidermal splits of the skin within the lamina lucida (LL) of the basement membrane and beneath the lamina densa (LD), respectively. Kindler syndrome (KS) is distinguished by mixed levels of skin cleavage. The insets show electron microscopy images of epidermal cell–cell and cell–matrix adhesions (courtesy of Dr. Ingrid Hausser, Department of Pathology, University of Heidelberg, Germany). Additional abbreviations: AF, anchoring fibril; BMZ, basement membrane zone; D, desmosome; HD, hemidesmosome; KIF, keratin intermediate filaments.

twentieth century, the major clinical entities were defined and the distinction was made between inherited and acquired forms of skin fragility. Many clinical variants were described and considered to reflect distinct etiologies, not simply variable expressions of one disorder. Later, in the 1960s, ultrastructural studies led to the first classification of EB into three major types—EB simplex, junctional EB, and dystrophic EB—based on the precise level of tissue separation (52, 96, 105).

In the 1980s, the generation of antibodies and development of immunofluorescence techniques resulted in the identification of the first proteins causally involved in EB and the establishment of the first molecular criteria for diagnosis using immunofluorescence mapping (36, 54). Molecular tools combined with immuno-electron microscopy allowed the identification of crucial adhesion proteins such as laminin-5 (previously kalinin, now laminin-332) and collagen VII as structural components of the hemidesmosomes and the anchoring fibrils, respectively (16, 17, 79, 80, 110). The new knowledge and diagnostic advances acquired during this decade were reflected in the revised clinical and laboratory criteria for EB published in 1991 (37).

The 1990s were marked by rapid progress in dermatogenetics, which was based on the development of molecular genetic methods, including gene cloning, linkage analyses for gene mapping, and efficient DNA sequencing. The mapping and discovery of genetic defects underlying several

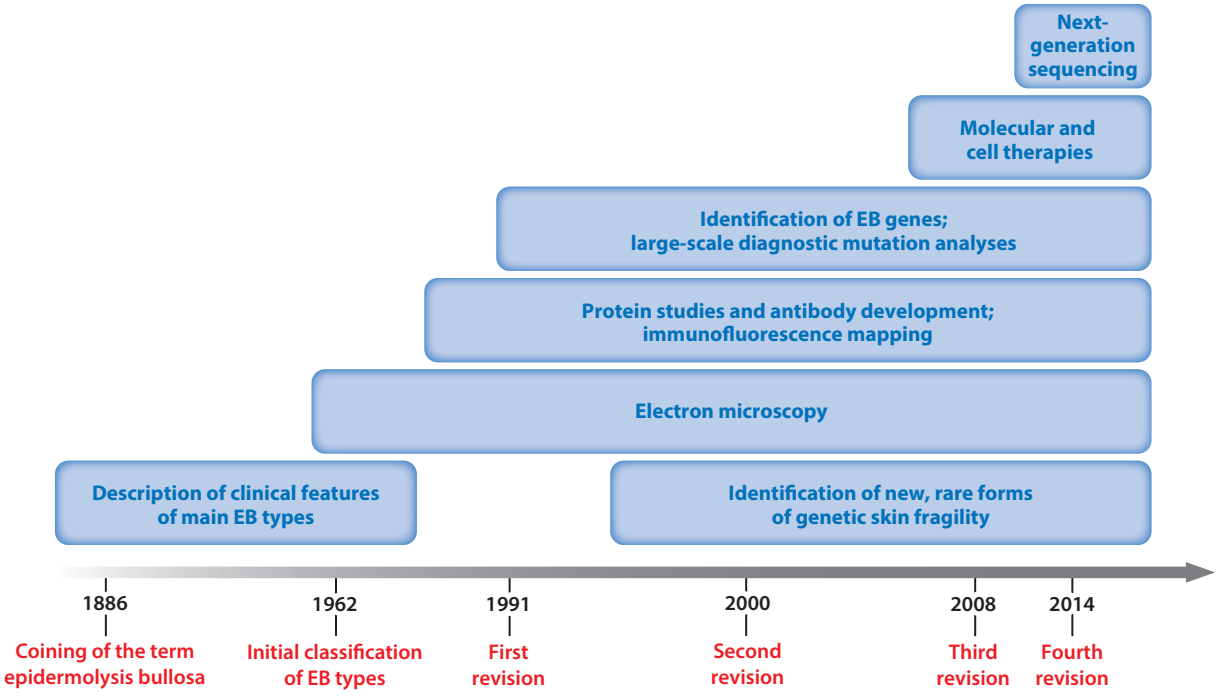

Figure 3

Schematic representation of the evolution and present state of the field of genetic skin fragility. Abbreviation: EB, epidermolysis bullosa.

EB subtypes were reported in rapid succession (23, 53, 56, 83, 107–109). At the beginning of the twenty-first century, substantial advances in understanding of the molecular basis of many old and new skin fragility disorders led to the tendency to avoid splitting the disease into too many subentities (38). Mutation analyses in a large number of patients with EB revealed that mutations in the same gene may cause a spectrum of phenotypes and that particular clinical features do not correlate with specific mutations.

In spite of the progress of the past decades, the field remains challenging and dynamic. Although novel therapies are being developed for dystrophic and junctional EB (the most severe and well-established EB types), the number of inherited disorders with skin fragility continues to expand. This is not surprising if one reflects on the complexity of the adhesion structures in the skin. The advances of the novel sequencing technologies have allowed investigators to elucidate the genetic defects of very rare conditions (22), revealing that previously unknown proteins play a role in keratinocyte biology and are associated with skin fragility (85).

