

## IN THIS ISSUE...

**Phenotypic Complexity of Epidermolysis Bullosa: The Paradigm of the Pruriginosa Subtype**

The heritable forms of epidermolysis bullosa (EB) comprise a group of mechano-bullous disorders with skin blistering as the unifying diagnostic feature (1). The clinical severity can be highly variable: in milder cases EB can present with life-long blistering tendency with no significant impact on the longevity of the affected individual, while at the other end of the spectrum, affected children often die at birth or shortly thereafter. In addition to skin findings, a number of extracutaneous manifestations can be found in EB; these include corneal erosions, enamel pitting, nail dystrophy, fragility of the respiratory epithelium, esophagus strictures, pyloric atresia and late-onset muscular dystrophy, among others. This phenotypic variability, as reflected by complex classification schemes riddled with eponyms, has often been confusing and presented a diagnostic challenge to the practitioners. Adding to the complexity is the fact that both autosomal dominant and autosomal recessive forms of EB can be recognized.

Traditionally, EB has been divided into three broad categories depending on the level of tissue separation at the cutaneous basement membrane zone (BMZ) (Table I). In the simplex forms tissue separation occurs within the basal cells of the epidermis; in the classic junctional forms tissue separation is evident within the lamina lucida of the dermal–epidermal basement membrane; and in the dystrophic forms tissue separation occurs below the lamina densa within the upper papillary dermis at the level of anchoring fibrils (1). It is evident, however, that within each of these three categories considerable heterogeneity exists. For example, we have previously suggested a fourth subgroup, so-called hemidesmosomal variants of EB, where tissue separation occurs at the basal keratinocyte plasma membrane–lamina lucida interface at the level of hemidesmosomes (2). Identification of this subtype has led to direct identification of hemidesmosomal gene/protein systems as targets for mutation analysis in these variants of EB.

Table I. *Clinical/molecular classification of epidermolysis bullosa*

Type	Level of tissue separation	Mutated genes
Simplex	Basal cell	<i>KRT5, KRT14</i>
Hemidesmosomal		
GABEB		<i>COL17A1</i>
EB-PA	Basal cell/lamina lucida	<i>ITGB4, ITGA6, PLEC1</i>
EB-MD	interface	<i>PLEC1</i>
Junctional		
(classic)	Lamina lucida	<i>LAMA3, LAMB3, LAMC2</i>
Dystrophic	Sub-lamina densa	<i>COL7A1</i>

EB: epidermolysis bullosa; GABEB: generalized atrophic benign EB; EB-PA: EB with pyloric atresia; EB-MD: EB with muscular dystrophy.

An example of both clinical and genetic heterogeneity is offered by the dystrophic forms of EB (DEB) (3). First, both autosomal dominant and autosomal recessive inheritance can be identified within this subgroup of EB. In general, the autosomal dominant forms manifest with milder blistering tendency, while the most severe forms of autosomal recessive, the Hallopeau-Siemens type of DEB, manifest with extreme fragility of the skin leading to characteristic scarring, fusion of the digits, joint contractures, and eventually squamous cell carcinomas. Within the autosomal dominant forms of EB, blistering tendency can be relatively localized or more generalized. A distinct subtype of the autosomal dominant variant of DEB is the pruriginosa subtype, characterized by blistering and erosions, associated with intense pruritus and often localized to the pretibial areas of the skin (1). This phenotype eventually evolves into skin lesions with resemblance to lichen simplex chronicus, nodular prurigo or hypertrophic lichen planus. The reasons for pruritus, in spite of extensive investigations into the immunologic, endocrinologic and nutritional factors, have remained undisclosed.

Significant progress has been made in molecular genetics of EB, and it is now known that ten different genes can harbor mutations which underlie the blistering phenotype in different variants of EB (4) (Table I). Examination of the mutation database has revealed some general genotype/phenotype correlations. Specifically, the level of expression of the mutated genes within the cutaneous BMZ, the types and combinations of the mutations and their consequences at the mRNA and protein levels, when placed on the individuals' genetic background and exposure to environmental trauma, they all explain the tremendous phenotypic variability in EB.

