Substantiating atypical phenotypes of epidermolysis bullosa

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Chapter 1

Introduction

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The Centre for Blistering Diseases, Department of Dermatology, University of Groningen, University Medical Centre Groningen, Groningen is the national referral centre for both autoimmune and inherited blistering diseases in the Netherlands. Patients from the Netherlands and neighbouring countries affected with epidermolysis bullosa (EB) are here assessed, diagnosed and treated. The Dutch National EB Registry documents characteristics such as phenotype, laboratory diagnostics, and molecular analysis. The works presented in this thesis are the results of continual collaboration between the departments of Dermatology and Genetics in diagnosis and research of EB.

EPIDERMOLYSIS BULLOSA

Butterfly child disease, cotton wool baby syndrome or crystal skin syndrome are all terms which refer to epidermolysis bullosa (EB), a group of inherited blistering diseases manifesting with skin and mucous membrane blistering after trivial trauma. [1] With no preference for gender or race, the incidence and prevalence of EB is 8.2 and 19.6 per 1 million births respectively, ranking it as a rare or orphan disease. [2,3] The phenotypic spectrum of EB is incredibly diverse, owing to the fact that presently mutations in 18 different genes resulting in impaired structural and functional cellular adhesion are known. [1] Next to skin and mucous membrane blistering, certain forms of EB show gastrointestinal, respiratory and genitourinary involvement, highly aggressive squamous cell carcinoma and involvement of skin adnexa, hair, enamel, and nail dystrophy. [1] Presently, there is no cure for EB and treatment is limited to rigorous wound care and chronic pain management. [4] Worldwide, different therapies are being developed, including cell, protein and gene therapies, and some of them are currently being tested clinically. [4-9]

EB Classification

Out of the 18 already identified genes in EB one can find those coding for enzymes of epidermal differentiation, constituents of keratin filaments, adhesion cytolinker proteins, desmosomes, hemidesmosomal plaque proteins and anchoring fibrils in skin and mucosa. [1] There are four major subtypes of EB, grouped based on the level of blistering present in the skin (Figure 1). [1] Visualization of blistering level is achieved with immunofluorescence microscopy (IF) and transmission electron microscopy (TEM). [1,10] In general, severity ranges from mild to lethal and the main subtypes are further divided into more than 30 specific variants. The broad phenotypic spectrum is dictated by: the mutated gene, pattern of inheritance, type and location of mutation, mutational effect on mRNA, posttranslational modification and expression levels of the encoded protein. [1,11-13] The immense clinical spectrum has provoked the development of a classification system based on international consensus. [1,14,15] The EB classification was updated in 2014 and structured by an ''onion skin'' approach, taking into account the EB type, mode of inheritance, phenotype, immunofluorescence antigen mapping findings, and mutation(s) present in each patient. [1,14,15]

The following three major subtypes of EB shall be briefly introduced as they are studied in this thesis: epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JEB) and dystrophic epidermolysis bullosa (DEB). Additionally, the fourth and last subtype of EB, Kindler syndrome (KS), is characterized by skin blistering with cleavage in different levels of the epidermis, poikiloderma, skin atrophy and photosensitivity. [16] Autosomal recessive mutations in *FEMRT1* are responsible for KS.

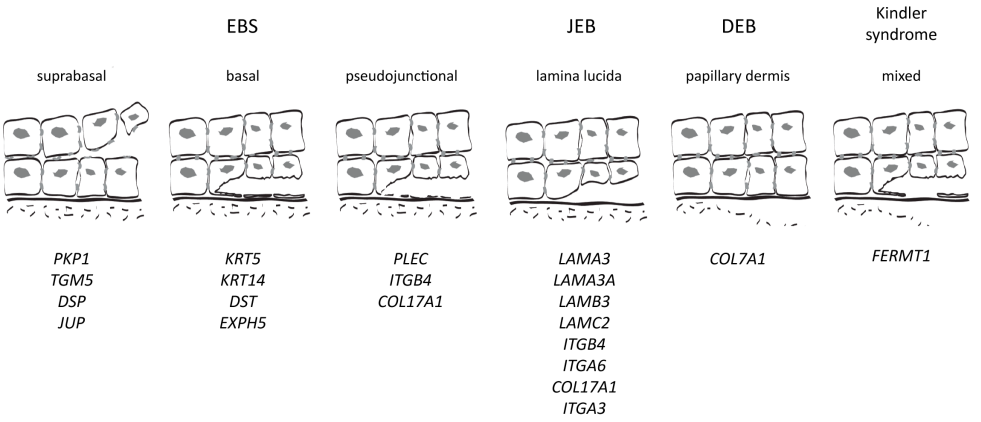

Figure 1. Graphic representation of the level of blister formation in major subtypes of epidermolysis bullosa: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB) and Kindler syndrome. Below listed genes are involved in the corresponding subtype. Courtesy of Prof. dr. M.F. Jonkman (with modifications).

