Cold cases in epidermolysis bullosa: not the usual suspects
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Blistering disease: insight from the hemidesmosome and other components of the dermal-epidermal junction

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Abstract

The hemidesmosome is a specialized, transmembrane complex that mediates the binding of epithelial cells to the underlying basement membrane. In the skin, this multiprotein structure may be regarded as the chief adhesion unit at the site of dermal-epidermal junction. Focal adhesions are additional specialized attachment structures located between hemidesmosomes. The integrity of the skin relies on well-assembled and functional hemidesmosomes, and on focal adhesions. However, if these adhesion structures are impaired, e.g., because of circulating autoantibodies, or inherited genetic mutations, the mechanical strength of the skin is compromised, leading to blistering and/or tissue inflammation. A particular clinical presentation will emerge subject to which molecule is targeted. All these junctional complexes and are not simply compounds of adhesion molecules, they also play a significant role in signalling pathways involved in the differentiation and migration of epithelial cells such as during wound healing, and in tumour invasion. In the following, we will summarize our current knowledge about hereditary and acquired blistering diseases emerging from pathologies of the hemidesmosome and its neighbouring proteins, components of the dermal-epidermal junction.

Introduction

The attachment of epithelial cells to the underlying basement membrane is of crucial importance for maintaining tissue structure and integrity. Hemidesmosomes are specialized multiprotein, junctional complexes that play a pivotal role in this attachment in stratified and other complex epithelia, e.g., in the skin, parts of respiratory and gastrointestinal tract, cornea, and the amnion.\(^1\)\(^-\)\(^3\) The name of hemidesmosome derives from its appearance in the electron microscope as half desmosome, an epithelial intercellular adhesion. Both the desmosome and the hemidesmosome have similar multilayered electron-dense cytoplasmic plaques for keratin bundles attachment. Regardless of their seeming resemblance, their components are rather different. The structural composition of hemidesmosomes is relatively well defined. They contain at least the following proteins: plectin, 230 kDa-bullous pemphigoid antigen (also known as BPAG1, BP230), integrin α6β4, type XVII collagen (also known as BPAG2, BP180), and a tetraspanin protein termed CD151 (Fig. 1).\(^4\)\(^-\)\(^7\) The hemidesmosomal cytoplasmic plaque contains plectin and BP230. These proteins mediate the attachment of keratin intermediate filaments to the hemidesmosomes. There are two hemidesmosomal transmembrane proteins: integrin α6β4 and type XVII collagen. They connect through their extracellular domains with
laminin 332 in fine thread-like filaments, thus providing cell anchorage to the basement membrane.2,8 The epidermal basement membrane consists of lamina lucida and lamina densa and is mainly composed of two independent but physically connected laminin and type IV collagen networks, linked by perlecan-containing aggregates. Nidogens 1 and 2 are integral parts of both networks and modulate their surfaces.9 Finally, semicircular anchoring fibrils, consisting of type VII collagen, attach the basement membrane to the papillary dermal connective tissue.1,10 Pathologies in any of the major components of the hemidesmosome or the dermal-epidermal junction may result in disruption of skin integrity. Several hemidesmosome- and junction-associated molecules have been identified as targets in hereditary bullous skin diseases or as autoantigens in autoimmune bullous skin diseases. This review provides an outline of our current knowledge on the hereditary and acquired blistering skin diseases of the hemidesmosome and other components of the dermal-epidermal junction. For a summary of relevant disorders see Table 1.

1. Inherited skin disorders of dermal-epidermal junction complex

Mutations in genes coding for proteins involved in the composition of the hemidesmosome and its associated filaments, as well as components of the focal adhesion units cause certain types of epidermolysis bullosa (EB). EB comprises a group of hereditary mechanobullous diseases, characterized by fragility of skin and mucous membranes. As the knowledge in the molecular background of EB increased, and research and diagnostic techniques improved, a classification system was developed. Through the years, the EB classification was further extended and adjusted according to new insights. The latest consensus meeting, held in London, in June 2013, lead to the publication of an updated classification of EB subtypes.11 There are 4 major EB types based on the level of tissue cleavage: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome (KS). EBS is characterized by intra-epidermal tissue cleavage and is further subdivided in suprabasal and basal EBS, with separation above and in the basal keratinocytes, respectively. In JEB the blister formation takes place in the lamina lucida and in DEB within the sublamina densa region of the upper papillary dermis. Finally, a mixed cleavage plane characterizes KS. The major EB types enclose a total of 29 minor subtypes, involving 18 different genes.11 Following is a review of the dermal-epidermal junction molecules targeted in EB, with the hemidesmosome as the focal point.
**Keratins 5 and 14**

The intermediate filament (IF) cytoskeleton provides structural stability and mechanical resilience to the basal keratinocytes both through the formation of a cell scaffold, and their connection to desmosomes and hemidesmosomes. The term intermediate derives from the relative size (10 nm in diameter) of these filaments, which is between microfilaments (6 nm) and microtubules (23 nm). They are composed of acidic type I keratin (such as keratin 14 (K14)) and basic type II keratin (such as keratin 5 (K5)). The molecular organization of K5 and K14 is a highly conserved three-elemental structure, which includes a central α-helical coiled-coil rod domain and the bordering non α-helical globular N- and C-termini, respectively. These keratins organize in obligate, parallel, coiled-coil heterodimers, via their central rod domains, thus providing basic building blocks for further assembly. Mutations in genes coding for K5 (KRT5) and K14 (KRT14) interfere with the proper assembly of the tonofilament cytoskeleton and the connection of IFs to desmosomes and hemidesmosomes. However, other processes, such as protein turnover and signalling functions may also be disrupted, thus contributing to the pathophysiology in EB. Mutations in K5 and 14 account for 70-75% of patients with basal EBS. This disease is predominantly inherited in a dominant autosomal manner. Missense mutations and small in-frame deletions or insertions in the KRT5 and KRT14 genes are the most common mutations, having a dominant negative effect and causing disruption of the basal keratinocytes. The resultant phenotype may vary significantly, ranging from mild localized acral skin fragility to severe generalized blistering. To some extent, there is a correlation between the location of the mutation and the resultant phenotype. As mentioned above, the central rod domains of keratins are involved in the assembly of coiled-coil keratin heterodimers, the building blocks of IF. The helix boundary motifs at the N- and C-termini of the rod domain play a significant role in initiating this process. This explains why mutations affecting the helix boundary motifs (HBM) tend to associate with the most severe phenotype, EBS generalized severe, formerly known as Dowling-Meara. This EB subtype presents with neonatal generalized circinaria or herpetiform grouped blisters, mucosal membranes are frequently involved. Subungual blistering leads to onycholysis and later on, these patients develop palmo-plantar keratoderma (PPK). Nevertheless, certain missense mutations in HBM of KRT5 gene produced milder, EBS localized phenotypes. Mutations in the central part of the rod domain and in the linker domains present with a milder phenotype, such as EBS, localized (EBS-loc), former EBS Weber-Cockayne, or EBS generalized intermediate (EBS-gen intermed), formerly known as EBS Koebner. EBS-loc is the mildest form and manifests mainly by acral blistering starting in infancy or early childhood. EBS-gen intermed is
defined by acral blistering but also significant generalized skin fragility from birth. When mutations occur in the non α-helical globular head and tail domains of K5 and K14 they may lead to a clinical picture characterized by pigmentary disturbances and/or inflammatory features. This phenomenon is well exemplified in the rare EBS migratory circinate (EBS-migr) and EBS with mottled pigmentation (EBS-MP) subtypes, but also in the following disorders: dermatopathia pigmentosa reticularis (DPR), Naegeli-Franceschetti-Jadassohn syndrome and Dowling-Degos disease (DDD). Autosomal recessive EBS (EBS-AR) cases caused by missense and nonsense/frameshift mutations in KRT14 have also been reported in the literature, however, these are rare entities.33-36