In different forms of DEB, mutations in the gene encoding type VII collagen (*COL7A1*) have been identified (3). In general, dominant-negative mutations, as exemplified by glycine substitution mutations in the collagenous domain of the protein, underlie the autosomal dominant forms, while the recessive subtypes are primarily due to loss-of-function mutations, characteristically stop codon mutations resulting in truncation of the translated polypeptide. Glycine substitution mutations have also been identified in a number of patients with the pruriginosa subtype of DEB (5, 6). Somewhat surprisingly, however, the nature of the pathogenic mutations in *COL7A1* in these patients seems not to differ from those found in patients with dominant DEB without the pruritus phenotype. Therefore, the potential roles of contributing factors, both genetic and environmental, have been postulated (7).

In this issue, the investigative dermatology group directed by Dr John McGrath at St John's Institute of Dermatology, London, UK (p. 6–11), has explored the

molecular basis of the blistering phenotype in a relatively large cohort of patients with EB pruriginosa ( $n=27$ ) and compared it with non-itchy dominant DEB ( $n=23$ ), recessive DEB ( $n=25$ ), and normal controls ( $n=50$ ) (8). First, these investigators identified heterozygous dominant-negative glycine substitution mutations in the type VII collagen gene in all 27 individuals with the pruriginosa subtype, and six of these mutations were previously unreported. These observations supported the autosomal dominant inheritance in this subtype and confirmed type VII collagen mutations as the underlying cause of the disease. Again, however, the types of mutations did not differ appreciably from those in non-itchy dominant DEB patients.

Secondly, the investigators explored the possibility that the pruriginosa phenotype might result from high level of expression of matrix metalloproteinase-1 (MMP1), a proteolytic enzyme with broad spectrum of substrates, including type VII collagen. This enzyme is synthesized by fibroblasts and keratinocytes in the skin, and its expression is under transcriptional control. Relevant to transcriptional regulation are previous demonstrations that *MMP1* promoter region contains a single nucleotide insertion polymorphism at position -1607 (1G/2G) upstream from the transcription initiation site (9). This SNP in case of an allele with two Gs forms a recognition site (5'-GGA-3') for *Ets* transcription factor, which has been shown to result in higher level of MMP1 expression in cells from individuals with 2G/2G or 2G/1G genotype; conversely, low level of expression is noted in cells from individuals with 1G/1G alleles. The level of expression of MMP1 has also previously suggested to modify the clinical severity of recessive DEB in three siblings with the same *COL7A1* mutation – a homozygous p.R2063W (10). However, the work by Almaani et al. (8) in this issue failed to demonstrate significant differences in the frequency of this polymorphism between the pruriginosa patients and those with non-itchy dominant DEB or controls. They concluded, therefore, that this variant of functional polymorphism in the *MMP1* gene promoter does not account for the itchy skin phenotype in pruriginosa patients. One further observation highlighted by this study in the group of individuals with recessive forms of DEB was the possible association of the 2G allele with early onset squamous cell carcinoma, a devastating complication in this subtype of EB. Squamous cell carcinoma is the major cause of premature mortality in recessive DEB, although its precise pathophysiology is still unknown. The preliminary observations made by Almaani et al. (8) suggest that presence of the 2G allele may make individuals with recessive DEB more susceptible to this complication, an observation that clearly needs to be followed up in larger cohorts since it could have important implications for management of susceptible patients.

The pruriginosa phenotype of EB remains unexplained, and further work is necessary to disclose the pathomechanisms leading to this unusual presentation. The importance of further work is predicated on the fact that no effective treatment is currently available for the treatment of EB in general or the pruriginosa phenotype in particular. The primary genetic lesion, mutations in the type VII collagen gene, is clearly the primary genetic cause of this disease, but the modifying factors and the pathomechanistic details remain to be disclosed.

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