Epidermolysis bullosa simplex

Intraepidermal blister formation characterizes epidermolysis bullosa simplex. The 2014 EB consensus continues to classify EBS into basal and suprabasal subtypes according to the level of blistering within the epidermis. [1]

Suprabasal EBS is defined by superficial blistering occurring above the basal cell layer of the epidermis (Figure 1). [1] This group includes extremely rare and severe autosomal recessive variants resulting from mutations in genes encoding desmosomal proteins plakoglobin (*JUP*), plakophilin-1 (*PKP1*) and desmoplakin (*DSP*). [17-19] Another milder, recessive variant affecting an enzyme involved in terminal differentiation of the cornified envelope in the epidermis, transglutaminase 5 (gene *TGM5*) causing acral peeling skin syndrome is also included in this group. [20,21] Patients show subtle to extensive erosions, abnormal keratinization and rarely intact blisters due to the high level of epidermal cleavage. [1,17,19,21-23]

The most frequent EBS subtype is basal EBS, occurring when blistering affects the basal keratinocytes. [24-27] Although not distinguished in the official EB consensus, the level of cleavage in basal keratinocytes can be further divided into two different levels: (i) cortical cleavage meaning within the basal keratinocyte cortex and (ii) lower basal cleavage termed 'pseudojunctional' meaning very low in the basal keratinocyte, which can be distinguished from cortical by the epithelial remnants visible on the blister floor seen in TEM (Figures 1 and 2b). [28,29] Collectively, there are five genes known to be responsible for basal EBS: *KRT5*, *KRT14*, *DST, EXPH5* (all resulting in basal cleavage) and *PLEC* (resulting in pseudojunctional cleavage). [30-33] Basal EBS is usually inherited as an autosomal dominant condition, caused in 75% of cases by mutations in *KRT5* and *KRT14*, of these more than 85% are autosomal dominant missense mutations. [27,34,35] An autosomal dominant skin-only form of basal EBS is seen in a rod domain specific missense mutation in *PLEC* in the variant EBS-Ogna (EBS-Og, MIM: 131950, named after the village in Norway where the first family was described). [36,37] Recessive genotypes in *KRT14* have been described, but are rare. [32,38-44] Clinically, basal EBS is regarded as the mildest form of EB due to the moderate blister formation improving with age and relatively mild extracutaneous complications (excluding plectin subtypes). [1,45]

In the neonatal period, blistering can be generalized and quite extensive, which is not predictive of the resulting phenotype later in life. [46] Acral blistering, sometimes in a circinate distribution, late onset palmoplantar keratoderma, onychodystrophy, post inflammatory hyperpigmentation and mucous membrane erosions are common. [1] Severe forms of EBS are seen when autosomal recessive mutations in *PLEC* occur (see section **Plectin**). [40,41,47]

Junctional epidermolysis bullosa

JEB is seen with blister formation within the lamina lucida, the 'clear' layer of the basal membrane zone (BMZ) localized on the epithelial side (Figure 2c). [1] Rarely, pseudojunctional cleavage can also occur (mutations in *ITGB4 and COL17A1*). [28,48,49] Autosomal recessive mutations in *COL17A1, ITGA3*, *ITGA6*, *ITGB4, LAMA3, LAMB3,and LAMC2* are responsible for generalized and localized phenotypes. [50-56] An isoform specific deletion in the N-terminal domain of the 'a' isoform encoded by *LAMA3A* leads to a rare entity called laryngo-onycho-cutaneous (LOC) syndrome (MIM: 245660). [57] JEB patients show a spectrum of extracutaneous features including pyloric atresia, urinary tract stenosis, anaemia, nephrotic syndrome, interstitial lung disease, enamel pitting and failure to thrive in different clinical variants. [1] All JEB patients have dental abnormalities, referred to as amelogenesis imperfecta (AI). There have been reports that carriers of single defective alleles in genes causing JEB also display dental abnormalities ranging from enamel hypoplasia to AI. [58-60] We observed this feature in carriers of *LAMA3* mutations and describe our findings in **chapter 5**.

Dystrophic epidermolysis bullosa

DEB is characterized by the deepest skin blistering at the level of the lamina densa, the electron dense layer of the BMZ on the side of the dermis (Figure 2d). [1] Autosomal dominant mutations in *COL7A1* encoding type VII collagen generally result in much milder disease (DDEB) than recessive mutations (RDEB). [61] Patients with severe RDEB show lifelong impaired would healing, blistering with resulting skin fibrosis, impaired mobility, pseudosyndactyly (mitten deformity of the hands), oesophageal strictures and aggressive squamous cell carcinoma. [1,62,63] One particular variant of DEB manifesting with chronic pruritus is the pruriginosa subtype (MIM:604129), seen in both recessive and dominant forms. [64-67] A new phenotypic observation seen in patients with DEB-pruriginosa is described in **chapter 6** of this thesis. [68]

The basement membrane zone

The BMZ is the central skin segment connecting the epidermis with the underlying dermis. Also referred to as the dermo-epidermal junction, the linear BMZ consists of two groups of molecules that secure the basal keratinocyte cytoskeleton to the dermal matrix: the hemidesmosome adhesion complex and focal adhesion complex (Figure 2). Hemidesmosomes (HD) are protein complexes, which anchor basal keratinoctyes to the BMZ. [69,70] There are two types of HD: type I are found in stratified epithelia such as the skin and contain α6β4 integrin, type XVII collagen (BP180 or bullous pemphigoid antigen-2, BPAG-2), tetraspanin (CD151), plectin and dystonin (bullous pemphigoid antigen-1, BPAG-1). [71] Type II are found in simple epithelia such as the intestine and uroepithelium and consist of α6β4 integrin and plectin. Together with desmosomes, adherens junctions, and tight junctions they form the

