GENETICS OF STRUCTURAL SKIN DISORDERS

# Molecular Heterogeneity of Blistering Disorders: The Paradigm of Epidermolysis Bullosa

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## INTRODUCTION

Inherited epidermolysis bullosa (EB) represents a clinically and genetically heterogeneous group of genodermatoses characterized by skin fragility, i.e., blisters and erosions of skin and mucous membranes in response to minor friction or mechanical trauma (Figure 1a–e). EB comprises a broad spectrum of phenotypes, ranging from severe cutaneous and extracutaneous involvement caused by severely compromised dermal–epidermal or intra-epidermal adhesion to discrete traits caused by subtle molecular defects. On the basis of our current knowledge, mutations in 17 different genes account for the genetic and allelic heterogeneity of EB (Figure 1). The EB-associated genes code for intracellular, transmembrane, or extracellular proteins involved in cytoskeleton, cell–cell or cell–matrix adhesion (Figure 1f). The main adhesive structures in the skin, i.e., desmosomes, hemidesmosomes, basement membrane, and anchoring fibrils represent supramolecular protein complexes and networks that not only assure the integrity and mechanical stability of the integument, but also regulate cellular functions by transmitting signals between the cells and their extracellular milieu. Therefore, the heterogeneity and the partial overlap between clinical and molecular EB subtypes are not surprising if one considers the high density of molecules and their complex interactions in cell–cell and cell–matrix adhesions.

## HISTORICAL PERSPECTIVE

The term EB (implying involvement limited to epidermis) was coined by Koebner more than 120 years ago (Koebner, 1886). It has remained in use until today, although this group of disorders now encompasses the entire spectrum of known skin fragility disorders, a concept sometimes difficult to comprehend by the patients and some clinicians as well. In the first half of the 20th century, the major achievements in the EB field consisted of defining clinical entities and distinguishing between inherited and acquired forms of bullous diseases. In the 1960s, ultrastructural studies led to the classification of EB into three major types—simplex, junctional, and dystrophic—based on the precise level of tissue separation (Pearson, 1962; Hashimoto *et al.*, 1975; Rodeck *et al.*, 1980; first milestone). In the 1970s and 1980s, a plethora of clinically distinct EB subtypes were defined, often designated by eponyms. In particular, the monograph published in 1971 by Tobias Gedde-Dahl included meticulously collected data on more than 100 patients with EB (Gedde-Dahl, 1971).

In the 80s, development of immunofluorescence techniques and generation of polyclonal and monoclonal antibodies resulted in the identification of the first molecules causally involved in EB and the establishment of first molecular criteria for diagnosis using immunofluorescence mapping of the dermal–epidermal junction ((Hintner *et al.*, 1981), reviewed in (Fine, 1987)). This was the second milestone in our pathogenetic understanding of this group of disorders.

In parallel, rapid improvements in protein biochemical methods and recombinant protein expression systems facilitated the isolation and molecular characterization of the proteins of the dermal–epidermal basement membrane zone or their functional domains. Using molecular tools, combined with immunoelectron microscopy, such pivotal adhesion proteins as laminin-332 (previously kalinin, laminin 5) and collagen VII were identified as structural components of the hemidesmosomes and the anchoring fibrils (Sakai *et al.*, 1986; Bruckner-Tuderman *et al.*, 1987, 1988; Marinkovich *et al.*, 1992, 1993; Urban, 2012). All the new knowledge and diagnostic advances acquired during this decade were reflected in the revised clinical and laboratory criteria for EB, published in 1991, which split EB into numerous clinical subtypes (Fine *et al.*, 1991).

The 1990s were marked by rapid progress in genetics, which was based on the development of molecular genetic methods, including gene cloning, linkage analyses for gene mapping, and efficient DNA sequencing. In 1991, *KRT14* mutations were found to cause EB simplex, the most common EB type (Coulombe *et al.*, 1991). Thereafter, in rapid succession, mapping and discovery of the genetic defects underlying several different EB subtypes were reported (Ryynanen *et al.*, 1991a, 1991b, 1992; Hovnanian et al., 1992; Christiano *et al.*, 1993; Hilal *et al.*, 1993; McGrath *et al.*, 1995; Li and Uitto, 2012). Molecular genetic diagnostics became available, but were still labor-intensive, expensive, and dependent on pre-screening. In