THE MOLECULAR BASIS OF CUTANEOUS ADHESION

The skin forms the outer barrier that protects the human body from external insults; it is highly resistant to physical, microbial, and chemical attacks and can tolerate mechanical stress. This results from a coordinated dynamic balance between proliferation, regeneration, differentiation, and shedding of the keratinocytes, the main constituents of the epidermis. In this context, epidermal cell–cell and cell–matrix adhesions are critical, not only from a purely mechanical point of view but

also in terms of their roles as hubs for signal transmissions by which cells sense their environment. To understand the structural and molecular basis of EB and related skin fragility disorders, basic knowledge of the epidermal cell–cell and cell–basement membrane adhesion is pivotal.

Epidermal Cell–Cell Adhesion

Epidermal cell–cell adhesions comprise desmosomes, adherens junctions, tight junctions, and gap junctions. The desmosomes are major intercellular junctions in many tissues exposed to mechanical stress, such as the skin, myocardium, bladder, and gastrointestinal mucosa (30). In the epidermis, they are easily recognized under an electron microscope, with an electron-dense midline in the intercellular space halfway between opposing keratinocyte plasma membranes, sandwiched by two pairs of electron-dense cytoplasmic plaques (43) (**Figure 2**). The stable molecular interactions between the desmosomal plaques and intermediate filaments provide a high degree of resistance to mechanical forces. Apart from the adhesion function, in vivo data suggest that desmosomal molecules also play a role in epidermal cell signaling (86).

The individual components of the desmosomal protein complex belong to three protein families: cadherins, armadillo proteins, and plakins. The desmosomal cadherins—desmogleins and desmocollins—are transmembrane proteins. The extracellular domains of these proteins consist of cadherin repeats separated by calcium-binding motifs, and the transmembrane stretch is followed by an intracellular domain at the cytoplasmic face of the plasma membrane (30). The extracellular domains interact to form the adhesive interface, whereas the cytoplasmic tails bind to the armadillo proteins. These proteins regulate the desmosomal assembly and attach desmoplakin and keratin filaments to the desmosome. Plakoglobin is found at both desmosomes and adherens junctions; it functions as a scaffold for several binding partners, including desmosomal cadherins, E-cadherin, and desmoplakin (86). Plakophilins are expressed in a tissue- and differentiation-specific manner, and plakophilin 1 and 2 are also found in the nucleus, suggesting specific regulatory roles. The N terminus of plakophilin 1 binds desmoglein 1, desmoplakin, and keratin intermediate filaments, and it plays a key role in the assembly of the desmosomes (5). Desmoplakin links the keratin cytoskeleton to the armadillo proteins and cadherins. It is the most abundant component of the desmosomes and binds through the N-terminal domain to plakoglobin and plakophilins. These highly orchestrated specific interactions secure tight cell–cell adhesion, and so it makes sense that genetic alterations in the genes encoding these proteins that cause entire or partial loss of their functions will result in a broad spectrum of epidermal fragility disorders.

Cell–Matrix Interactions

The epidermal BMZ in the skin and mucous membranes, also called the dermal–epidermal junction zone, is a highly specialized structure with a multitude of functions (123). It attaches the epidermis to the dermis; lends the skin resistance against mechanical forces; maintains tissue architecture during development, repair, and regeneration; and regulates epithelial–mesenchymal interactions (4, 15, 55, 122, 123). The BMZ is not visible by light microscopy but is easily recognizable under an electron microscope as a bilayer comprising the electron-lucent lamina lucida and the electron-dense lamina densa (**Figure 2**). Flanking structures include the hemidesmosomes on the plasma membrane of basal keratinocytes that face the lamina lucida, the anchoring filaments emanating from the hemidesmosomes through the lamina lucida, and the anchoring fibrils that extend from the lamina densa into the dermis. Next to the hemidesmosomes, focal adhesion complexes provide additional cohesion by binding the actin cytoskeleton to the basal cell plasma membrane and subsequently to the BMZ.

At the molecular level, the hemidesmosomes represent supramolecular assemblies of intracellular and transmembrane proteins. Intracellular BPAG1 and plectin are localized in the hemidesmosomal inner plaque that interacts with keratin intermediate filaments (132). The two transmembrane components are collagen XVII and α6β4 integrin (78). Collagen XVII is a type II transmembrane protein with a small endodomain (which interacts with plectin, BPAG1, and the β4 integrin subunit to link the keratin cytoskeleton into the hemidesmosome) and a large ectodomain with a collagenous structure (39, 49). The ectodomain binds laminin-332 and the α6 integrin subunit to ensure the adhesion of the cell to the basement membrane, and it can be proteolytically shed from the cell surface to release the cell from the basement membrane when needed (39). The α6 integrin subunit forms a functional integrin with the β4 subunit; its ligands include collagen XVII and laminin-332. The focal adhesions at the basolateral plasma membrane of basal keratinocytes contain integrin α3β1 and kindlin-1 (78). The α3 integrin subunit provides adhesion by binding to laminin-332 and laminin-511. Kindlin-1 is an epithelium-specific phosphoprotein and an adaptor protein linking filamentous actin into the cell cortex. It is an activator of β1 integrins and a member of the intracellular β1 integrin–associated signaling complex along with numerous other proteins. In vitro and in vivo studies of human and murine cells that lack kindlin-1 have revealed its functions in maintaining keratinocyte shape, polarization, adhesion, and proliferation.