**Plectin**

Plectin is a large protein of the plakin family with a molecular mass over 500 kDa. Structurally, this polypeptide consists of a central coiled-coil rod domain with a globular N-terminal head domain and a C-terminal tail domain at each end, respectively. The globular N-terminus includes binding sites for the cytoplasmic region of integrin β4, BP180, and actin filaments, whereas the globular C-terminus connects to keratin filaments. Also, plectin associates with BP230 in attaching IFs to the plasma membrane of the basal keratinocytes at the site of the hemidesmosome. Plectin, through its many tissue specific isoforms, is found among different mammalian cell types. It is extensively distributed in the stratified squamous epithelia, skeletal and cardiac muscle, and nerve tissue. In skin, its major function is to provide mechanical reinforcement by means of connecting the cytoskeleton to the desmosomes, hemidesmosomes, focal adhesions and cell organelles. In addition, plectin plays a role in signaling pathways involved in cell migration, e.g., plectin-null keratinocytes migrate faster than their wild-type equivalents. Plectin-knockout mice exhibit extensive skin fragility, but also cardiac and muscular deficits, all resulting in death 2-3 days after birth. Their skin contained hemidesmosomes with a normal structure, but they were reduced in number. Plectin deficiency in human, plectinopathies, may affect skin, muscle, nerve, heart, gut and mucous membranes. Plectin gene defects lead to various forms of epidermolysis bullosa simplex. Autosomal recessive mutations in the plectin gene (PLEC) causes subtypes of EBS associated with muscular dystrophy (EBS-MD), or pyloric atresia (EBS-PA). A different EBS subtype associated with plectin mutations is EBS-Ogna (EBS-Og); its inheritance is autosomal dominant. Dominant plectin mutations underlie epidermolysis bullosa simplex in 8% of patients. Generally, EBS-MD is associated with mutations in the central rod domain of plectin. Muscle dystrophy, when plectin deficiency disrupts the connection of the muscle cell cytoskeleton to the sarcolemma, presents several years after birth, but may manifest
as late as 30 years of age. EBS-PA is associated with mutations in the distal domains of plectin and usually results in fatality. EBS-Og is characterized by mainly acral involvement, bruising tendency, mild blistering and erosions healing with violaceous macules. Tooth pitting and mild focal palmo-plantar keratoderma may be additional features. Muscular dystrophy is not part of its clinical picture. The cleavage plane for these diseases is very low intraepidermal (‘pseudojunctional’), in close proximity to the plasma membrane of the basal keratinocytes.

**BP 230**

BP 230 (BPAG1-e), like plectin is also a member of the plakin family and involved in the organization of the cytoskeleton and the linkage of IFs to the plasma membrane at the site of hemidesmosome. This protein was originally discovered as one of the antigens targeted by autoantibodies in serum of patients with bullous pemphigoid. Structurally, BP230 is composed of central coiled-coil rod domain and flanking N- and C-termini. The N-terminal is involved in the integration of BP230 into the hemidesmosomes and has binding sites for BP180 and β4 integrin, whereas the C-terminus provides regions for attachment to intermediate keratin filaments. The recruitment of BP230 into hemidesmosome depends on the availability of BP180. Dystonin (DST) gene, by means of alternative splicing, generates several tissue specific isoforms (BPAG1-e, BPAG1-a, BPAG1-b) which are variably expressed in the skin, central nervous system and muscle tissue, correspondingly. Important insights in BP230 function came from the development of mice where this respective protein was ablated. Their clinical phenotype was characterized by mechanical fragility of the skin and, interestingly, dystonia musculorum which, in fact, might be well explained by the neuronal isoform of dystonin (DST) gene. The hemidesmosomes of DST-knockout animals lacked the inner plaque and had, strikingly, no IFs attached to them. Nevertheless, this aspect had not influenced the linkage of hemidesmosome to the extracellular matrix and the rest of its components had normal structure. In humans, not until recently two unrelated cases with autosomal recessive EB simplex due to homozygous nonsense mutations within the coiled-coil rod domain of BP230 (BPAG1-e, epithelial isoform) were reported. The immuno-histochemical analysis revealed a complete deficiency of BP230, and ultrastructurally an absence of the hemidesmosomal inner plaques was noted. The clinical phenotype of the affected individuals was characterized by generalized skin fragility and also mild, mainly acral skin blistering. Only one of the cases had additional neurological symptoms including: headaches, collapse, numbness and weakness. It is, however, not possible to ascertain that those symptoms are due to DST mutation since the subject had additional NOTCH3 gene pathology. Neurological features were clearly part of the clinical picture when
selective muscle and nerve tissue isoforms (BPAG1-b, BPAG1-a) were affected in another case. The patient had severe motor and mental delay, tracheo-oesophageal atresia, but no skin involvement.45

**Integrin α6β4**

The integrin α6β4 is a transmembrane polypeptide located at the core of the hemidesmosomes. Its primary functions are to link the intracellular hemidesmosomal plaque to the extracellular matrix. Additionally, it plays an important role in initiating signaling pathways involved in cell migration, differentiation and survival.51 These heterodimers are primarily found in stratified squamous and transitional epithelia, e.g. skin, mucous membranes, gastro-intestinal and urinary tract. Structurally, β4 integrin subunit has an unusually large intracellular domain that interacts with the cytoplasmic domain of BP180 and provides linkage to the keratin filaments via plectin and BP230.8,51 The extracellular domain of α6 and β4 subunits provide binding sites to different laminin isoforms, including laminin 332.52 Ultrastructurally, when the β4 integrin subunit was absent, the hemidesmosomes were rudimentary, reduced in number, lacking a sub-basal plaque and often also the inner hemidesmosomal plaque.53 Mutations in ITGA6 and ITGB4 genes encoding the respective subunit polypeptides of α6β4 integrin have been associated with EBS/JEB with pyloric atresia (PA)54-56 and JEB, localized.57,58 ITGB4 mutations are more common than ITGA6 mutations, nonetheless they all inherit in an autosomal recessive manner.54,56 The affected individuals present with a variety of phenotypes ranging from early death to mild skin fragility and nail dystrophy. Additional features, such as pyloric atresia and urinary tract involvement might be present; they are, however, not mandatory. In the lethal JEB with pyloric atresia (JEB-PA) the immunofluorescent staining of integrin α6β4 is substantially reduced or absent. Such severe cases usually result from premature termination codons or mutations affecting highly conserved amino acids.59,60 JEB-PA may present with congenital absence of skin (aplasia cutis congenita), particularly in the lower extremities (Fig. 2d), this feature is, however, not discriminatory from other subtypes of EB.11 The non-lethal phenotype with PA is characterized by mild acral and perioral blistering, enamel pitting and nail dystrophy.61 Finally, cases with milder phenotypes defined by a localized or generalized pattern of blistering, without PA and intra epidermal or intra lamina lucida cleavage planes have been reported.57,58,62
**Integrin α3β1**

Focal adhesions, also known as focal contacts, are structures between hemidesmosomes at the site of basal membrane zone and basal keratinocytes. They recruit integrin α1β3 into their constitution and function as adhesion devices for the actin cytoskeleton.63 Their molecular complexity is considerably higher than that of the hemidesmosomes. More than 100 proteins are involved in their composition, suggesting an extensive functional diversity.64 Initially, they were thought to play an insignificant role in the attachment of keratinocytes to the BMZ, and rather function as regulators of cell migration and cell-extracellular matrix signaling.65 However, interesting insights were born when integrin α3 ablated mice were developed. Lack of this integrin subunit resulted in neonatal death, possibly due to lung and kidneys defects,66 and in addition, these mice developed acral skin blistering.63 It was not until very recently that mutations in integrin α3 have been linked to disease in humans. Three patients were described with homozygous mutations in ITGA3 gene. They presented with a multiorgan disorder consisting of congenital nephrotic syndrome, interstitial lung disease, and skin blistering. The pulmonary and renal symptoms were the most prominent, and respiratory distress lead to death of the affected individuals. The skin fragility presented the clue to the diagnosis, even though it was mild.67 This subtype of EB is categorized as JEB with respiratory and renal involvement (JEB-RR).11