main types of junctions in the human epidermis.[72] Ensuring resistance to exogenic stress in the skin, HD contain some of the protein complexes affected in EB (Figure 2). They are visualized on the ultrastructural level as electron dense structures composed of an inner and outer plaque located at the base of basal keratinocytes.[69,71] The inner plaque (intercellular portion or cytoplasmic side), is the site of attachment of the intermediate filament system with plectin and dystonin.[69,73] Plectin and dystonin connect with the transmembrane β4 subunit of α6β4 integrin in the outer plaque of the HD (extracellular side). It is thought that this interaction is one of the first to occur during HD assembly and the most crucial for formation and stability of HD.[71] The basal keratinocytes are finally bound to the basement membrane through the α6β4 integrin complex, which attaches to extracellular portion of laminin-332, type XVII collagen and tetraspanin.[69,74] Ultimately, the laminin-332 anchoring filament bridges the plasma membrane and binds to type VII collagen through it's β3 chain in the lamina densa of the BMZ.[75] The anchoring fibrils of type VII collagen, loop from the BMZ to the papillary dermis and back.[75] Between anchoring HD adhesion complexes, the BMZ focal adhesion complexes connect actin and microtubule cytoskeleton to the dermis. The focal adhesion complexes contain proteins involved in the pathogenesis of EB (integrin α3 and kindlin-1).[76] In this thesis, we will focus on subtypes of EB caused by mutations in genes encoding the polypeptides **keratin 5**, **plectin**, **laminin-332** and **type VII collagen**.