The bilayered basement membrane underneath these cell-surface-associated protein complexes contains several molecular components. The electron-lucent lamina lucida is traversed by anchoring filaments that consist of laminin-332 and the ectodomain of collagen XVII. The filaments insert into the lamina densa, the lower layer of the basement membrane, and a specific molecular network containing collagen IV, nidogens, laminin-511, and perlecan (6, 33, 55, 126). From the lamina densa, anchoring fibrils extend into the dermis and link the basement membrane with it. Under an electron microscope, the fibrils appear as cross-striated condensed aggregates with frayed ends (**Figure 2**). Their only known molecular component is collagen VII, which undergoes a multistep procollagen-to-collagen maturation and assembly process to form the anchoring fibrils (123). Binding of collagen VII to its ligands, laminin-332 and collagen IV, secures the attachment of the anchoring fibrils to the basement membrane, and its binding to collagen I links the anchoring fibrils to dermal fibrils (128). Mutations in the genes encoding components of the BMZ perturb the functions of the BMZ and weaken the adhesion of the skin layers. The clinical consequences are mechanically induced skin blistering, delayed wound healing, and chronic skin fragility.

LESSONS FROM MUTATION ANALYSES

Over the past 20 years, extensive mutation analyses have identified more than 1,500 distinct mutations in 18 different genes causing skin fragility and have contributed significantly to a better understanding of the corresponding phenotypic spectrum. The broad range of clinical severity results mainly from a large number of allelic disease-causing variants. In addition, genetic, epigenetic, and environmental modifying factors, which remain largely unknown, are likely to contribute to intrafamilial phenotypic variability. For most types of EB, genotype–phenotype correlations apply in cases of complete loss-of-function mutations, which are associated with the most severe generalized forms of skin fragility. Apart from this clear-cut rule, partial expression or function of the target proteins seems to result in a multitude of mild and moderate phenotypes. In such cases, the molecular mechanisms are understood only in part. Ample biochemical knowledge has been derived from studies of the consequences of mutations and the resulting in vitro and in vivo phenotypes in humans and in animal models (13). However, replication of the results in larger

cohorts is mostly lacking, and our knowledge remains biased by the small number of observations and the lack of confirmation in additional cases.

This limitation could be overcome by the implementation of carefully curated gene-specific databases or patient registries in which investigators could include the genotypes and phenotypes of their unpublished cases. Such long-term, international efforts are the only basis for reasonable studies on epidemiology and genotype–phenotype correlations, for the identification of disease-modifying factors, and, from a more practical point of view, for genetic counseling and prognostication.

PTC: premature termination codon

The Phenotypic Diversity of Skin Fragility Disorders

Clinical manifestations of skin fragility in newborns and infants are rather monomorphic and do not allow disease classification or outcome prediction. Only later can secondary symptoms and complications suggest a diagnosis, e.g., severe generalized junctional or dystrophic EB (47) (**Figure 1*b*,*c***). Cases with late onset or minor skin fragility are even more difficult to assess and often remain unrecognized or misdiagnosed (50, 68, 85) (**Figure 1*d*–*g***). Molecular diagnostics by immunofluorescence mapping with antibodies to epidermal and BMZ adhesion molecules serves to determine the level of skin cleavage and to identify the defective protein (102) (**Figure 2**). This method assigns the EB type, especially if skin blistering is pronounced and mutations lead to a lack of protein expression, but usually fails to be informative in cases with mild skin fragility (47). Mutation analysis remains the diagnostic gold standard, and real-world practice has shown that analysis of several genes may be required in order to find the disease-causing mutation.

There is an interesting contrast of genotype–phenotype correlations between junctional and dystrophic EB. Junctional EB is molecularly heterogeneous, being associated with mutations in seven different genes, but the phenotypes are all similar regardless of the causative gene (12). In contrast, all forms of dystrophic EB are caused by mutations in one gene, but the phenotypes exhibit great variety. The genes involved in junctional EB encode membrane and structural proteins, such as collagen XVII, laminin-332, or the α6 and β4 integrin subunits. Structurally and functionally, these are intimately interconnected in the supramolecular networks at the BMZ. Therefore, it is not surprising that the main clinical features of different junctional EB subtypes are similar, encompassing skin and mucosal blistering, nail dystrophy and loss, and/or amelogenesis imperfecta (75, 137). Whereas loss-of-function mutations lead to severe phenotypes (i.e., generalized severe junctional EB, generalized junctional EB, or junctional EB with pyloric atresia), residual protein expression resulting from splicing or missense mutations usually yields more moderate clinical features (67, 113). In such cases, skin fragility may be mild and localized in childhood, mitigated during adulthood, and triggered again by advanced age or by acquired disorders (C. Has & L. Bruckner-Tuderman, unpublished cases). Approximately 15% of physiological BMZ protein levels seems to suffice for adequate mechanical stability of the skin and mucous membranes, but not for appropriate formation of the enamel or resistance of the nails to permanent minor trauma (67).

A particular constellation occurs with both *COL17A1* and *LAMB3* mutations, apparently leading to premature termination codons (PTCs) but with moderate phenotypes. In such cases, alternative regulation of splicing rescues the total loss of the gene product and leads to disease of moderate severity (40, 90, 106).

Although all subtypes of dystrophic EB are caused by mutations in the collagen VII–encoding gene (*COL7A1*), the molecular pathology remains challenging because of the large number and variety of mutations reported (e.g., in the Human Gene Mutation Database; **http://www.hgmd.org**). More than 650 mutations, both dominant and recessive, are known; these may occur in compound heterozygosity in some cases (1). The clinical features range from

skin and mucosal blistering to scarring, chronic wounds, loss or dystrophy of nails, and alopecia (10) (**Figure 1*c***). At one end of the phenotypic spectrum, dramatic blistering, wounds, scarring, joint contractures, and reduced life span are caused by the complete absence of collagen VII, whereas at the other end, sole nail dystrophy can reflect certain glycine substitution mutations.