**BP180 (type XVII collagen)**

The 180-kDa bullous pemphigoid antigen (BP 180) is a transmembrane glycoprotein expressed in skin, mucosa, teeth, central nervous tissue, cornea, placenta, umbilical cord and transitional epithelium of the bladder.68,69 Its intracellular domain contains the noncollagenous N-terminal, whereas its extracellular domain has a triple helical conformation and includes 15 collagenous repeats, hence the term type XVII collagen.70-72 The extracellular domain crosses lamina lucida and reaches its binding partner, laminin 332 in the lamina densa of basement membrane, through its C-terminal.73,74 The intracellular domain has regions that interact with α6β4 integrin and plectin,13,42,75 and plays a crucial role in integrating BP230 into the hemidesmosome.76 BP180 has great functional value in maintaining the integrity of dermal-epidermal junction. This fact was demonstrated when individuals with mutations in BP180 gene (COL17A1) developed subtypes of junctional epidermolysis bullosa.77,78,79 Ultrastructurally, their skin contained rudimentary hemidesmosomes with underdeveloped cytoplasmic plaques and received less IFs comparing to healthy controls. That lead to a frail attachment of basal keratinocytes to the basement membrane and development of blisters. The cleavage plane of such disorder is within lamina lucida.77,78
Nevertheless, there has been a report in the literature, where deletion of the cytoplasmic domain of type XVII collagen lead to both intraepidermal and junctional cleavage planes and the phenotype had predominant features of EBS.\textsuperscript{80} Also, mutations in the ectodomain of type XVII collagen lead to an intraepidermal cleavage plane and EBS phenotype in another report.\textsuperscript{81} According to the latest consensus, disorders caused by type XVII collagen mutations include: JEB, generalized intermediate (JEB-gen intermed) former GABEB, generalized atrophic benign epidermolysis bullosa; JEB, localized (JEB-loc); and JEB, late onset (JEB-LO). Skin biopsies of the affected individuals generally exhibit an absent or reduced staining for type XVII collagen, except in JEB-LO where the staining may be positive but with abnormal pattern (broadened BMZ).\textsuperscript{11,82} Additionally, mutations in \textit{COL17A1} result in reduction or loss of the apical-lateral staining of basal keratinocytes. This occurrence is evident even prior to a reduction in staining of the BMZ.\textsuperscript{83} The clinical phenotype of JEB-gen intermed patients is characterized by generalized blistering, sparse primary (Fig. 2a) and absent secondary hair, corneal scarring, whereas patients with JEB-loc have blistering mostly restricted to face and acrae, mild or absent nail dystrophy, their secondary hair is sparse, and primary hair is normal. Remarkably, they all have enamel pitting (Fig. 2b).\textsuperscript{77,78,83} This disease might be complicated by the development of squamous cell carcinoma and influence longevity.\textsuperscript{84,85} JEB-LO is caused by the missense mutation p.R1303Q in type XVII collagen. The affected individuals present with late-onset skin blistering, progressive skin atrophy with scarring, loss of dermatoglyphs and nail abnormalities. In some patients enamel pitting or carious teeth were reported, but no alopecia beyond the androgenetic type.\textsuperscript{82,86-88}

**\textit{CD151}**

\textit{CD151} is a member of the tetraspan superfamily of cell membrane proteins. This molecule is expressed in epithelia, endothelia, muscle cells, renal glomeruli, Schwann and dendritic cells, but also in platelets and megakaryocytes.\textsuperscript{89,90} In human skin, it co-distributes with \(\alpha 3\beta 1\) integrin in the focal adhesions and with \(\alpha 6\beta 4\) integrin in the hemidesmosomes. \textit{CD151} is believed to play a role in the organization and stability of hemidesmosomes by facilitating the formation of stable laminin-binding complexes with integrin \(\alpha 6\beta 4\), as well as being involved in cellular signaling.\textsuperscript{6,91} In humans, a homozygous nonsense mutation in \textit{CD151} in two siblings resulted in hereditary nephropathy, sensorineural deafness and pretibial epidermolysis bullosa.\textsuperscript{92} This single report awaits, however, more rigorous confirmation before it can be accepted as a separate EB subtype, mainly because recessive dystrophic epidermolysis bullosa pretibial (RDEB-pt) is also a possibility.\textsuperscript{11} The clinical phenotype of \textit{CD151} deficient mice is thus far inconclusive. In one report, \textit{CD151}-null mice were normal, healthy and fertile
with no skin or hemidesmosome pathology, whereas another research group reported CD151-null mice that had substantial renal disease, including focal glomerulosclerosis, disorganization of the glomerular membrane and tubular cystic dilatation. Their skin and hearing apparatus were not involved.

**Laminin 332**

Laminin 332 is a cross-shaped glycoprotein composed of α3, β3 and γ2 chains encoded by *LAMA3*, *LAMB3* and *LAMC2* genes, respectively. It is found in different epithelia such as stratified squamous, transition, and simple epithelia. This heterotrimer is assembled in the basal keratinocytes and secreted into the basement membrane where it self-organizes into polymer networks. Laminin 332 plays an essential role in the dermal-epidermal attachment and can be regarded as a bridge between the hemidesmosomal proteins (α6β4 integrin and type XVII collagen) and the anchoring fibrils on the dermal side, consisting of type VII collagen. In addition, laminin 332 modulates cell behaviour by participating in signaling via integrin α6β4 in the hemidesmosomes and integrin α3β1 in the focal adhesions. These interactions are key for several cell events, including survival, regeneration, migration, and carcinogenesis. Mutations in *LAMA3*, *LAMB3*, and *LAMC2* genes lead to several subtypes of JEB, such as JEB, generalized severe (JEB-gen sev); JEB, generalized intermediate (JEB-gen intermed); JEB, localized (JEB-loc); and JEB, inversa (JEB-inv; JEB-l). All these pathologies are inherited in an autosomal recessive manner. The cleavage plane is in the lamina lucida of basement membrane. Evidence for the functional importance of laminin 332 came from the severe clinical features in cases with absent staining for laminin 332 using monoclonal antibody GB3. Affected individuals develop JEB generalized severe, formerly known as Herlitz type of JEB. Extensive blistering of skin and mucous membranes marks the clinical phenotype. Also, abundant granulation tissue characteristically around the nails (Fig. 2c), nose, mouth and buttocks is almost pathognomonic for this disorder. Multiple complications arise in this setting, including vulnerability to infections, anaemia, dyspnoea, and failure to thrive. Their devastating effects result in early childhood fatality. JEB-gen intermed (former generalized atrophic benign EB, GABEB) manifests with milder symptoms and reduced staining for laminin 332 in the skin. The clinical picture is characterized by extensive skin fragility and blisters, which heal with slight atrophy and hypopigmentation. Mucous membranes may be involved, although not as extensively as in JEB-gen sev. Other symptoms include nail loss or dystrophy, granulation tissue, enamel defects and various degrees of hair loss. A comparable phenotype can be seen in JEB-gen intermed resulting from certain type XVII collagen mutations. JEB-loc and JEB-inv present with a milder phenotype distributed mostly acrally and intertriginously, respectively.
Parents of JEB cases who are carriers of truncating LAMA3 mutations have enamel hypoplasia.\textsuperscript{104} LAMA3 thus appears to display haploinsufficiency as far as enamel development is involved. Specific mutations in LAMA3A gene, encoding the laminin α3a isoform, result in the potentially lethal JEB laryngo-onycho-cutaneous syndrome (JEB-LOC syndrome). The clinical phenotype is characterized by slow healing skin erosions, nail dystrophy, exuberant granulation tissue in conjunctiva and larynx and dental anomalies.\textsuperscript{105,106} A remarkable clinical course has been described in several very rare cases with laminin 332 mutations leading to premature termination codons. The affected individuals had severe congenital skin fragility and absent immunofluorescence staining for laminin 332 in their skin. Mutation analysis revealed homozygous nonsense or frame-shift mutations in LAMA3, LAMB3 or LAMC2 genes.\textsuperscript{107,108} All these data advocates for the diagnosis JEB-gen severe, surprisingly, the phenotype of these patients improved with age and laminin 332 immunofluorescence staining was detected in their skin biopsies. This phenomenon has been attributed to the activation of cryptic splice sites that lead to the removal of the mutation-carrying exon,\textsuperscript{107} or spontaneous activation of read-through mechanisms.\textsuperscript{100,107,109}