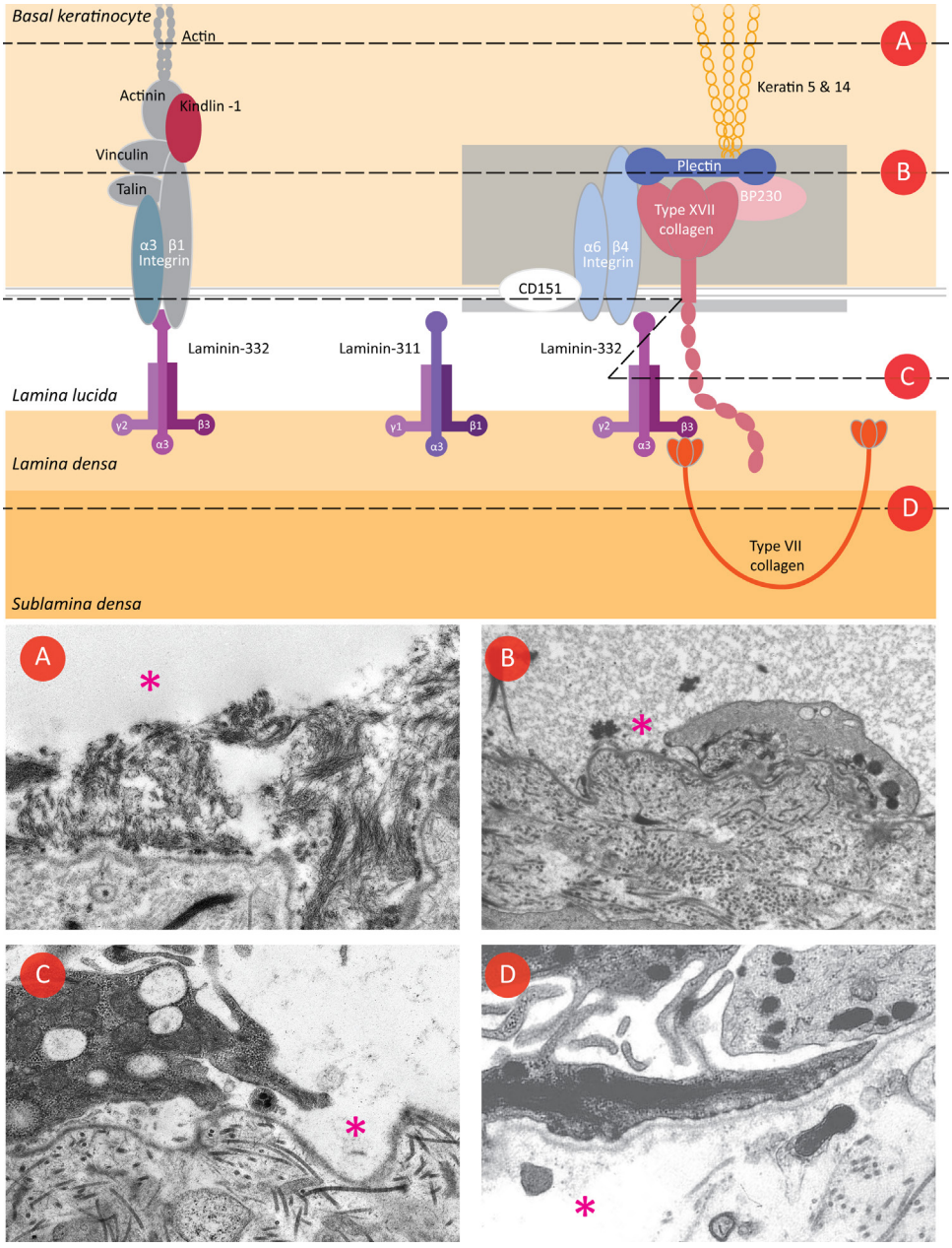

Figure 2. Schematic representation of the basement membrane zone (BMZ): hemidesmosome adhesion complex (right) and focal adhesion complex (left) with proteins associated with epidermolysis bullosa shown in colour. Level of blistering indicated and exemplified in TEM. Pink asterisk indicates blister cavity. **A**: Intraepidermal cleavage seen in basal EBS, **B**: Pseudojunctional cleavage seen in basal EBS and EB with pyloric atresia (JEB-PA), **C**: Cleavage in the lamina lucida seen in JEB (can occur high and low), **D**: Blistering in DEB occurs in the sublamina densa. Note: Supra-basal (EBS) cleavage occurring epidermally higher than the basal keratinocyte is not depicted as it extends beyond the scope of the BMZ. Cleavage seen in Kindler syndrome occurring with various levels of skin blistering is also not depicted.

Keratin 5

Keratin 5 is a type II (acidic) intermediate filament of the basal epidermis encoded by the *KRT5* gene. [77,78] Keratin 5 structure is in line with the classic composition of all intermediate filaments, consisting of a non-helical globular N- and C-termini with central α-helical coiled coil rod domain. The helical rod domain has four α-helical segments 1A, 1B, 2A and 2B, which are interrupted by non-helical linker segments, L1, L12, and L2. The 2B helical subdomain also contains a 'stutter' interruption. Together with keratin 14 (type I, basic filament encoded by gene *KRT14*), it forms the basal keratin cytoskeleton by arranging as obligate parallel dimers, then anti-parallel apolar tetramers in the basal epidermis. The intermediate filaments insert into plectin and BP230 in the outer plaque of the hemidesmosome. [69] Keratin filaments are joined together in a coiled coil formation by their α-helical domains with individual residues forming hydrophobic interactions labelled as heptad repeats (abcdefg). [35,79,80] Depending on the affected portion of the keratin protein, different severities of basal EBS result. [34,81] The described point mutations common in basal EBS compromise intermediate filament scaffolding in the basal cells causing keratin to clump. [82] This phenomenon is well visualized with TEM of skin biopsies and is often a pivotal clue when considering EBS candidate genes (Figure 2a). **Chapter 4** describes how a novel genotype of in-frame exon skipping in *KRT5* affects keratin filament assembly in a four-generation family affected with the subtype EBS generalized severe (EBS-gen sev, MIM:131760).

Plectin

Plectin is a universal cytolinker protein, expressed as several isoforms, encoded by a single gene. [83-85] As a member of the plakin group, plectin exerts linking functions in the skin, muscle, myocardium, gut and neuronal tissue. In the skin, it acts together with α6β4 integrin as the dominant anchor of the hemidesmosome (Figure 2). [70,84,86] It also takes part in desmin and desmoplakin binding in the upper epidermal layers. [87] Structurally, plectin consists of a central rod domain flanked by two globular domains (Figure 3). The N-terminus actin-binding domain (ABD) is the site of binding with integrin β4 subunit of α6β4 and type XVII collagen while the C-terminus of plectin mediates insertion of intermediate filaments such as keratin 5 and 14. [47,85,88] The protein functions as a dimer through it's α-helical rod domain stimulating parallel homodimerization or heterodimerization with other plectin isoforms. [84] The encoding *PLEC* gene is well known for alternate physiological splicing, justified by eight isoforms produced from alternate splicing of individual first exons of the gene at the N-terminus (Figure 3). [89,90] The isoforms are tissue specific, which is best seen when isoform specific mutations occur resulting in different disease phenotypes. [47,86,90-92] In addition to the eight unique tissue isoforms, an isoform lacking the coiled coil rod domain occurs from physiological splicing in exon 31. [93,94] To date, the function of this rodless plectin variant is not yet fully understood. [94] Mutations in the *PLEC* gene including the isoform specific mutations give rise to a large spectrum of disease collectively referred to as the 'plectinopathies'. [47] Probably the most well known and studied are the different forms of basal EBS occurring from biallelic frameshift or nonsense mutations in *PLEC*. [95-97] Autosomal recessive null-mutations in exon 31 encoding the plectin rod domain result in EBS with muscular dystrophy (EBS-MD, MIM:226670), whereas recessive null-mutations in exons outside of exon 31 lead to EBS with pyloric atresia (EBS-PA MIM:612138), a severe subtype resulting in death shortly

after birth. [95,97] Patients express little to no plectin protein in skin in EBS-PA, in contrast to EBS-MD patients, who express some plectin protein, often the rodless variant. [94,98] **Chapter 3** explores a newly identified plectin isoform produced by alternate splicing in exon 8 (Figure 3).