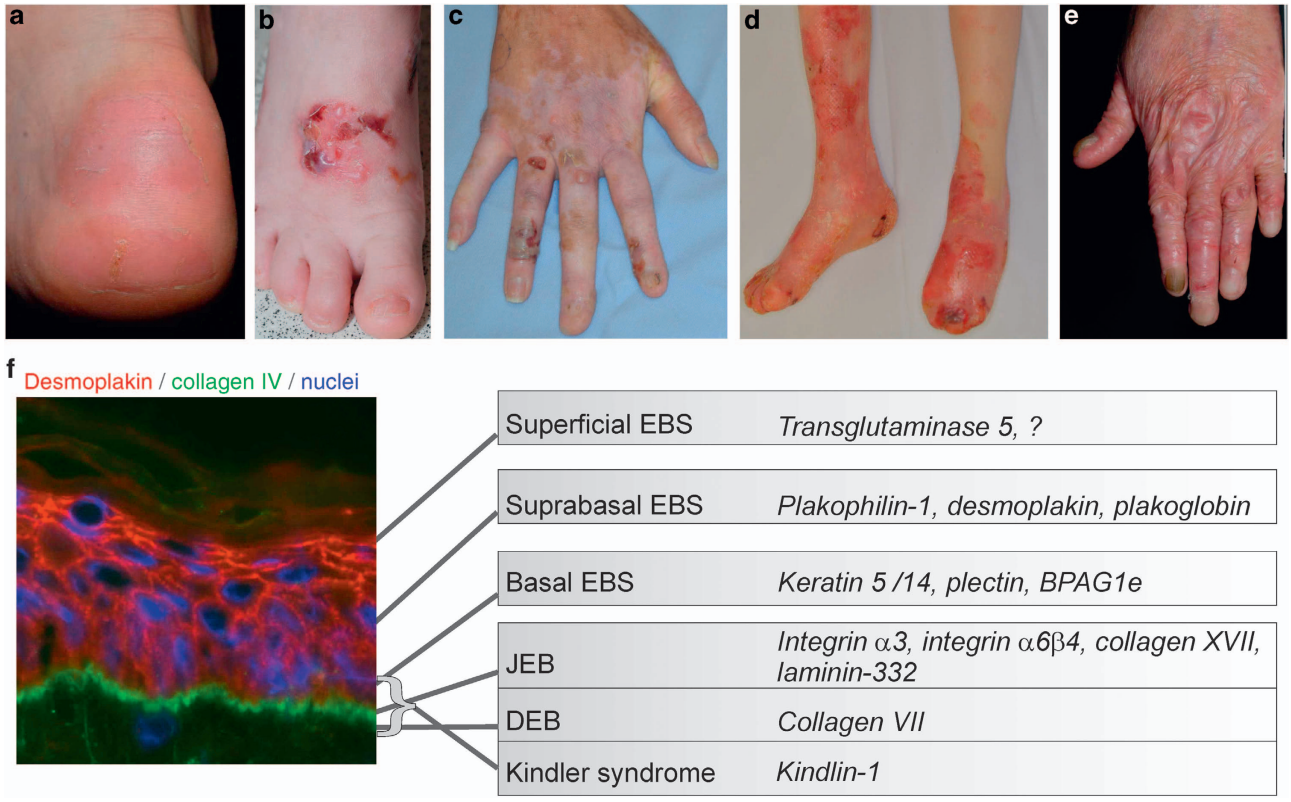


**Figure 1. Clinical and molecular heterogeneity of epidermolysis bullosa (EB).** (**a**) Residual superficial erosions after blistering on the heel of a boy with acral peeling syndrome and transglutaminase 5 mutations, p.[G113C];[L214CfsX15]. (**b**) Grouped blisters and crusts on the foot of a girl with EB simplex Dowling-Meara caused by the keratin 14 mutation, p.R125C. (**c**) The right hand of a woman with junctional EB–other and collagen XVII mutations, p.[M1T];[R1226X], showing blisters, erosions, crusts, hypopigmentation, and nail loss. (**d**) Feet of a boy with dystrophic EB with *COL7A1* mutations (c.[425A>G];[3276G>A]) demonstrate crusts, extensive scarring, webbing of the toes, and nail loss. (**e**) The left hand of a young man with Kindler syndrome homozygous for the frameshift mutation in the *FERMT1* gene p.[D153RfsX3];[D153RfsX3], demonstrates pronounced skin atrophy, incipient webbing of the finders, and nail dystrophy. (**f**) The left panel shows immunofluorescence staining of normal human skin with antibodies to desmoplakin (red) and collagen IV (green); nuclei are in blue. The levels of skin cleavage, which correspond to the main EB types and subtypes, and the defective proteins are indicated on the right side of the figure.

parallel to the elucidation of new genetic defects and different kinds of mutations in the known genes, researchers started to explore the molecular mechanisms underlying keratinocyte fragility in EB simplex and dermal–epidermal destabilization in junctional and dystrophic EB. In 1997, revertant mosaicism, i.e., genetic reversion of inherited mutations was recognized clinically in patients with junctional EB and demonstrated on molecular genetic level (Jonkman *et al.*, 1997). For many years, this phenomenon of natural healing was considered rare and described only in isolated cases. Recently, however, it has become clear that revertant mosaicism occurs in most, if not all EB types, and is more widespread than expected (Lai-Cheong *et al.*, 2011; Kiritsi *et al.*, 2012; McLean and Irvine, 2012).