MMP1: matrix metalloproteinase 1

Glycine substitutions in the triple helix of collagen VII have a particular significance because they can disrupt the local helix stability and the salt bridges that function to stabilize subregions of the triple helix (136). Depending on the localization of the mutated glycine within the large triple helix, altered protein folding renders the collagen VII molecule more or less unstable and sensitive to degradation. Glycine substitution mutations in collagen VII may be inherited in a dominant or recessive manner, and genotype–phenotype correlations are difficult to predict (1). The rare subtype of dystrophic EB inversa affects mainly the great folds and was hypothesized to be caused by specific arginine and glycine substitutions in the collagenous subdomains, although only a few patients were investigated (124). Although the mechanisms underlying this predilection of blistering in the folds remain unknown, the hypothesis was that glycine substitutions located near the borders of collagenous subdomains exert milder changes than those located in the centers of collagenous subdomains (21) and that the pathophysiology is temperature dependent (124). More than 100 *COL7A1* splicing mutations are known at the canonical splice sites as well as within regulatory regions in introns or exons (26, 34, 63, 131). Most of them are recessive, but some cause in-frame skipping of exons and may act in a dominant manner (70, 138). In dystrophic EB, clinical variability between cases with the same mutation, or within the same family (31, 103, 118), is not rare and may be due to disease modifiers (see below).

Phenotypic Modifiers of Skin Fragility

The mechanical fragility of the skin is strongly dependent on the interactions between genetic susceptibility and environmental factors. The degree of the symptoms is related not only to the molecular defect and its consequences but also to a great extent to external factors, such as the mechanical load applied to the skin or infections, as well as to the nutritional status of the patient and the quality of medical care. Modifier genes, an integral part of the genetic landscape, have been less well explored in this context. A growing number of modifier genes are emerging from studies with mouse disease models, and new technical advances promise many more to come (45). Skin fragility could conceivably result from a primary gene defect that is modulated by variants in the same gene or in genes coding for molecular interaction partners, or by enzymes involved in protein processing or degradation. Although clinicians commonly observe variability within families with different EB forms (27, 103), only a few molecular mechanisms have been uncovered.

The first example of a modifier gene in skin fragility was *MMP1*, encoding matrix metalloproteinase 1 (MMP1) (118). Collagen VII is a substrate of MMP1, and an imbalance between its synthesis and degradation could conceivably worsen the dystrophic EB phenotype. The initial study addressed three siblings that had the same *COL7A1* genotype but great variations in disease severity. The variations correlated not with collagen VII synthesis levels but with protein levels at the BMZ, suggesting increased degradation. This idea was supported by increased transcript and active MMP1 levels in the most severely affected children, who carried a known single-nucleotide polymorphism (1G/2G) in the *MMP1* promoter. This polymorphism creates a functional ETS binding site, resulting in transcriptional upregulation. Subsequently, in a cohort of 31 unrelated dystrophic EB patients that had at least one in-frame *COL7A1* mutation and mild, moderate, or severe phenotypes, Titeux et al. (118) found a strong genetic association between the 2G variant and the severe disease phenotype; however, this result was not replicated in independent cohorts (2, 63).

Another interesting example is a monozygotic twin pair discordant for recessive dystrophic EB. The phenotypic variation did not correlate with collagen VII synthesis and deposition, demonstrating that other genes influence the phenotypic severity in this disease. Genome-wide expression profiling showed that twin fibroblasts differentially express genes encoding components of the TGF-β signaling pathway or molecules involved in inflammation and extracellular matrix remodeling (31, 89).

BPAG1-e: bullous pemphigoid antigen 1

Bubier et al. (18) impressively documented the potent impact of genetic modifiers on the strength of dermal–epidermal adhesion and on the clinical severity of junctional EB in the context of the *Lamc2$_{jeb}$* mutation in a hypomorphic mouse model with low levels of the BMZ protein laminin-332. Through an unbiased genetic approach involving a combination of quantitative trait locus mapping and positional cloning, the authors elegantly demonstrated that *Col17a1* is a strong genetic modifier of the junctional EB that develops in *Lamc2$_{jeb}$* mice. This modifier is defined by variations in 1–3 neighboring amino acids in the noncollagenous 4 (NC4) domain of the collagen XVII protein, which is a physiological ligand and binding partner of laminin-332. The allelic variants alter the strength of dermal–epidermal adhesion in the context of the *Lamc2$_{jeb}$* mutation and, consequently, broadly impact the clinical severity of junctional EB (116).

New Forms of Skin Fragility

The majority of the well-defined and more common EB subtypes have been recognized and characterized on the clinical and molecular levels. Still, very rare entities emerge. Some are clinically unspectacular and difficult to discriminate from the classical subtypes. Others remained unrecognized for a long time, probably because they lead to early demise.

The EB simplex surprise. EB simplex with skin cleavage within the basal epidermal layer is the most common form of skin fragility, with the vast majority of cases caused by mutations in the genes encoding keratin 5 or 14. Surprisingly, this EB type has recently grown extremely complex, with many more new subtypes and genes than expected.