Of interest, one specific heterozygous missense mutation in the rod domain of *PLEC* results in the autosomal dominant form of EBS, EBS-Ogna characterized by skin-only fragility. [99] The Ogna mutation substitutes a basic and positively charged arginine to a neutral and hydrophobic tryptophan, rendering the rod susceptible to proteolytic degradation. [100] A second skin-only EBS plectinopathy occurs from homozygous mutations in the 1a isoform and is reported for the first time in this thesis in **chapter 2**. [101] The significance of plectin attachment function in the hemidesmosome is exemplified in plectin null mice, which exhibit extensive blistering of epithelia, generalized myopathy of skeletal muscle and ultrastructural changes of myocardium. Plectin null mice die shortly after birth. [102]

While the hemidesmosome is targeted in skin fragility from plectin mutations, blistering in the suprabasal layers, at the ultrastructural location of plectin involvement in desmosomes has yet to be reported. The observation that carriers of heterozygous mutation in plectin show cardiomyopathy due to desmosomal fragility and that plectin knockdown in mice leads to cardiac pathology has inspired further research into the exact role of plectin mutations in cardiomyopathy. A cluster of plectin missense variants located on exon 31 has been located in both Dutch and British patient cohort populations, however the exact mechanism how these variants lead to cardiomyopathy is unknown. [103]

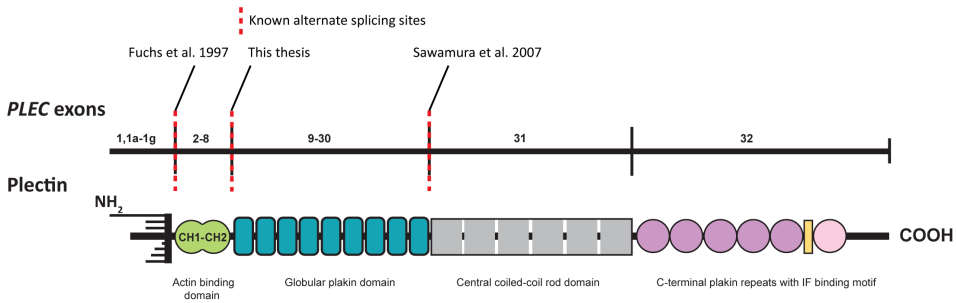

Figure 3. Graphic representation of the *PLEC* gene and encoded plectin protein with different domains. Dotted red line at exon 1, 8 and 31 indicate sites of alternate splicing in *PLEC*. (Figure modified and used with permission of Dr. M.C. Bolling, 2010).

Laminin-332

Laminin-332 is an adhesion heterotrimer polypeptide produced by basal keratinocytes structurally bridging the lamina lucida to the lamina densa. [104,105] As all laminins, it is composed of an α, β, and γ chain, which collectively join as a cross shape forming a trimer. [105] The α, β, and γ chains are encoded for by individual genes, *LAMA3*, *LAMB3*, and *LAMC2* respectively and autosomal recessive mutations result in various JEB subtypes. [1,48,49,51] Complete absence of functional laminin-332 results in the most severe subtype of JEB-generalized severe (JEB-gen sev, MIM: 226700, formerly JEB-Herlitz) resulting in early death at an average age of 5.8 months. [13,106] The presence of laminin-332 in mucous membranes and enamel results in

extracutaneous features when mutations affect this protein in JEB. [107-110] The significance of biallelic laminin-332 expression for proper enamel formation is discussed in **chapter 5**.

Type VII collagen

Type VII collagen is the affected protein in DEB, consisting mostly of anchoring fibrils, as its main component located in the lamina densa. Type VII collagen is synthesized mainly by keratinocytes, first as a pro-alpha 1 (VII) precursor polypeptide. Pro-alpha 1 is composed of a non-collagenous 1 (NC1) domain, triple helix domain and non-collagenous 2 (NC2 domain) at the C terminus. [111] The triple helix domain consists of glycine repeats (Gly-X-Y) interrupted by bouts of non-collagenous sequences. [112] Patients with glycine missense mutations in *COL7A1* in this domain often suffer from the pruriginosa variant of DEB. [67] The pro-alpha 1 peptide undergoes post translational modification to form a homotrimer, which then becomes secreted. [111] The homotrimers join to form anchoring fibrils as homodimers, which in turn bind to laminin-332 and integrins. [69,112] Visualization of type VII collagen with immunofluorescence antigen mapping allows for semi-quantitative assessment of expression levels roughly correlating with severity of DEB disease phenotype. [12,61,113] Anchoring fibrils ultrastructure can be visualized with transmission electron microscopy, as loop structures in the subepidermal lamina densa (Figure 2d). [114,115]

DIAGNOSTIC WORK-UP OF SUSPECTED EB

The described vast clinical spectrum of EB is a diagnostic challenge for the treating dermatologist. The diagnostic work-up is traditionally composed of the following elements:

Macroscopic analysis

Clinical work up of patient phenotype including: age and form of onset of cutaneous and mucosal blistering, provocation factors, seasonal influence of symptoms, extracutaneous symptoms and subjective health complaints, precise family history

Microscopic analysis

Immunofluorescence antigen mapping (IF) of skin biopsies (perilesional and healthy) with monoclonal antibodies targeting various epidermal and BMZ protein constituents for identification of cleavage plane and assessment of protein expression (e.g. normal, absent, reduced, increased) [10,116]

Transmission electron microscopy (TEM) of skin biopsies (perilesional and healthy) for assessment of ultrastructural findings including but not limited to: split level, number and size of hemidesmosomes (EBS, JEB), tonofilament clumping (EBS), absence of anchoring fibrils (DEB) [115]

Mutation analysis: Currently, it is still standard practise in many centres to employ Sanger sequencing of candidate genes based on the aforementioned clinical workup, IF and TEM. This form of DNA sequencing detects single nucleotide (missense, nonsense, insertion/deletion, splice site and intronic mutations (but only in the approx. 20 bp splice site-flanking regions) in candidate genes [117,118]

Due to the increasing number of genes involved in EB, clinical heterogeneity and overlap of EB subtypes with other genodermatoses especially in the neonatal period, targeted gene panels are becoming employed. These panels use next generation sequencing allowing for efficient parallel analysis of all disease related genes in one test. [119,120] The current and future application of this method specifically used in EB and a discussion on its advantages and disadvantages is presented in **chapter 7**.