## CLINICAL CLASSIFICATION

In the beginning of the 21st century, a rich body of molecular genetic data on EB was already available. Mutation databases (http://www.interfil.org, https://portal.biobase-international.com/hgmd/) and patient registries (https://grenada.lumc.nl/LOVD2, http://www.deb-central.org/molgenis.do, http://www.col7.info) facilitated both the study of genotype–phenotype correlations and the genetic counselling. Together with the knowledge acquired on biochemical and cell biological disease mechanisms, these data served as prerequisite for rational disease classifications. During the first decade of the 21st century, two revisions of the EB classification were needed to reflect the complexity and significant developments in the field (Fine *et al.*, 2000, 2008). Substantial advances in understanding the molecular basis of many old and new EB forms led to the tendency to avoid splitting of the disease into too many sub-entities and to reduce the use of eponyms. However, this is in part counteracted by continuous progress relating to discovery of new genes in rare EB subtypes.

## CLINICAL AND GENETIC FEATURES OF EB SUBTYPES

The most extensive changes have concerned EB simplex. Although the vast majority of EB simplex cases is caused by keratin 5/14 mutations (Figure 1b), several new causative genes have been identified. BPAG1e (dystonin isoform 4) and plectin mutations can cause cytolysis of basal keratinocytes and mild blistering, and may account for

at least some of the molecularly unsolved EB simplex cases (Rezniczek *et al.*, 2010; Liu *et al.*, 2012). Furthermore, the spectrum of EB simplex has extended to include subtypes with cleavage in the suprabasal epidermal layers, e.g., the plakophilin deficiency and the lethal acantholytic EB. These very rare new forms are caused by genetic defects of desmosomal proteins (McGrath *et al.*, 1997; Jonkman *et al.*, 2005; Pigors *et al.*, 2011). Hence, our vision on EB has extended to include conditions with perturbed cell–cell adhesion, in which the clinical picture is dominated by skin erosions rather than blisters. The molecular basis of EB simplex superficialis has remained unclear (Fine *et al.*, 2008), as a mutation in the collagen VII gene, associated with dominant dystrophic EB, was identified in the original family (Fine *et al.*, 1989; Martinez-Mir *et al.*, 2002). In a number of patients with superficial blisters and erosions (Figure 1a), transglutaminase 5 mutations indicative of acral peeling skin syndrome have been disclosed (Kiritsi *et al.*, 2010; Pigors *et al.*, 2012). We believe that it is time to classify acral peeling skin syndrome as a skin fragility disorder, rather than a form of ichthyosis as it is now (Oji, 2010).

The classification of the junctional EB was simplified by distinction of the lethal (Herlitz) subtype from the others, which were collectively designated as junctional EB–other. Junctional EB Herlitz is defined by loss of laminin-332, and it remains one of the most severe forms of EB. Junctional EB–other encompasses diverse phenotypes, in which blistering ranges from lifelong, generalized (Figure 1c), to late onset, mild, and localized, depending on the residual expression or activity of the mutated protein. The laryngo-onycho-cutaneous syndrome was included as a junctional EB variant, as it has similar clinical features and is associated with mutations in the α3-chain of laminin-332. Very recently, integrin α3 mutations were discovered in three patients with a new complex phenotype, including junctional EB, congenital nephrotic syndrome, and interstitial lung disease (Has *et al.*, 2012).

All dystrophic EB subtypes are caused by mutations in the gene encoding collagen VII, the main component of the anchoring fibrils. The clinical presentations vary from severe generalized blistering and scarring (Figure 1d) to sole nail dystrophy without skin blistering. Although more than 600 *COL7A1* gene mutations are known to date, the genotype–phenotype correlations are understood only in part. The heterogeneity of the allelic subtypes is likely to result from the variable expression and function of mutated collagen VII, with the complex interplay of genetic and environmental modifiers.

Because of the variable (mixed) level of skin cleavage, the Kindler syndrome was recognized as a distinct type of EB. It is clinically characterized by skin blistering during childhood and progressive poikiloderma, skin and mucosal scarring, and predisposition to epithelial skin cancer in adulthood (Figure 1e). Mutations in the *FERMT1* gene encoding kindlin-1 underlie this rare autosomal recessive genodermatosis. From a molecular point of view, the Kindler syndrome and the EB with integrin α3 mutations may be classified separately as focal adhesion disorders, because they impair the functions of focal adhesions, adherence, and signalling platforms, which are needed for epidermal cell adhesion and migration.

## CLINICAL PERSPECTIVE

By the end of 2011, more than 1250 different mutations in 17 genes responsible for EB were included in the Human Gene Mutation Database Professional release (2011.4; Harel and Christiano, 2012). In several countries, mutation analysis is now routinely available, every patient having the right to know his/her own mutation. This development expanded the spectrum of disorders encompassed by the term EB and represents the basis for individualized molecular therapeutic approaches (Cho *et al.*, 2012; Uitto, 2012).

Many preliminary studies regarding translational approaches in EB have been published during the last decade (Mavilio *et al.*, 2006; Fritsch *et al.*, 2008; Wong *et al.*, 2008; Remington *et al.*, 2009; Wagner *et al.*, 2010; Cho *et al.*, 2012). In the future, the implementation of novel biologically valid therapies must take into account the balance between the gain and risk. No unique solution will be available for all patients, but the success will rather come with personalized therapies adapted to the individual molecular and clinical constellation (Uitto, 2012).

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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