Mutations in the genes encoding BPAG1-e (dystonin isoform 4), exophilin 5 (Slac2-b), and plectin can cause skin cleavage within the basal layer and mild blistering and may account for at least some of the molecularly unsolved EB simplex cases. BPAG1-e has long been known as bullous pemphigoid antigen 1, the target in bullous pemphigoid, an autoimmune disorder with severe acquired skin fragility. The phenotype of the corresponding genetic disorder had remained mysterious for many years, and finally it proved to be quite unspectacular. Groves et al. (44) identified a homozygous nonsense mutation in the gene encoding BPAG1-e (*DST*) in a Kuwaiti individual with autosomal recessive EB simplex. The mutation occurred within the gene segment encoding the coiled-coil rod domain of BPAG1-e. The main symptom was mild trauma-induced acral blistering, although there was also some generalized skin fragility. Liu et al. (77) subsequently reported an unrelated family with mild skin blistering resulting from a different homozygous nonsense mutation in the *DST* gene. The clue to diagnosis lies in the lack of hemidesmosomal inner plaques visible under transmission electron microscopy and in the negative BPAG1-e immunostaining of the skin (77).

Astute observation of distinctive clinical features in a consanguineous family coupled with genomic searches led to recognition of mutations in the gene encoding exophilin 5 (*EXPH5*) (85). Whole-exome sequencing revealed a homozygous frameshift mutation in *EXPH5* in three siblings (85). The clinical features comprised generalized scale-crusts and occasional blisters, mostly induced by trauma, as well as mild diffuse pigmentary mottling on the trunk and proximal limbs (85, 101). Exophilin 5 is a Rab27B GTPase effector protein. Rab proteins form part of the

Ras superfamily of monomeric G proteins, which regulate many key steps of cell membrane traffic, including vesicle formation, vesicle movement along actin and tubulin networks, and membrane fusion (85). A role for exophilin 5 in keratinocyte biology was supported by findings of cytoskeletal disruption and decreased cell adhesion both in keratinocytes from an affected subject and in normal keratinocytes after small hairpin RNA knockdown of exophilin 5 (85).

In 2002, Koss-Harnes et al. (71) reported that a site-specific mutation in the gene encoding plectin (*PLEC*), p.R2000W, causes a rare autosomal dominant EB simplex, designated Ogna, in two families. This unremarkable phenotype subsequently remained underrecognized and was revisited only recently (7, 68). It is difficult to clinically distinguish such cases from EB simplex caused by *KRT5* or *KRT14* mutations (**Figure 1*d***). Surprisingly, in a Dutch cohort, *PLEC* mutations accounted for 8% of the autosomal dominant or sporadic EB simplex cases (7). The diagnosis is indicated by electron microscopy analysis revealing abnormal hemidesmosomes and by indirect immunofluorescence with antibodies to the rod domain of plectin revealing strongly reduced or negative staining. The p.R2000W mutation renders plectin's rod domain more vulnerable to cleavage by calpains and other proteases activated in the epidermis but not in skeletal muscle, explaining this particularly mild phenotype (130).

The most puzzling molecular pathology concerns the skin fragility disorders associated with defects of desmosomal proteins. These EB simplex subtypes with cleavage in the suprabasal epidermal layers are very rare (61, 84, 100). Mutations in the genes coding for desmoplakin, plakoglobin, and plakophilin 1 cause similar clinical pictures, comprising skin erosions, palmoplantar keratoderma, and anomalies of nails and hair (98) (**Figure 1*e***). Importantly, they also cause anomalies of cardiac desmosomes, leading to disrupted cell–cell adhesion, defects of desmosome-mediated intracellular signaling, and arrhythmogenic right ventricular cardiomyopathy/dysplasia (25). The most severe phenotypes of desmosomal skin fragility disorders evolve with congenital generalized erosions, loss of skin barrier function, and poor prognosis in the neonatal period (82), whereas in subtypes with milder skin fragility, the cardiac disease may determine the prognosis (8). Genotype–phenotype correlations are difficult to predict. For example, recessive mutations leading to PTCs in the desmoplakin gene have been reported in cases with very different clinical severity, namely lethal acantholytic EB or palmoplantar keratoderma, woolly hair, and arrhythmogenic right ventricular cardiomyopathy/dysplasia. In the future, a better understanding of the range of clinical phenotypes in combination with the inherent desmosome gene mutation(s) in more patients will be helpful in managing and counseling patients as well as in providing insights into the biological functions of specific components of desmosomes in the skin and other tissues (98).

The molecular basis of EB simplex with superficial blisters, peeling, and subcorneal skin cleavage has remained unclear for many years. Recently, mutations in the gene encoding transglutaminase 5 that are associated with acral peeling skin syndrome have been found in several of these patients (65, 99) (**Figure 1*f***), and this disorder is now considered a subtype of EB simplex (37a).

Skin fragility resulting from mutations in genes encoding proteins related to focal adhesions. Focal adhesions are integrin-containing, multiprotein assemblies that span the plasma membrane and link the cellular cytoskeleton to the surrounding extracellular matrix (35). Focal adhesions can be observed in cultured cells in vitro but not in tissues in situ, and their functional significance in the organism has therefore been questioned. Support for the physiological role of these multiprotein assemblies in the skin has been provided by two skin fragility disorders caused by mutations in genes encoding two focal adhesion proteins: kindlin-1 and the α3 integrin subunit (51, 59). Kindler syndrome, caused by mutations in the gene encoding kindlin-1 (*FERMT1*), is considered a distinct type of EB because the level of skin cleavage is mixed above, along, or below

the basement membrane, and it is typically associated with reduplications of the basement membrane. The clinical phenotype progresses from skin blistering in childhood to increasing pigment anomalies and skin atrophy (poikiloderma), soft tissue scarring, and a predisposition to epithelial skin cancer in adulthood (48) (**Figure 1*g***).