THE COLD CASES

The journey to gene discovery and mutation identification in EB has been a long and continuous process and despite considerable progress, certain cases remain unsolved. When work began on this thesis, the largest group of unsolved EB cases known in the Center for Blistering diseases in Groningen were patients diagnosed with intraepidermal blistering and a clinical suspicion of basal EBS comprising approximately 15-20% of the EBS cohort. [27,121] Following standard Sanger sequencing of candidate genes no genetic variants in known EB genes were identified. The identification of the underlying genetic mutation is of high importance for a number of reasons. Firstly, it allows complete diagnosis of disease and proper prognostic advice. This is crucial in a disease exhibiting such a wide phenotypic spectrum as EB. Tying into this, complete genetic diagnosis with inheritance pattern is important for family planning. In the more severe EB subtypes such as JEB-gen sev and RDEB, carrier identification is a reason for pre-implantation counselling should the family request it. Lastly, genotype-phenotype correlations, as discussed in **chapters 2-6** of this thesis, allow further

understanding of molecular biology of epidermal proteins and can give insights for specific targeted therapies in the future.

IDENTIFICATION OF EB GENES

As one of the aims of the studies presented in this thesis was to solve genetically undiagnosed cases of EBS, it is interesting to consider a few examples that have in the past led to the discovery of EB genes. Functional biological assessment of skin biopsies with IF and TEM seems to predominate as the first and foremost clue for the level of epidermal skin fragility and indicator of abnormal skin proteins. Other clues include correlation of clinical characteristics to that seen in mouse models, and genome wide sequencing where an identified genetic locus can be correlated with specific epidermal expression.

In 1995, Jonkman et al. first connected type XVII collagen (180-kD bullous pemphigoid antigen, BP180) with JEB generalized intermediate, formerly referred to as JEB, non-Herlitz or generalized atrophic benign epidermolysis bullosa (GABEB).[122] Preceding studies had shown variable or normal staining of JEB skin when tested with serum from bullous pemphigoid (BP) patients in whom staining for laminin-332 was normal. Once monospecific antibodies for both dysytonin (BP230) and type XVII collagen antigens became available, type XVII collagen, and not dystonin was deficient in patient skin in IF. This was further supported by a reduced amount of normal length type XVII collagen mRNA from cultured keratinocytes of one the studied patients. The first mutations in the gene encoding type XVII collagen, *COL17A1,* were described by McGrath et al. in the same year.[56]

In the second example, Pigors et al (2010) pinpointed the molecular defect to the desmosome in a newborn presenting with generalized erosions and massive fluid loss.[17] IF revealed abnormal staining of desmosomal and adherens junction proteins (desmoplakin, desmoglein-3, plakophilin-1, β-catenin). TEM further confirmed remnants of desmosomes in basal cells with absent desmosomes on the apical surface of basal keratinocytes and in the epidermal spinous layer. Based on these findings, plakoglobin, which takes part in both desmosome and adherens junction adhesion was suspected. Sequencing of the plakoglobin encoding *JUP* gene revealed a homozygous nonsense mutation and to the identification of a new form of suprabasal EBS.

The most recent EB gene to be discovered was *EXPH5* in 2012 by McGrath et al. while studying a consanguineous family presenting with minor skin fragility and diffuse mottled pigmentation of the trunk and limbs.[33] Slight deviations in keratin 14 and tetraspanin staining were seen in IF, however, no mutations were found in *KRT14* and *CD151*. The real clue was revealed in the increased number of perinuclear vesicles witnessed in TEM. Whole exome sequencing showed a 1bp deletion in *EXPH5* and co-segregation in affected individuals was later confirmed by linkage analysis. Immunolabelling of patient keratinocytes pinpointed the location of the encoded protein Slac2b to be at intracellular vesicles. Using retroviral delivered shRNA, keratinocytes from both an affected and healthy individual with knocked down Slac2-b were studied. They ultimately connected the disrupted keratin filament network with the mutated Slac2b protein which impaired vesicle transport. The biological evidence of accumulation of perinuclear vesicles and a disrupted tonofilament network was seen in TEM during the initial diagnostic process.

A complete chronological bullet style illustration of gene discovery in EB is presented in Figure 4.