**Type VII collagen**

Type VII collagen is the main, if not the exclusive, constituent of anchoring fibrils, which play an integral role in the structural integrity of the anchoring complex of the dermal-epidermal junction. These semicircular structures attach the lamina densa of the basement membrane to the underlying papillary dermis.\textsuperscript{110} Similar to other collagen molecules, type VII collagen consists of three identical α-chains which self-organize into a triple-helical collagenous structures.\textsuperscript{111} Each triple helical domain is flanked by a non-collagenous N-(NC1) and C-terminal(NC2), respectively. Type VII collagen is abundantly expressed in the basement membrane of skin, oral and cervical mucosa, cornea and chorioamnion.\textsuperscript{112} Immunofluorescence staining for type VII can be normal or reduced. The affected individuals develop generalized or localized trauma induced blisters which heal with atrophic scarring and milia, their nails are dystrophic and eventually lost. The presence of milia is not a pathognomonic sign for DEB, although frequently so believed. Additional clinical features such as scalp abnormalities, “albopapuloid lesions”, ocular and gastrointestinal tract involvement may occur. These patients have generally a relatively good quality of life and prognosis.\textsuperscript{11,113} When loss of function mutations in both alleles occur, type VII collagen and anchoring fibrils are entirely absent. These patients exhibit a much more severe phenotype characterized by congenital generalized muco-cutaneous blistering (Fig. 2e), which resolves with extensive scarring, milia and mutilating pseudo syndactyly that leads to “mitten formation” of hands (Fig. 2f) and feet. Eventually, functionally invalidating acral contractures
develop.\textsuperscript{114} This disorder is termed recessive DEB (RDEB)-generalized severe, the former RDEB Hallopeau-Siemens. Due to the severity of blistering, involvement of the mucosa, pain, chronic blood loss, inflammation, and poor nutrition, these patients develop anaemia, infections, growth retardation and failure to thrive.\textsuperscript{115} Renal complications, sepsis, and aggressive squamous cell carcinomas at the site of chronic wounds are the main reasons why the affected individuals have a reduced life expectancy.\textsuperscript{84,84,116,117} Variants of RDEB due to missense mutations or in-frame deletions present with milder phenotypes. The affected individuals lack the extensive scarring and mutilation seen in the severe generalized forms of RDEB, having to some extent a better prognosis.\textsuperscript{11}

\textbf{Kindlin-1}

Although not a hemidesmosomal disease, Kindler syndrome (KS) deserves a mention in this review given that it is also a disorder of the junction. KS is caused by autosomal recessive “loss of function” mutations in \textit{FERMT1} gene encoding for kindlin-1.\textsuperscript{118} The cleavage plane is dermal as well as intra-epidermal. KS was included within the EB spectrum disorders in 2007, during the Third International Consensus meeting on Diagnosis and Classification of EB.\textsuperscript{119} Kindlin-1, the targeted molecule, is involved in linking the actin cytoskeleton to the extracellular matrix at the site of focal adhesions.\textsuperscript{120} The clinical picture of the affected individuals is manifested by trauma induced blistering resolving with scarring and pigmentation defects. Photosensitivity, poikiloderma and “cigarette paper” like atrophy are characteristic for this syndrome.\textsuperscript{121-123} Additional features such as keratoderma, nail dystrophy, dental caries, skeletal abnormalities, as well as gastrointestinal and urogenital involvement have also been reported.\textsuperscript{124,125} KS is associated with an increased risk of developing squamous cell carcinomas (SCC).\textsuperscript{122} Very recently transgenic mice lacking kindlin-1 have been developed. These mice exhibited the characteristic features of KS and increased skin tumour susceptibility. Researchers suggested that kindlin-1 may contribute to the risk of developing SCC in a \(\beta1\) integrin independent manner through regulation of Wnt and TGF-\(\beta\) signaling.\textsuperscript{126}
Recent developments in epidermolysis bullosa

An intriguing phenomenon termed revertant mosaicism was depicted for the first time in 1997 in a JEB patient with mutations in COL17A1 gene. Later on, it was also reported in several cases with mutations in KRT14, LAMB3, COL7A1 and FERMT1 genes. The affected individuals have islands of skin that appear normal and display improved mechanical integrity in comparison to the neighbouring skin (Fig. 2g). Such “natural gene therapy” results from a subpopulation of cells that regained their wild-type phenotype through spontaneous somatic reversemutations. These corrected revertant keratinocytes offer promising prospects for autologous cell therapies and development of patient specific induced pluripotent cells. Interestingly, a recent study has established the presence of circulating autoantibodies in sera of small cohort of EBS and DEB patients. Anti-type VII collagen, but also anti-BP180 and anti-BP230 autoantibodies titres were higher in RDEB patients than in EBS patients. Their pathogenic role has not been established yet, their occurrence might simply be an epiphenomenon.

2 - 35
2. Acquired skin disorders of dermal-epidermal junction complex

Circulating autoantibodies targeting structural constituents of the dermal-epidermal junction is the hallmark of pemphigoid diseases spectrum. The clinical picture, consisting of tense bullae and muco-cutaneous erosions, results from their specific binding to essential structural molecules that connect the cytoskeleton of the basal keratinocytes to the extracellular matrix and the later to the papillary dermis underneath. The knowledge about the factors that set off the production of autoantibodies is limited. All the acquired diseases of the dermal-epidermal junction are characterized by subepidermal cleavage plane and a negative Nikolsky sign, meaning friction of the non-lesional skin doesn’t result in visible erosion. The precise diagnosis of this group of disorders is critical, since the treatment modality and prognosis can differ considerably. Clinical presentation, although heterogeneous is, however, not always conclusive for the exact diagnosis, hence techniques for detecting skin- and mucosa-bound autoantibodies as well as circulating serum antibodies have been employed. Histopathology is aimed at identifying the type of infiltrate, structural changes, and the cleavage level. Direct immunofluorescence studies (DIF) are directed at detecting tissue bound autoantibodies in skin or mucosa, whereas indirect immunofluorescence studies (IIF) target the detection of antigen specific autoantibodies in patients’ sera. Characteristic for DIF are linear deposits of immunoglobulins (Igs) and C3 complement along the basement membrane zone (BMZ) which often follow an n-shaped or an u-shaped pattern.\textsuperscript{134-136} This is termed serration pattern and results from the relative position of the autoantigen in the BMZ; eg., if the target antigen is within or above lamina densa, the result is an n-serrated immunofluorescence pattern, whereas if located in the sublamina densa zone the result will be an u-serrated pattern.\textsuperscript{135} IIF analysis is carried out on standard substrates such as salt-split skin (SSS), monkey oesophagus and rodent bladder. SSS is a product of incubation of human skin in 1M NaCl solution which leads to separation of the dermis from the epidermis through lamina lucida. This allows for the discrimination between epidermal and dermal autoantigens through binding of autoantibodies to either the epidermal, or dermal side of the blister. Collective results from DIF serration pattern and IIF on SSS allow distinction between certain subepidermal autoimmune blistering diseases (sAIBD).\textsuperscript{135,136} Additional techniques, including western blot, enzyme-linked immunosorbent assay (ELISA), and immunoprecipitation have been employed for serum analysis, each with their own specificity and sensitivity. Often, a combination of these immunoassays is required to ascertain the diagnosis.\textsuperscript{134,137} In this review we will discuss the immunobullous diseases related to the disruption of dermal-epidermal junction. (see Table1.). Dermatitis herpetiformis, although an autoimmune
blistering disorder, has not been included because the targeted protein, transglutaminase, is not a constituent of the junction. Also, elusive entities, such as 105kDa-, 125kDa-, and 168kDa-pemphigoid await more robust validation. Autoantibodies against the above-mentioned antigens have been found in sera of patients affected by pemphigoid diseases. Their role has not yet been elucidated.\textsuperscript{138,139} It is important to note that patients with acquired blistering disorders can have multiple target molecules. Moreover, a phenomenon called “epitope spreading” may occur. That implies the development of autoimmunity to new epitopes resulting from the exposure of self-antigens during a chronic autoimmune or inflammatory reaction.\textsuperscript{140} All classes of lgs, except IgD have been linked to AIBD. Autoimmune reactivity may be expressed by one single class as well as by multiple lg classes. The clinical presentation may be influenced depending on which Ig class is involved, e.g., if IgA autoantibodies are the sole or major players, the result is a higher degree of mucosa involvement.\textsuperscript{141-143}

