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Dystrophic epidermolysis bullosa

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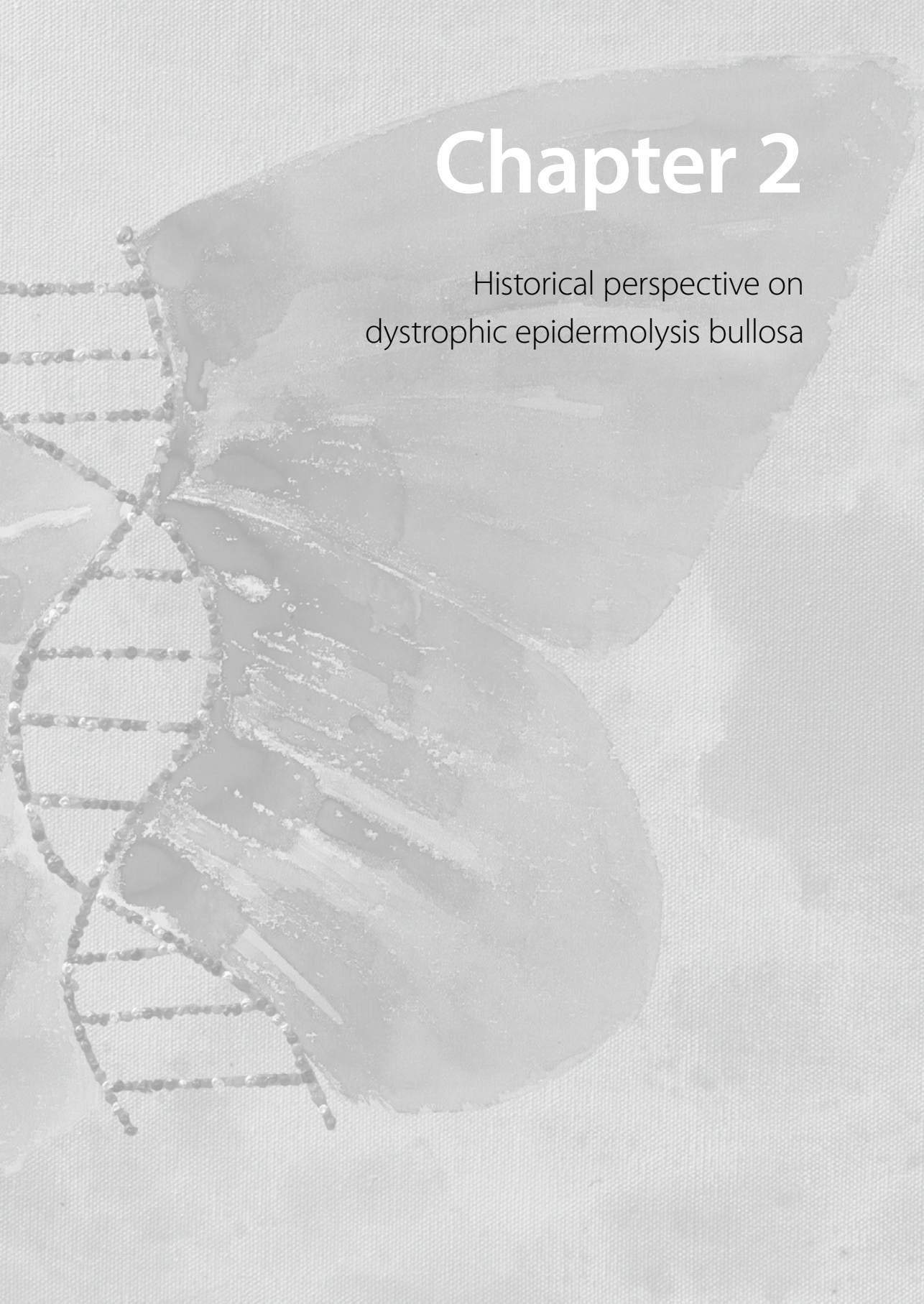
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Chapter 2

Historical perspective on
dystrophic epidermolysis bullosa



This chapter gives a historical perspective on dystrophic epidermolysis bullosa (DEB). It summarizes the key observations and discoveries that led to the current disease classification and what we understand about its genetic background. It covers the period from the first publications on DEB in the late 19th century to the discovery of the *COL7A1* gene as the disease-causing gene in the early 1990s. This review explains many of the names and concepts frequently found in the DEB literature and throughout this thesis.

THE ORIGIN OF THE TERM EPIDERMOLYSIS BULLOSA

The first reports of hereditary blistering disorders date back to the second half of the 19th century and were published mainly in the German and French literature.^{1,2} In 1870, Von Hebra was the first to report a familial blistering disorder using the term *erblichen Pemphigus* [inherited pemphigus],³ although it was Goldscheider who was the first to speak of a hereditary condition under the name “Hereditäre Neigung zur Blasenbildung” [inherited tendency of friction blisters] in 1882,⁴ followed shortly thereafter by Valentin in 1885, who used the name *Dermatitis bullosa hereditaria* [inherited bullous dermatitis].⁵ In 1886, Köbner described a dominant pedigree under the name “Hereditäre Anlage zu Blasenbildung” [inherited tendency of friction blisters] for which he coined the term *epidermolysis bullosa hereditaria*,⁶ the term that became established to describe this disorder and that is still used today.