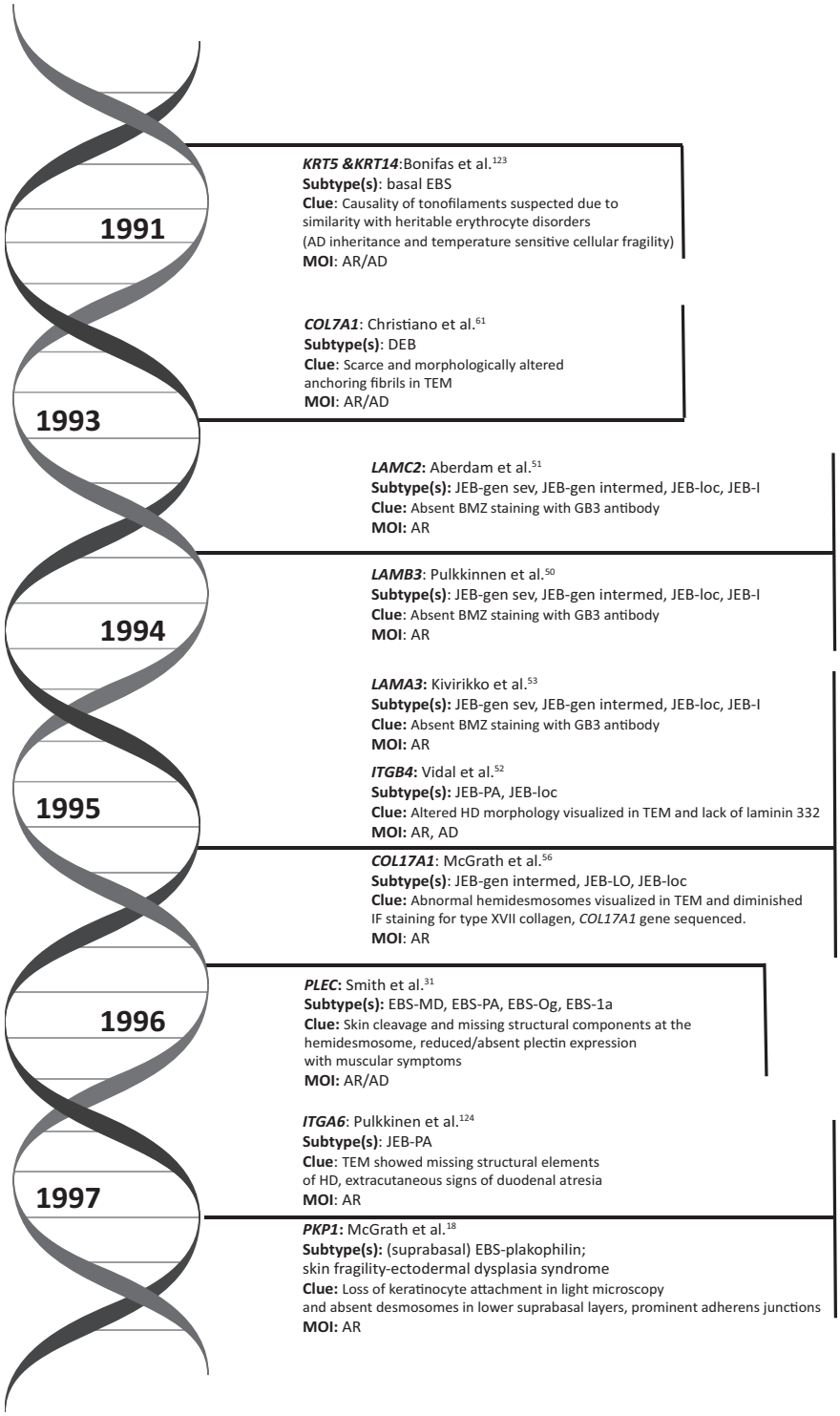

Figure 4. Timeline of gene discovery in EB

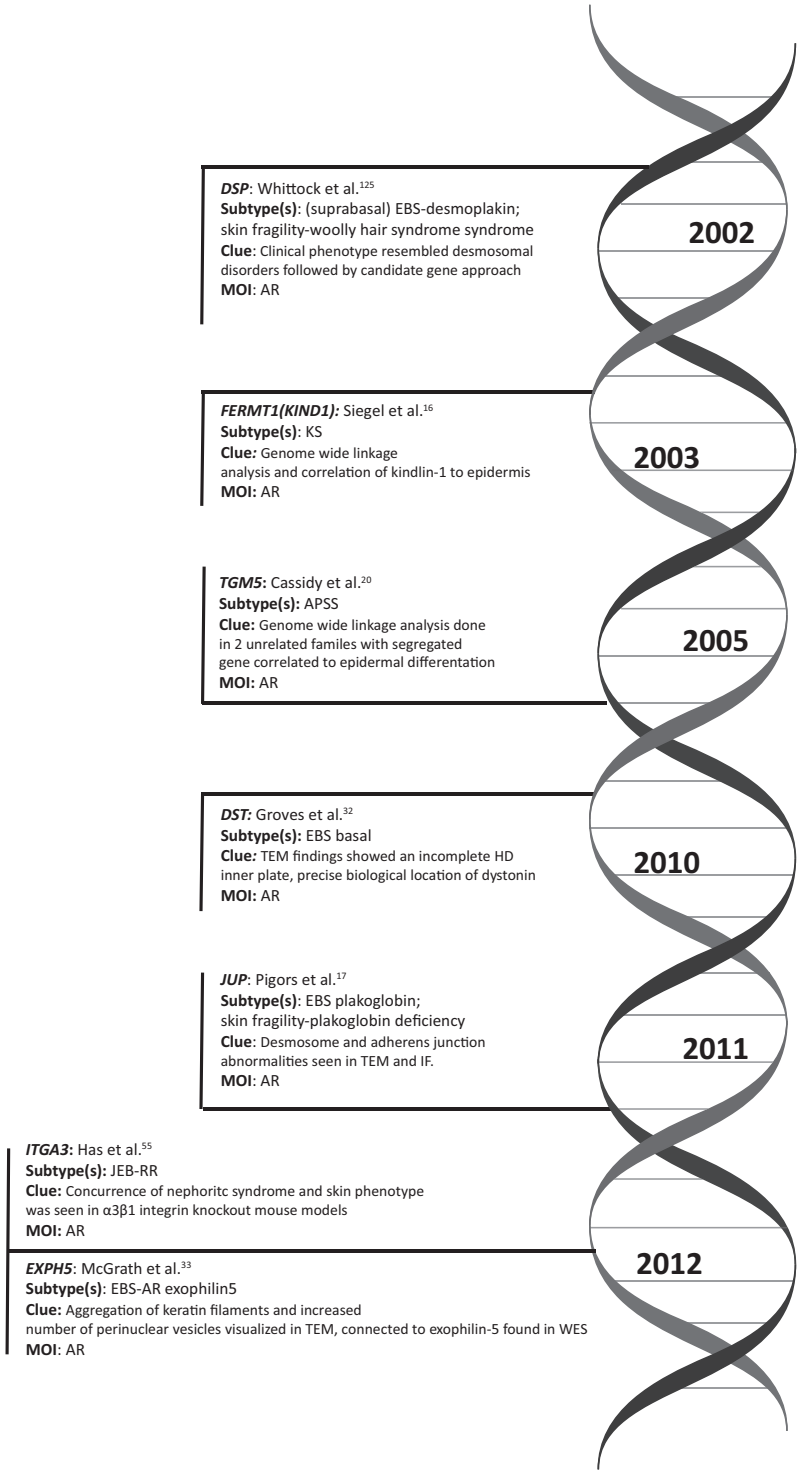

Figure 4. Timeline of gene discovery in EB

Legend Figure 4:

AR: autosomal recessive

AD: autosomal dominant

APSS: acral peeling skin syndrome

BMZ: basement membrane zone

DEB: dystrophic epidermolysis bullosa

DSP: desmoplakin gene

EBS: epidermolysis bullosa simplex

EBS-AR exophilin 5: epidermolysis bullosa autosomal recessive exophilin 5

EBS-MD: epidermolysis bullosa simplex with muscular dystrophy

EBS-Og: epidermolysis bullosa simplex Ogna

EBS-PA: epidermolysis bullosa simplex with pyloric atresia

EBS-1a: epidermolysis bullosa simplex isoform 1a

HD: hemidesmosome

IF: immunfluoresence antigen mapping

JEB: junctional epidermolysis bullosa

JEB-gen intermed: junctional epidermolysis bullosa generalized intermediate

JEB-gen sev: junctional epidermolysis bullosa generalized severe

JEB-I: junctional epidermolysis bullosa inversa

JEB-LO: junctional epidermolysis bullosa late onset

JEB-loc: junctional epidermolysis bullosa localized

JEB-PA: junctional epidermolysis bullosa with pyloric atresia

JEB-RR: junctional epidermolysis bullosa respiratory and renal

KS: kindler syndrome

MOI: mode of inheritance

TEM: transmission electron microscopy

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis was twofold. The first aim was to elucidate uncharacteristic phenotypes of EB with meticulous clinical description, supported by functional and molecular studies in order to increase the understanding of specific adhesion molecules involved in EB. The second aim was to determine the genetic background of the unresolved EB cases focussing specifically on the largest group in the Dutch registry, basal EBS.