Because loss of integrin α3 in transgenic mouse models caused abnormal kidney and lung organogenesis and skin blisters, the gene encoding this integrin was a candidate gene for junctional EB for a long time (32, 72). In 2012, the corresponding human disease was identified: Homozygous loss-of-function mutations in the integrin α3 gene cause a lethal multiorgan disorder, including congenital nephrotic syndrome, interstitial lung disease, and EB (51, 87). The renal and respiratory features predominated and determined the course of the disease. Although skin fragility was mild, it provided clues to the diagnosis. In all affected organs, disrupted basement membrane structures compromised barrier functions. This explained the pathogenesis in part, but further patients must be investigated for a full understanding of the disease mechanisms.

REVERTANT MOSAICISM IN SKIN FRAGILITY DISORDERS

Spontaneous gene repair, also called revertant mosaicism or natural gene therapy, has been documented in several genetic disorders involving organs that undergo self-regeneration, e.g., Lesch–Nyhan syndrome, Wiskott–Aldrich syndrome, and Bloom syndrome (93). Through its visibility and accessibility, the skin is particularly well suited for the recognition and study of somatic mosaicism. In 1997, Jonkman et al. (62) observed revertant mosaicism in junctional EB and demonstrated it on a molecular genetic level. For many years this phenomenon was considered rare, but recently it has become clear that it is more widespread than expected and occurs in all EB types (93). Because of the therapeutic potential of revertant cells, the interest of researchers has strongly increased.

The literature abounds in case reports on revertant mosaicisms in simplex, junctional, and dystrophic types of EB, with single or a few revertant skin spots. Various repair mechanisms of the disease-causing mutation—e.g., back or second-site mutation, mitotic recombination, or deletion—have been identified (28, 60, 91, 94, 95, 112, 115, 125). Notably, Kindler syndrome patients with *FERMT1* duplication mutations demonstrate a particular disseminated pattern of revertant mosaicism (66) (**Figure 4**). Back mutations arising from slipped mispairing in direct nucleotide repeats were found in all investigated revertant skin spots from two patients (66). The sequence around the mutations demonstrated a high propensity for mutation, favoring both microinsertions and microdeletions. Additionally, in some revertant patches, mitotic recombination generated areas with homozygous normal keratinocytes. Restoration of kindlin-1 expression led to clinically and structurally normal skin in terms of epidermal stratification and proliferation as well as BMZ morphology. Because loss of kindlin-1 severely impairs keratinocyte proliferation, revertant cells have a selective advantage that allows their clonal expansion and, consequently, the improvement of the skin condition (66).

Some fundamental scientific questions regarding revertant mosaicism remain to be answered using systematic investigations, analysis of larger cohorts of patients, and probably the establishment of models. For example, what is its incidence in skin fragility disorders? What are the mechanisms of mutation repair? When do revertant mutations occur? And what is the natural history of the revertant areas (93)? That mosaicism is a spectrum seems conceivable. The idea is that patients acquire revertant cells at some point, and the frequency, diversity, and functionality of the revertant cells depend on various factors, including the strength of selection (29).

From a more pragmatic perspective, two important challenges must be solved: how to recognize revertant skin and how to expand the patient's revertant cells to provide significant material for cell

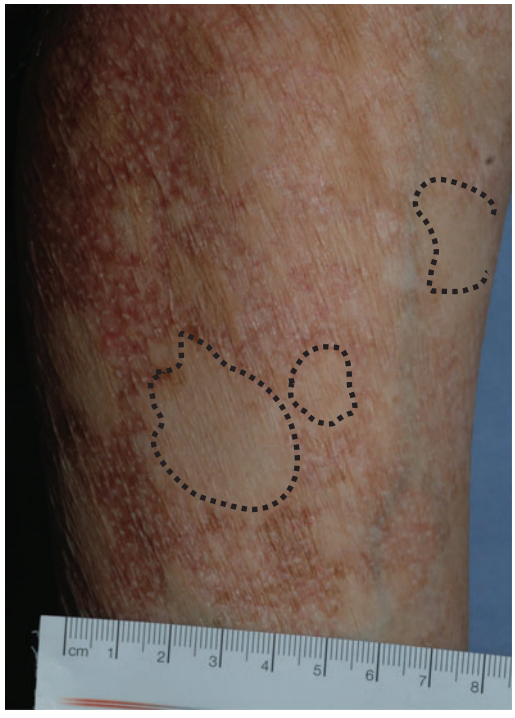

Figure 4

Revertant mosaicism on the arm of a patient with Kindler syndrome. Against the contrast of the erythematous, atrophic background of the affected skin, numerous revertant areas (of which three are outlined) with normal-appearing skin color and texture can be distinguished.

therapies (see below). Clinically, revertant skin areas demonstrate a "normal" or improved texture and mechanical resistance compared with the surrounding affected skin (**Figure 4**). The "normality" can be rapidly confirmed through indirect immunofluorescence staining with antibodies specific to the mutant protein. Reversion will lead to an observable and significant increase in protein levels, especially in cases with PTC mutations. By contrast, in cases with mild skin fragility and residual protein expression, differences between revertant and diseased areas are difficult to appreciate on both a clinical and a molecular level. Defining the reversion mechanisms of the mutations is more labor intensive and requires special skills and equipment, e.g., laser-dissection microscopy (92).