**BP180 and BP230**

**Bullous pemphigoid (BP),** with its main antigenic target the hemidesmosomal molecules BP180 and/or BP230, is the most common variant of pemphigoid. Mostly affecting the elderly, this disorder has an annual incidence between 13.4-21.7 cases/million people in Europe.\textsuperscript{144-146} With the prospect of increasing age of the general population, use of multiple medication, and better diagnostic techniques, the incidence is expected to rise.\textsuperscript{147} BP has been associated with significant morbidity and increased mortality rates of three to six times greater compared to age- and sex-adjusted general population,\textsuperscript{146,148} Predictors for poor outcome are: generalized disease, increased age, high doses of corticosteroids, and low serum albumin levels.\textsuperscript{149} Recently, an intriguing association has been noted between BP and neurological disorders, including dementia, Parkinson’s disease, stroke, and epilepsy.\textsuperscript{150} This occurrence has been attributed to the finding that both BP180 and a BP230 isoform are, in fact, expressed in the central nervous system.\textsuperscript{45,151} Although less common, cases of bullous pemphigoid in children have also been reported, where half of the cases developed the disease in the first year of life.\textsuperscript{152} The clinical picture of BP is characterized by large, tense blisters that may arise from erythematous macules or urticarial plaques, but also on non-erythematous skin (Fig. 3a). Typical sites are the flexural areas of upper and lower limbs, abdomen and flanks; a small group of patients can transiently exhibit oral lesions. Severe itching is a cardinal sign.\textsuperscript{153} Interestingly, a subset of patients will have pruritus and immunopathological features of BP, but no blistering. Additional dermatological features such as eczema, urticaria, papulonodular skin lesions may also be present and result in misdiagnosis, such as toxic drug reaction, eczema, xerosis cutis, or pruritus due to liver or renal impairment. Up to date,
there is no consensus on how to name this variant of BP. Recently the term “nonbullous cutaneous pemphigoid” has been proposed. Generally, more than 90% of BP patient sera contain IgG against BP180 and/or BP230 molecules, but IgE autoantibodies are also commonly found. They typically target tightly clustered epitopes in the noncollagenous 16A domain (NC16A) of the BP180 molecule. Autoreactivity is, however, not exclusively restricted to the NC16A domain, antigenic sites on the intracellular domain of BP 180 have also been reported. Notably, disease activity positively correlates with the serum levels of specific autoantibodies. In BP230, several antigenic reactive sites have been found, mostly located in the globular C-terminal domain of BP230. Owing to its intracellular localization, this molecule is believed to play a subordinate role in initiating the inflammatory response in BP. Pathogenic autoantibodies targeting BP180 and BP230 act together with innate immune system players to produce the clinical and immunopathological alterations seen in BP. Autoreactive T cells produce a Th1/Th2 mixed cytokine profile. Among others, TNFα, IL-6, IL-8, IL-15 and CCL18 are secreted and parallel the disease course.

In addition, lesional skin specimens and blister fluid from BP patients contain high level of proteolytic enzymes, particularly neutrophil elastase (NE), and eosinophil-derived matrix metalloproteinase-9 (MMP-9), thought to be involved in the cleavage of BP180 and loss of cell-matrix attachment at the site of dermal-epidermal junction. The diagnosis of BP is established based on clinical symptoms and a combination of immunopathologic studies. That includes: DIF with linear depositions of IgG and/or C3 complement along the BMZ in an n-serrated pattern and characterization of circulating autoantibodies through western blot and/or ELISA and IIF using SSS. The later will exhibit a linear staining of the epidermal side of the blister. Mild, localized cases of BP may be successfully treated with potent topical corticosteroids. The management of generalized and/or severe cases includes treatment options such as: high-potency topical corticosteroids, systemic corticosteroids, azathioprine, mycophenolate mofetil, antimicrobials (tetracycline, nicotinamide), methotrexate, and dapsone.

**Mucous membrane pemphigoid (MMP)**, formerly known as cicatricial pemphigoid, is a chronic progressive disorder predominantly affecting any mucous membranes, though the skin may also become involved. The term cicatricial pemphigoid (CP) is, according to new consensus, restricted to the clinical variants of pemphigoid with scarring involving solely the skin (without mucous membranes). MMP is thus a separate entity, distinct from CP. The predilection sites for MMP include: oral cavity, followed by conjunctivae, skin, nasal mucosa, anogenital area, pharynx, larynx and the esophagus. Oral disease presents with erosions, blisters, sometimes desquamative gingivitis (Fig. 3b), and damage of the periodontium that may lead to
loss of teeth. Patients who have restricted oral mucosa lesions tend to have a milder disease phenotype and better prognosis. Healing with scarring is a clinical characteristic of MMP, nonetheless, oral mucosa lesions may re-epithelize without scarring. \cite{168,170} Ocular involvement tends to follow a progressive pattern, unfortunately. It generally begins unilaterally with conjunctivitis, later on, repeated tissue injury causes shortening of fornixes, symblepharon, entropion and corneal neovascularization (Fig. 3c). \cite{171} Due to these severe complications, 53% of eyes in a series of 28 MMP patients developed visual loss. \cite{172} Tracheal and laryngeal involvement may result in airway obstruction, and genital disease can cause urinary and sexual dysfunction. \cite{173,174} MMP has an annual incidence estimated between 1.3 and 2.0 cases per million. \cite{145,175} Specific HLA class II alleles, such as DQB1*0301, DRB1*04 and DRB1 were detected more frequently with this form of pemphigoid and associated with severe disease phenotype. \cite{176,177} Several target antigens have been involved in MMP, such as BP180 with its soluble ectodomains LAD-1 and LABD97 antigens (in an estimated 75% of patients), BP230 (in 27%; often together with BP180), \cite{177,178} followed by laminin 332 (α3, β3 and γ2 chains), laminin 311 (α3 chain), type VII collagen and α6β4 integrin. \cite{138} IgA reactivity has been established in approximately 63% of MMP cases. Patients with more severe clinical phenotype had combined IgG and IgA reactivity to several BP180 antigens, whereas a milder clinical course was noted when just a single BP180 antigen was involved. \cite{177} The major antigenic regions are the NC16A domain and the C-terminal tail of the BP180. \cite{178,179} As a result, the IgG autoantibodies are deposited in lamina lucida and lamina densa, the later well explaining the susceptibility for scar formation in MMP patients. Diagnosis of MMP is established based on predominant mucous membrane involvement and DIF analysis of a perilesional specimen. Similar to BP, linear n-serrated depositions of IgG or C3 and sometimes IgA along the epithelial BMZ are essential immunopathologic features. Unfortunately, 20% of patients with ocular MMP will have a negative DIF analysis. \cite{169} IIF studies on SSS may aid de diagnosis, although the sensitivity is low. Typically, the epidermal side of the blister will be stained in cases where BP180, B230, or β4 integrin are the target antigens. In cases where dermal reactivity is detected, investigations toward anti-laminin 332 MMP or epidermolysis bullosa aquisita should be initiated. Although skin lesions have a tendency for rapid remission, in general, MMP is distinctly refractory to therapy. Patients with exclusively oral mucosa involvement, and thus a milder disease, can be managed with topical corticosteroids, \cite{168,170,180} whereas the severe cases will require long-term immunosuppressive treatments including: dapsone, systemic corticosteroids, azathioprine, mycophenolate mofetil, and cyclophosphamid. \cite{168,181} Recently, several severe or refractory cases of MMP have been successfully managed with a novel biologic, rituximab, sometimes in conjunction with intravenous immunoglobulin. \cite{182,183} Surgical intervention may also be used in cases
of limited functionality due to scarring.\textsuperscript{172,184} Referral to the appropriate specialists is essential when clinically indicated.

**Brunsting-Perry pemphigoid** is a rare variant of cicatricial pemphigoid, usually manifesting in older men.\textsuperscript{185} The original report dates to 1957 when Brunsting and Perry described seven patients with persistent circumscribed vesicobullous lesions limited to head, forehead, and nuchal region of the neck which heal with scarring and atrophy (Fig. 3d).\textsuperscript{186} Distinct from MMP, mucosal involvement is uncommon and usually mild.\textsuperscript{187,188} The major antigenic sites in Brunsting-Perry pemphigoid are the C-terminal domain of BP180;\textsuperscript{189} single studies report also LAD-1,\textsuperscript{190} laminin 332,\textsuperscript{191} BP230, and desmoplakins I/II.\textsuperscript{192} The later are intracytoplasmic components of desmosomes and their antigenicity is probably a consequence of the epitope spreading phenomenon.\textsuperscript{192} Histological features include subepidermal blistering and mixed inflammatory infiltrate consisting of lymphocytes, eosinophils and neutrophils.\textsuperscript{193} Later stages will exhibit fibrosis. DIF analysis of perilesional skin usually demonstrate linear IgG and/or C3 depositions, sometimes in conjunction with IgA and IgM along the BMZ. IIF studies on the sera of these patients may, however, not always detect circulating autoantibodies.\textsuperscript{187,194} Topical treatment with ultrapotent corticosteroids may control the disorder to some extent; many patients will require, however, systemic immunosuppressive medication or dapsone.\textsuperscript{194}