In **Chapter 2**, we report for the first time the disease phenotype occurring from an autosomal recessive mutation in the isoform specific first exon of the 1a isoform of *PLEC*. This novel mutation found in two sisters resulted in skin-only EBS lacking signs of muscular dystrophy or cardiomyopathy. With supporting functional studies, we were able to draw conclusions as to the significance of specific plectin isoforms in the basal epidermis. **Chapter 3** continues investigation of the plectin gene with identification of a new physiologic plectin isoform discovered while studying a genetically unsolved case of EBS-MD in a young woman. The second cold case studied is presented in **Chapter 4** as an example of classic EBS-generalized severe arising from a mutation in *KRT5*. Interestingly, it was once again the intron that harboured the pathogenic mutation leading to the discovery of the first reported case of in-frame exon skipping in *KRT5*. In **Chapter 5** we investigate enamel pathology in patients with JEB due to mutations in *LAMA3*. This form of nonsyndromic amelogenesis imperfecta in the absence of skin fragility was confirmed to arise from haploinsufficiency of *LAMA3* in carriers of heterozygous functional null mutations. Comprehensive understanding of phenotypic traits in the immense clinical EB spectrum is again revisited in **Chapter 6**, this time in a subtype of DEB presenting with severe pruritus. We report two female patients affected with glycine substitution mutations in *COL7A1* presenting with unbearable pruritus on clinically healthy skin, which differs from itch seen commonly in DEB-pruriginosa patients. Finally, in **Chapter 7** our findings are discussed in the light of the newest genetic sequencing techniques. In this chapter we also suggest a comprehensive clinical approach to unsolved cases of EB.

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