FUTURE ISSUES

Improved Diagnostic Methods

The strong impact of novel DNA sequencing technologies on the diagnostic and basic research of genetic skin fragility is clear and expected to increase in the future. Although diagnostic algorithms for EB are well established and validated as described above and elsewhere (20, 102), the application of next-generation sequencing has led to the identification of new genes (85) and to the rapid diagnosis of unusual phenotypes (50, 73; reviewed in 22, 111).

Targeted sequencing of panels of genomic regions has been successfully applied to the diagnosis of genetic skin diseases (114) and is also well suited for skin fragility disorders. It is more

affordable than large-scale exome or genome sequencing, yields much higher coverage of the genomic regions of interest, and reduces sequencing cost and time (42). This accelerates diagnosis by circumventing skin biopsies, avoiding inconclusive findings from skin immunofluorescence staining, and eliminating the need to sequence several genes. In addition to revealing disease-causing mutations, this approach will enable investigators to identify sequence variants in other genes associated with skin fragility that could possibly act as genetic modifiers and account for phenotypic particularities. However, the interpretation and biological validation of such findings will remain challenging.

Furthermore, analyses of the molecular mechanisms of somatic mosaicism, in particular those of revertant mosaicism, would strongly benefit from next-generation sequencing. Targeted deep sequencing of the gene harboring the disease-causing mutation would allow the identification of low-coverage variants potentially involved in mutation repair in a small population of cells. This procedure would bypass the current laborious and error-prone procedures comprising tissue microdissection, nested polymerase chain reaction, subcloning, and sequencing of a large number of clones (92).

Because most of the genes associated with skin fragility are known and the candidate gene can be determined in the majority of cases, it remains doubtful that whole-exome sequencing will be established as the diagnostic procedure for this group of disorders (139). However, it will certainly be the best choice for unusual phenotypes that remain unexplained after targeted sequencing of skin fragility gene panels. In such cases, either mutations in new genes or two disease-causing mutations may be expected.

Perspectives for Molecular and Cell Therapies

Both the urgency of high unmet medical need and the rapid advances in elucidating the molecular mechanisms of skin fragility disorders have led to a new major research focus in the field: the development of biologically valid therapies. Indeed, the skin is an ideal organ to study novel therapeutic approaches because it is easily accessible both for the treatments and for macroscopic, microscopic, cellular, and molecular analyses. Functional restoration of components of epidermal or BMZ adhesion complexes in human skin is obviously a huge task, but preclinical research has delivered positive information that may reduce the challenge at least to some extent. Analysis of both collagen VII–deficient mice and collagen XVII–deficient human keratinocytes indicated that clinical benefit does not require full restoration of a missing protein; approximately 15–35% of the physiological collagen levels could be sufficient for adequate dermal–epidermal adhesion (67, 88). Many laboratories worldwide are involved in preclinical testing of therapies using genes, cells, and proteins both in animal models and in pilot trials with individual patients (**Table 3**), as described below.

The first pilot study on treatment of laminin-332-deficient junctional EB with keratinocyte gene therapy was successful (81). Several years of follow-up demonstrated the clinical stability of the graft and persistent laminin-332 expression at the BMZ (19). However, the limited capacity and inadvertent genomic insertion of viral vectors and the moratorium on the use of retroviral vectors for gene therapy in Europe have hampered further development of this approach. These problems can possibly be circumvented by the use of skin or keratinocyte grafts derived from the patient's skin patches with revertant mosaicism (41). In the future, when the issues relating to suitable vectors for gene transfer have been resolved, the ex vivo gene therapy approach could also employ genetically corrected keratinocytes or fibroblasts differentiated from induced pluripotent stem cells derived from the patient's own cells (14, 57, 120).

Because gene therapy for skin fragility disorders turned out to be substantially more complicated than initially anticipated, scientists were forced to assess alternative treatment modalities,

Table 3 Novel therapy approaches for skin fragility disorders

Disorder	Therapeutic modality	Reference(s)	Comments
EB simplex	Small interfering RNA	3	In vitro approach
Junctional EB	Keratinocyte gene therapy	81	Human pilot trial, functional keratinocyte grafts, use of retroviral vectors problematic
	Bone marrow transplantation	129	Severe complications
	Small skin grafts with revertant mosaicism	41	Graft take successful, sustained skin integrity
Dystrophic EB	Intradermal fibroblast injections	64, 97, 127, 134	Preclinical, clinical pilot trials with individual patients, no long-term follow-up
	Intradermal injection of mesenchymal stem cells	24	Clinical pilot trial with one patient, no long-term follow-up
	Bone marrow stem cells	117, 119	Preclinical
	Bone marrow transplantation	120, 129	Transition of the phenotype to a milder form
	Protein replacement therapy	104	Preclinical
	Induced pluripotent stem cells	58, 121	In vitro, preclinical
Pachyonychia congenita	Small interfering RNA	76	Human pilot trial, highly painful administration

Abbreviation: EB, epidermolysis bullosa.

including cell-based and protein replacement therapies. Dystrophic EB is an ideal model to test these approaches because *COL7A1* is the only causative gene, and the fact that collagen VII is synthesized by both keratinocytes and fibroblasts means that different options are available. Intradermal injections of allogeneic fibroblasts or bone marrow–derived mesenchymal stromal cells have been evaluated in mouse models and in pilot experiments in individual patients with dystrophic EB. The cell injections led to the accumulation of collagen VII protein at the BMZ, and although the cells underwent apoptosis within four weeks, the collagen exhibited high stability and persisted for several months (24, 64, 97, 127, 133). These experiments provided proof of principle of the feasibility of topical cell therapies, but—more important—they demonstrated that a therapeutically administered structural protein of the BMZ, collagen VII, remains stable and functional in the skin for extended periods. This is also likely to be the case for other BMZ proteins, e.g., laminin-332.