**Pemphigoid gestationis (PG),** formerly known as herpes gestationis, is an autoimmune blistering disorder related to pregnancy or a rare paraneoplastic occurrence described in trophoblastic tumours, hydatiform mole and choriocarcinoma.\textsuperscript{195,196} Its annual incidence is estimated at approximately 20 cases per million.\textsuperscript{197} PG may occur in every trimester or even in puerperium, but is usually seen in the second or third trimester. The clinical picture initiates with intense pruritus followed by the development of erythematous urticarial papules and plaques with a targetoid or polycyclic appearance. These lesions tend to progress to tense clustered vesicles and bullae. PG typically begins in the umbilical area with subsequent spreading to the whole abdomen and extremities. Involvement of face and mucosa is unusual, but not excluded.\textsuperscript{198} The clinical course normally reaches spontaneous remission within weeks to months after the delivery. Nevertheless, there have been reports of refractory PG cases that continued for years after parturition, thus becoming chronic or conversing to BP.\textsuperscript{199-201} PG may recur with the following pregnancies, the clinical course is, generally, more severe and has an earlier onset. Also, hormonal determinants associated with the use of oral contraceptives or menstruation may also cause recurrences. The fetal and neonatal prognosis is generally good, however, there is risk for prematurity and intrauterine growth restriction. Up to 10% of the new-borns may exhibit transient skin
The exact pathophysiological mechanism of PG is still unclear. Its development is associated with the presence of the maternal MHC class II HLA antigens DR3 and DR4. It is hypothesized that aberrant placental expression of MHC class II initiates an allogenic reaction through presentation of 180 kDa antigen to the maternal immune system. In placenta of PG patients, type XVII collagen is presented in the context of paternal MHC molecules, as a result it may not be recognized as self-antigen and an immune response may be set off. Autoreactivity is typically directed against the NC16A domain of BP180, although about 10% of PG patients also have autoantibodies targeting epitopes on the BP230 molecule. IgG class anti-placental antibodies cross-react with BP180 and BP230 in the skin, form immune complexes, activate the complement cascade, and engage an inflammatory response which leads to tissue injury and blistering. Also, cases with IgE and IgA antibodies to either BP180 or BP230 molecules have been reported. The diagnosis is established based on clinical features and laboratory studies. DIF on perilesional skin will reveal pronounced linear deposits of complement component C3 and in some cases of IgG. Histopathological findings are similar to BP. Certain cases may require additional studies such as IIF, western blot and ELISA. Treatment modalities for PG are restricted due to potential fetal or neonatal risks. The main target is to reduce pruritus and prevent new blister formation, which in mild cases can be achieved with potent topical corticosteroids with or without H1-receptor antagonists. Oral prednisolone is appropriate in cases where the clinical severity outweighs the risks.

**Lichen planus pemphigoides (LPP)** is a rare autoimmune entity with an estimated annual incidence of 2.5 cases worldwide. Clinically, it manifests as a mixture between BP and lichen planus (LP). Presence of LP is, in fact, a prerequisite for the development of LPP. The appearance of LP lesions usually precedes the development of vesicobullous lesions of LPP, however in some cases, the presentation may be concomitant. The upper and lower extremities are predilection sites for lesion distribution, often with the involvement of palms and soles. Oral mucosa can also become affected and develop bullae, erosions, and white streaks, termed Whickham striae. The pathogenesis of LPP is not completely elucidated; an intriguing theory is that the lichenoid infiltrate from LP induces damage to the BMZ and basal keratinocytes thus exposing new antigens. An important distinction should be made between bullous LP and LPP, the former develops the blisters restricted to the LP lesions, whereas in LPP blisters can also occur on previously uninvolved skin. BP230 and the C-terminal part of NC16A domain of BP180 were identified as targets in LPP. However, it has been proposed that in LPP, the target epitope in the C-terminal NC16A domain of BP180 is different from the one targeted in BP. These findings may explain the different clinical course of both diseases. LPP has usually an earlier onset and tends
to be less severe than BP. Histopathology of LPP skin will reveal a band-like lymphocytic infiltrate in the upper dermis, liquefactive degeneration of basal keratinocytes and subepidermal blister formation. DIF studies will demonstrate linear n-serrated deposits of IgG and/or C3 in perilesional skin at the site of BMZ and IIF aims the detection of circulating IgG autoantibodies against the NC16A domain of BP180. Treatment of lichen planus in conjunction with the treatment algorithm for bullous pemphigoid represent the cornerstone in the management of LPP.

**BP180, BP230, LAD-1, LABD-97**

**Linear IgA dermatosis (LAD)** is a pemphigoid disease characterized by exclusively IgA deposition alongside the BMZ of the skin and mucosa with stratified squamous epithelia. Its annual incidence is circa 0.5-2.3 cases per million. Although all ages are prone to the development of LAD, two peaks of onset have been observed; the childhood-onset LAD begins before the age of 5, whereas the adult-onset form usually starts after age 60. In children, LAD is the most frequent form of pemphigoid and in most cases has a self-limiting clinical course; nevertheless some cases persisted into adulthood. The clinical spectrum of this disorder is heterogeneous, sometimes resembling other blistering disorders. Some patients can suffer from severe pruritus. Lesions consisting of clear or hemorrhagic tense vesicles and/or bullae develop on an erythematous urticarial background or on normal appearing skin. Occasionally a distinctive configuration of lesions, such as “crown of jewels” or “string of pearls” can be seen (Fig. 3e). An estimated 70% of patients exhibit mucosal involvement which can cause significant comorbidity. Oral cavity and eyes are the most frequently affected sites. Ocular disease if not treated promptly may result in blindness, through scarring. Use of certain medication may become a triggering factor in the development of LAD. The most reported culprit is vancomycin. Drug-related LAD typically resolve rapidly and spontaneously upon drug withdrawal. Genetic investigations have revealed associations between LAD and the HLA Cw7, HLA B8 and DR3 haplotypes. Also, a significant association with the rare tumour necrosis factor-2 (TNF-2) has been established, such finding predicts longer disease duration. The complex pathophysiological process in LAD engages the humoral and cellular responses, activation of complement, inflammatory cells recruitment, and release of proteolytic enzymes from neutrophils and eosinophils. The majority of LAD patients have IgA1 autoantibodies targeting the 120-kDa (LAD-1) and 97-kDa (LABD-97) antigens. These molecules are localized in lamina lucida and are, in fact, products of proteolytic cleavage of the BP180 ectodomain. The NC16 domain of BP180 and BP230 may also become an antigenic target in a subgroup of patients. Diagnosis is established through a combination of clinical presentation, histopathologic and
immunopathologic studies. Histopathologic findings include subepidermal blistering, upper epidermis infiltrate mainly consisting of neutrophils, and sometimes also eosinophils and mononuclear cells.\textsuperscript{227,232} DIF on perilesional mucous membrane or skin biopsies will reveal linear n-serrated depositions of IgA at the site of BMZ.\textsuperscript{135} Serum IgA autoantibodies may be detected through IIF studies on human salt-split skin; the binding will be on the epidermal side of the blister. Western immunoblotting may be an additional test used to reach an accurate diagnosis. The two most employed treatment options include dapsone or sulfapyridine in combination with potent topical corticosteroids.\textsuperscript{138,233}

\textit{Plectin}

Plectin, a cytoplasmic hemidesmosomal protein, can also become a target for autoimmunity. Such event, although uncommon, was seen in sera from BP patients where anti-plectin autoantibodies were detected. It is plausible that due to its large molecular weight, estimated at >500 kDa, the detection of plectin through conventional immunoblotting or immunoprecipitation techniques can be challenging, as a result, autoantibodies targeting this polypeptide might have been overlooked. Nevertheless, in a study where more sensitive techniques combining immunoprecipitation and immunoblotting were employed, the occurrence of autoantibodies to plectin remained a rare phenomenon in BP.\textsuperscript{234} The reported prevalence in a series of 282 consecutive patients with sAIBD was established at 3.9%. Epitope mapping revealed that the immunodominant hotspot is usually located on the central coiled-coil rod domain of plectin molecule. Presence of exclusively anti-plectin autoantibodies is rarely the case, as the majority of patients have also autoantibodies targeting other pemphigoid antigens.\textsuperscript{235} The clinical manifestation is similar to BP. Plectin has also been reported in the literature as an autoantigen in paraneoplastic pemphigus (PNP), with a higher prevalence than in BP. PNP is a rare autoimmune muco-cutaneous blistering disease associated with an underlying neoplasm. The autoimmunity is directed against multiple antigens, generally from the plakin family proteins, including plectin, but also against desmogleins, adhesion molecules at the site of desmosomes\textsuperscript{236} It is hypothesized that anti-desmoglein antibodies engage the primary role in initiating the pathology by damaging the cell membranes, thus providing intracellular access for anti-plakin autoantibodies.\textsuperscript{236,237}
Integrin α6β4

Both α6 and β4 integrin subunits have been recognized as antigenic sites in MMP. Although located in close proximity, these autoantigens cause distinct pathologies. The α6 integrin subunit has been associated with exclusively oral MMP, whereas β4 integrin subunit is targeted in MMP with predominantly ocular involvement. Some studies support the pathogenicity of anti-β4 integrin autoantibodies in a subgroup of MMP patients. While autoantibodies targeting BP180 and/or BP230 have also been detected in patients’ sera, their presence was temporary and did not parallel the clinical course. Anti-α6 and -β4 integrin antibodies have, however, been found to correlate with the disease activity and severity. Notably, anti-α6 integrin antibodies have also been detected in the sera of classical BP patients. Nevertheless, the affected individuals have not developed any mucosal involvement. The existence of these anti-α6 autoantibodies may represent an epiphenomenon. Distinguishing between the causal autoantigen has significant clinical importance; patients with oral MMP have a better prognosis and some can be managed with local corticosteroids, while individuals with ocular MMP require prompt and adequate systemic immunosuppressive treatment and have a poor prognosis.

Laminin 332

Anti-laminin 332 pemphigoid is a form of MMP, discovered in 1992, when a group of patients developed IgG autoantibodies targeting a disulfide-linked protein, then known as epiligrin. This molecule had a diverse nomenclature throughout the time including nicein, kalinin, BP600, and ultimately laminin 332. Circulating IgG autoantibodies recognize the G domain on the α3 chain of laminin 332 in almost all patients. Their pathogenic role has been demonstrated when the injection of anti-laminin 332 antibodies in neonatal mice and into a human skin graft model caused subepidermal cleavage without inflammation. Autoantibodies against the γ2- and β3 subunits of laminin-332 are rare, but have also been reported. Notably, previous investigations have confirmed that autoantibodies from a subset of MMP patients recognize the α3 chain in both laminin 332 (former lamin 5) and laminin 311 (former laminin 6), thus laminin 331 is co-targeted in anti-laminin 332 pemphigoid. Up to a fifth of all MMP cases is represented by anti-laminin 332 MMP patients. Clinically, they develop predominantly mucous membrane blistering and erosions which have a propensity for scarring and tissue destruction. Mucosa of mouth, eyes, nose, pharynx, larynx, oesophagus, and anogenital area may become affected, creating significant comorbidities, such as gingival destruction, loss of teeth, potential blindness, hoarseness, dysphagia, airway obstruction, and other complications. If lesions develop
on the skin, the predilections sites are the head and upper trunk. Anti-laminin 332 pemphigoid is associated with an increased relative risk for cancer of either lung, stomach, colon, or endometrium. Approximately a third of anti-laminin 332 MMP cases in a cohort were found to have or developed later a single solid malignancy. Hence, careful cancer screening should be performed in individuals affected by this disorder. On the other hand, a French study found no association with internal malignancy. Diagnosis of anti-laminin 332 pemphigoid is based on the clinical picture, histopathologic, and immunopathologic studies. Light microscopy will reveal subepidermal blistering and a dermal infiltrate composed of lymphocytes. Fresh lesions might also display a mixture of neutrophils and eosinophils in the infiltrate, whereas older lesions have sparse cellularity and some degree of lamellar fibrosis. DIF analysis will show linear n-serrated IgG and frequently C3depositions along the epithelial basement membrane. IIF studies on SSS show binding of autoantibodies to the dermal side of the blister. Comparative analysis of methods for detecting anti-laminin 332 antibodies identified immunoblotting of human keratinocytes extracellular matrix or human keratinocytes extract as the most practical technique. Due to potential severe scarring with resulting complications, anti-laminin 332 pemphigoid requires prompt and adequate treatment. Therapy options include, among others: systemic corticosteroids, dapsone, tetracycline, cyclophosphamide, mycophenolate, rituximab.

\[ \text{p200/ laminin } \gamma 1 \]

**Anti-p200 pemphigoid** was described for the first time in 1996 when investigators identified a sAIBD with a novel antigenic target: a 200-kDa glycoprotein, component of the lower lamina lucida. The clinical spectrum of this disorder can be heterogeneous resembling BP, dyshidrotic eczema, the inflammatory form of epidermolysis bullosa aquisita, and LAD. Interestingly, in 30% of the cases, coexistence of psoriasis was noted. The affected individuals develop erythematous urticarial plaques, tense blisters and vesicles on the limbs and trunk (Fig. 3f). Mucous membranes may also become involved. Autoreactivity against anti-laminin \( \gamma 1 \) was found in approximately 90 % of patients’ sera affected by anti-p200 pemphigoid. Laminin \( \gamma 1 \) is a protein chain entering into the heterotrimer composition of several laminins, including laminin 311, 321, and 511, all found at the site of the dermal-epidermal junction. Nonetheless, *in vivo* and *ex vivo* studies have not established a pathogenic role for anti-laminin \( \gamma 1 \) autoantibodies yet. The results demonstrated, however, that patients’ sera depleted of anti-laminin \( \gamma 1 \) antibodies and nondepleted sera caused subepidermal cleavage in an *ex vivo* model of autoantibody-mediated leukocyte-dependent neutrophil activation on cryosections of human skin.
This data suggests that the presence of anti-laminin γ1 antibodies can be viewed as a diagnostic marker for a majority of anti-p200 pemphigoid cases. The factual, pathogenically involved autoantigen has yet to be elucidated.\textsuperscript{269} Histopathologic features of anti-p200 pemphigoid are not very specific and can mimic LAD or dermatitis herpetiformis and include: subepidermal blistering, neutrophilic infiltrate in the papillary dermis with sometimes a mixture of eosinophils. Micro abscesses at the tips of dermal papillae may also be found.\textsuperscript{262,270} DIF studies on perilesional skin will reveal linear n-serrated IgG and C3 deposition along the BMZ. IIF studies on SSS will demonstrate binding on the dermal side of the blister. Western blotting will show reactivity against 200-kDa protein in the human dermis.\textsuperscript{262} Also, autoantibodies against the C-terminus of laminin γ1 may be detected by immunoblotting or ELISA.\textsuperscript{264,271} In a subgroup of patients coexistent autoantibodies targeting BP180, BP230, laminin 332 and type VII collagen have been reported.\textsuperscript{262,272,273} This might be a consequence of epitope spreading. The treatment of anti-p200 pemphigoid consists of topical or systemic glucocorticoids and others: cyclosporine, azathioprine, mycophenolate mofetil, intravenous immunoglobulin, or rituximab.\textsuperscript{138}