Systemic stem cell therapy has been assessed in a clinical trial of bone marrow transplantation in individuals with dystrophic EB (120, 129). A follow-up study demonstrated some increased deposition of collagen VII at the BMZ and some degree of clinical benefit that in optimal cases has lasted up to four years after the transplantation. However, the high cost and 25% morbidity and mortality rate (120) associated with this procedure must be weighed against the long-term benefits. Moreover, the nature of the bone marrow–derived cells and other factors generating the clinical responses remains elusive (120). Therefore, both the international scientific community and patients are awaiting more accurate data on the outcome of the complex treatments. New clinical trials with informative end points are needed to determine the true risk–benefit ratio and the clinical applicability of this treatment modality.

An intriguing approach to counteract blistering in EB involves protein replacement therapy. Based on ample published data concerning the protein biochemistry, suprastructural assembly, and structural biology of basement membranes, it was unexpected that both intradermal and intravenous injections of recombinant human collagen VII into collagen VII–deficient mice

resulted in deposition of the collagen at the BMZ and partial correction of the blistering phenotype (104, 135). These data suggest that collagen VII protein replacement therapy could become a realistic option. If so, this approach will have substantial clinical advantages, because no gene manipulation, allogeneic cells, or subsequent immunosuppressive therapies will be needed (14 and references therein). This line of protein replacement therapy for dystrophic EB is currently being developed by the pharmaceutical industry.

Small-molecule compounds—e.g., chemical compounds and drugs—have not received much attention as potential therapeutic agents for skin fragility disorders. They should be reconsidered, because although such compounds will not correct the gene defect, they may be valuable in inhibiting tissue alterations and alleviating symptoms. A further advantage is that, particularly in the case of repurposed drugs, the regulatory requirements should be significantly less extensive than they are for gene-, cell-, or protein-based therapies.

Preliminary in vitro evidence for the therapeutic potential of small-molecule compounds has been reported for some skin fragility disorders, including the use of small interfering RNA for dominantly inherited EB simplex (3) and PTC read-through aminoglycosides for several types of genetic skin disorders (74). In dystrophic EB, progressive soft tissue scarring and subsequent skin cancer are feared complications, and inhibitors of these processes can alleviate symptoms. Repurposing two well-known and approved drugs may turn out to be helpful. Losartan, an antagonist of TGF-β signaling (9), and ruxolitinib, an inhibitor of JAK/STAT signaling pathways (46), have the potential to reduce scarring and contractures and to block proinvasive extracellular matrix remodeling in vivo. Both are currently under investigation in animal models of dystrophic EB (L. Bruckner-Tuderman, unpublished results).

In summary, preclinical testing of biologically valid therapies for some skin fragility disorders has generated the first feasibility data that can serve as a basis for future clinical trials on gene-, cell-, and protein-based therapies. However, clinicians should avoid giving patients unrealistic expectations, because the hurdles in the development of novel therapies for clinical implementation are huge. For example, delivering and distributing therapeutic agents into the entire skin and controlling possible immune reactions to the therapeutic cells and proteins remain large challenges. In light of these issues, the scope of combinations of different therapy approaches and/or interval therapies will have to be optimized for each individual case. For the whole group of skin fragility disorders, which encompasses so many genes and different kinds of mutations, success will come with personalized therapies adapted to the individual molecular and clinical constellations (14).

SUMMARY POINTS

1. Genetic skin fragility disorders, collectively designated epidermolysis bullosa (EB), manifest with trauma-induced blistering and erosions of the skin; the most severe forms also involve the mucous membranes. Residual manifestations of skin fragility comprise pigment anomalies, epidermal atrophy, or scarring. The appendages of the skin, nails, hair, and teeth, as well as other organs such as the muscular system, gastrointestinal tract, heart, kidney, and urogenital tract, may also be affected. Severe EB subtypes evolve as systemic diseases with secondary multiorgan involvement and premature death.
2. Based on the level of skin cleavage, four EB types are distinguished: EB simplex, junctional EB, dystrophic EB, and Kindler syndrome. Disease-causing mutations in 18 distinct genes lead to the defective mechanical stability of keratinocytes and reduced intraepidermal or dermal–epidermal adhesion.

3. Extensive mutation analyses have provided substantial new information on the spectrum of skin fragility disorders and the biology of epidermal adhesion and have paved the way for the development of specific molecular therapies.
4. New subtypes of skin fragility continue to emerge. These may be due to mutations in new genes or to particular alleles in known genes. Revertant mosaicism appears to be more common than expected and has become a therapeutic option.
5. The differential diagnosis of genetic skin fragility encompasses other genetic disorders manifesting with cutaneous inflammation, metabolic anomalies, or, in the case of dermal or vascular fragility, connective tissue diseases, such as certain types of the Ehlers–Danlos syndrome.
6. Next-generation sequencing is a powerful tool that enables the rapid diagnosis of highly heterogeneous EB types and unusual cases as well as the identification of novel mutation constellations and genes.
7. Among genetic skin disorders, EB is a perfect candidate for the development of novel therapy strategies. Ex vivo gene therapy was successfully applied as early as 2006, with cell- and protein-based therapies only later becoming the main focus of researchers.
8. Bone marrow transplantation provides some alleviation of skin fragility but is marked by high mortality rates. The application of fibroblast or mesenchymal stem cell injections increases collagen VII deposition at the basement membrane zone but is limited to relatively small skin areas. Protein therapies are attractive but still in preclinical development.

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