\textit{Type VII collagen}

\textbf{Epidermolysis bullosa acquisita} (EBA) is a rare sAIBD with a reported annual incidence of 0.25 cases per million. Both children and adults can become affected.\textsuperscript{175} This disorder is characterized by autoantibodies targeting type VII collagen in the anchoring fibrils at the site of dermal-epidermal junction. The autoreactivity in EBA is directed mainly against the NC1 domain of α chain in type VII collagen.\textsuperscript{274,275} In some EBA cases the NC2 and triple helical domains have been described as targeted region.\textsuperscript{276,277} The pathogenicity of autoantibodies targeting type VII collagen is emphasized by the observation that a neonate of an EBA mother developed congenital blisters due to passive placental transfer of maternal IgGs.\textsuperscript{278} Furthermore, \textit{ex vivo} studies\textsuperscript{279} and passive or active transfer mouse models involving anti- type VII collagen autoantibodies have demonstrated blister formation.\textsuperscript{280-282} Clinically, EBA can manifest with either inflammatory or mecanobullous phenotype. The classical mecanobullous phenotype resembles DEB, and the less severe cases can mimic porphyria cutanea tarda where acral blistering resolves with atrophic scarring, milia and pigmentedary changes (Fig. 3g).\textsuperscript{283} When the scalp, neck, and shoulders are the only affected sites, the clinical picture can be reminiscent of Brunsting-Perry pemphigoid.\textsuperscript{284} The inflammatory phenotype of EBA, on the other hand, can resemble BP or MMP. Patients develop vesicles and bullae mainly affecting the flexural areas, with no scaring or milia. Also, grouped circinary vesicles and arciform erythema, configurations seen in LAD, may be witnessed in inflammatory EBA.\textsuperscript{285,286} Several studies have suggested an
association between EBA and inflammatory bowel disease (IBD), particularly Crohn’s disease. The connection is explained by the fact that type VII collagen is also expressed in the colon, although in lower concentrations than in the skin. Typically the onset of gastrointestinal symptoms precedes the skin involvement, thus EBA may be regarded as a complication of IBD. In addition, EBA is associated with an increased frequency of HLA-DR2 haplotype, mainly DRB1*15 in black patients of African descent and DRB1*13 in Korean patients. Histopathology of inflammatory EBA shows subepidermal blistering and a mixed inflammatory infiltrate consisting of lymphocytes, neutrophils and eosinophils, whereas the infiltrate of the mechanobullous variant has a more sparse cellularity. DIF studies on perilesional skin biopsies will show linear u-serrated depositions of IgG, IgA, and C3 at the site of BMZ. Serration pattern analysis in EBA has considerable significance as it allows differentiation from other pemphigoid disorder which all have an n-serrated pattern. Also, the importance of serration pattern analysis is supported by the fact that less than a half of EBA patients’ sera will show reactivity by IIF on SSS by binding on the dermal side of the artificial blister. Several other laboratory studies including immunoblotting, immuno electron microscopy and ELISA may confirm or aid establishing the diagnosis. Type VII collagen ELISA test may also be employed for monitoring the clinical activity in EBA. Its value is however limited in SSS-non-reactive EBA sera. A subgroup of patients will exhibit exclusively IgA reactivity on the dermal side of SSS or in the sublamina densa zone by indirect or direct immuno electron microscopy. Such, entity is known as IgA-mediated epidermolysis bullosa aquisita (IgA-EBA). Its clinical manifestation resembles the classic “lamina lucida type” LAD or the inflammatory variant of IgG-mediated EBA. Some investigators, in fact, view IgA-EBA as a subtype of LAD where the IgA autoreactivity is directed against type VII collagen. Treatment options of EBA include systemic corticosteroids in conjunction with colchicine or dapsone. Severe or refractory cases may require, among others: cyclosporine, azathioprine, mycophenolate mofetil, intravenous immunoglobulin, or rituximab.
**Figure 1.** Schematic representation of the dermal-epidermal junction. Molecules or their subunits targeted by genetic mutations and/or circulating autoantibodies are shown in colour (excluding grey).
Table 1 Targeted molecules and their corresponding hereditary and/or acquired disease at the site of dermal epidermal junction

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<th>Molecule</th>
<th>Inherited disease</th>
<th>Acquired disease</th>
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<td><strong>Intermediate filaments molecules</strong></td>
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<td>Keratin 5, 14</td>
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<td>EBS with mottled pigmentation (K5)</td>
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<td></td>
<td>EBS, migratory circinate (K5)</td>
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<td></td>
<td>EBS, autosomal recessive K14</td>
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<td><strong>Hemidesmosomal molecules</strong></td>
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<tr>
<td>Plectin</td>
<td>EBS with muscular dystrophy</td>
<td>Anti-plectin pemphigoid*</td>
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<td></td>
<td>EBS with pyloric atresia*</td>
<td>Paraneoplastic pemphigus*</td>
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<tr>
<td></td>
<td>EBS-Ogna*</td>
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<td><strong>BP230</strong></td>
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<td></td>
<td>EBS, autosomal recessive-BP230 deficiency*</td>
<td>Bullous pemphigoid</td>
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<td>Mucous membrane pemphigoid</td>
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<td>Pemphigoid gestation</td>
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<td>Lichen planus pemphigoid</td>
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<td>Linear IgA disease</td>
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<td>Brunsting-Perry pemphigoid</td>
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<td><strong>BP180</strong></td>
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<td></td>
<td>JEB, generalized intermediate</td>
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<tr>
<td></td>
<td>JEB, late onset*</td>
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<tr>
<td></td>
<td>JEB, localized</td>
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<tr>
<td><strong>LAD-1, LABD-97</strong></td>
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<tr>
<td>Integrin α6β4</td>
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<td></td>
<td>EBS with pyloric atresia (integrin α6; β4) *</td>
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<td></td>
<td>JEB with pyloric atresia (integrin α6; β4) *</td>
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<td>JEB, localized (integrin β4)</td>
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<td><strong>Other junctional molecules</strong></td>
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<tr>
<td>Laminin 332 (α3,β3,γ2 chain)</td>
<td>JEB, generalized severe</td>
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<td></td>
<td>JEB, generalized intermediate</td>
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<td></td>
<td>JEB, localized</td>
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<td></td>
<td>JEB-LOC syndrome (isoform α3 chain)*</td>
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<td>Laminin 311 (α3 chain)</td>
<td>JEB</td>
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<td>p-200/ laminin γ1</td>
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<td>Type VII collagen</td>
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<td>DDEB</td>
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<td>DDEB/RDEB, bullous dermolysis of the newborn*</td>
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<td>RDEB, generalized severe</td>
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<td>RDEB, generalized intermediate</td>
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<td>RDEB, localized</td>
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<td>RDEB, all other subtypes</td>
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<td><strong>Focal adhesion molecules</strong></td>
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<td>Kindlin-1</td>
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<td>Kindler syndrome*</td>
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<td>Integrin α3</td>
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<td></td>
<td>JEB with respiratory and renal involvement*</td>
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*, rare diseases; EBS, epidermolysis bullosa simplex; K5, keratin 5; K14, keratin 14; JEB, junctional epidermolysis bullosa; DDEB, dominant dystrophic epidermolysis bullosa; RDEB, recessive dystrophic epidermolysis bullosa.
Figure 2. Clinical features in various inherited blistering diseases of the dermal-epidermal junction. **a** Blistering and scarring alopecia in a patient with junctional epidermolysis bullosa, generalized intermediate resulting from type XVII collagen mutations. **b** Enamel pitting in a patient with junctional epidermolysis bullosa, generalized intermediate due to type XVII collagen mutations. **c** Exuberant granulation tissue involving the nail area of hand digits in a child with junctional epidermolysis bullosa, generalized severe. **d** Cutis aplasia (congenital absence of skin) in the lower leg in a child with junctional epidermolysis bullosa. **e** Extensive blistering, erosions and chronic wounds in a child with recessive dystrophic epidermolysis bullosa, generalized severe. **f** “Mitten-like” hand deformity with encapsulated digits in a patient with recessive dystrophic epidermolysis bullosa, generalized other. **g** Island of normally pigmented and mechanically resilient skin on the dorsal side of hand as consequence of revertant mosaicism in a patient with junctional epidermolysis bullosa generalized intermediate.
Figure 2. Clinical features in various inherited blistering diseases of the dermal-epidermal junction. 

a Blistering and scarring alopecia in a patient with junctional epidermolysis bullosa, generalized intermediate resulting from type XVII collagen mutations.

b Enamel pitting in a patient with junctional epidermolysis bullosa, generalized intermediate due to type XVII collagen mutations.

c Exuberant granulation tissue involving the nail area of hand digits in a child with junctional epidermolysis bullosa, generalized severe.

d Cutis aplasia (congenital absence of skin) in the lower leg in a child with junctional epidermolysis bullosa.

e Extensive blistering, erosions and chronic wounds in a child with recessive dystrophic epidermolysis bullosa generalized severe.

f “Mitten-like” hand deformity with encapsulated digits in a patient with recessive dystrophic epidermolysis bullosa, generalized other.

g Island of normally pigmented and mechanically resilient skin on the dorsal side of hand as consequence of revertant mosaicism in a patient with junctional epidermolysis bullosa generalized intermediate.

Figure 3. Clinical symptoms in various acquired blistering diseases of the dermal-epidermal junction. 

a Generalized bullous pemphigoid, with autoantibodies targeting BP180, featuring erythematous urticarial papules and plaques with tense blisters and erosions.

b Desquamative gingivitis in a patient with mucous membrane pemphigoid with autoantibodies against laminin 332.

c Conjunctivitis and symblepharon in a patient with mucous membrane pemphigoid with autoantibodies against BP180.

d Scalp erythema, erosions, crusting, and extensive alopecia with scarring in a patient with Brunsting-Perry pemphigoid with autoantibodies targeting BP180.

e Bullous lesions in “string of pearls” configuration on the lower abdomen of a patient with linear IgA disease with autoantibodies targeting LAD-1.

f Localized monomorphic blistering of the arm in a patient with p200 pemphigoid.

g Tense blisters and milia on the dorsal side of the hand in a woman with epidermolysis bullosa aquisita, mechanobullous variant with autoantibodies against type VII collagen.
References