THE IDENTIFICATION OF THE MAJOR EPIDERMOLYSIS BULLOSA TYPES

In 1898, in his “Nouvelle note sur la dermatose bulleuse héréditaire et traumatique” [Novel note on the hereditary and traumatic bullous dermatosis], Hallopeau was the first to distinguish several types of epidermolysis bullosa (EB).⁷ Earlier, in 1890 and 1896, he had already described two DEB families under the name “dermatose bulleuse congénitale avec cicatrices indélébiles, kystes épidermique et manifestations buccales” [congenital bullous dermatosis with permanent scarring, epidermal cysts and buccal manifestations]. In his 1898 note, summarizing the work of his own group and other reported cases, Hallopeau recognized that most of the bullous phenotypes described under different names in fact represented different forms of the same disease, the “hereditary and traumatic bullous dermatoses”. He separated EB simplex (EBS) (*forme bulleuse simple*) from DEB (*forme bulleuse dystrophique*) and concluded that there also seemed to be a *forme fruste* of the disorder. Notably, after this publication it took nearly 40 years before Herlitz described the third major EB group. He reported a case of lethal EB that he believed was different from EBS and DEB and that he named *EB letalis*;⁸ this is the EB type currently known as the Herlitz type of junctional EB (JEB-H).^{9,10} Kindler syndrome, the fourth major EB subtype, was first described in 1954 by Kindler as “Congenital poikiloderma with traumatic bulla formation and progressive cutaneous atrophy”.¹¹

RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA

Hallopeau gave detailed clinical and histological descriptions of the three phenotypes he had recognized. He summarized the clinical phenotype of DEB as the occurrence of bullous eruptions caused by mechanical trauma, followed by “trophic disturbances of varied and complex

nature that consisted of nail dystrophy, scarring atrophy of the integument and epidermal milium cysts". The nails were either absent or dystrophic, resembling "the claws or beaks of parrots" which he called onychogryphosis; the scarring atrophy was mainly present on the knees and elbows, but could also be seen over the rest of the body and intra-orally; the milia occurred after the blisters had disappeared, mainly on the hands, feet, elbows and pinnae, vanishing spontaneously within several weeks. Histologically, he reported scar tissue in the dermis. For several years after Hallopeau's work had appeared, DEB was reserved for forms of EB with dystrophic nails or a post-blistering scarring atrophy.²

In 1921, Siemens meticulously described the disease in a 12-year old boy and carefully reviewed the literature available to him in his paper "Zur Klinik, Histologie und Ätiologie der sog. Epidermolysis bullosa traumatica (Bullosis mechanica), mit klinisch-experimentellen Studien über die Erzeugung von Reibungsblasen" [On the clinical aspects, histology and etiology of the so-called epidermolysis bullosa traumatica (mechanical bullosa) with clinical-experimental studies on the development of friction blisters].¹² The boy was the sixth child of unrelated healthy parents. Skin blistering had started 5 weeks after birth, occurred either spontaneously or after mild trauma, and was restricted to his hands, feet, elbows, knees and lower legs. There was no difference in severity between summer and winter. He was able to walk bare-foot in the summer and do handwork without blistering. He had atrophic, cigarette paper-like scars on the elbows, knees and ankles, and small atrophic scars on his extremities, shoulders and back. He had lost most of his finger nails and all of his toe nails, and had milia on the hands. There was blistering of the oral mucosa. In addition, he had numerous hyperkeratotic, verrucae planae-like lesions on the dorsal aspect of the hands, fingers, lower legs, feet, and face. Histological examination of four skin biopsies revealed detachment of the complete epidermis from the dermis. The family history was negative for blistering or other cutaneous disorders and Siemens made a diagnosis of recessive DEB. Based on current knowledge, the most likely diagnosis in this boy would now be either mild RDEB (RDEB-O) or *de novo* DDEB, perhaps of pretibial subtype (Table 1).

From his review, Siemens concluded that there seemed to be a correlation between the level of blistering in the skin and the clinical phenotype: in the 11 patients with DEB, the disorder was associated with deep, 'epidermolytic' blistering, whereas in three EBS patients there was more superficial, acantholytic or keratinolytic blistering. The mode of inheritance in the DEB patients seemed to be recessive in all of them, whereas it was dominant in the EBS cases. Of note, Siemens did not cite the work of Hallopeau, who had reported that there were affected persons in multiple generations in almost all families, hinting at a dominant mode of inheritance.⁷

Table 1. 2007 DEB consensus classification.¹⁰

Phenotype ^a	
DDEB	
DDEB, generalized	DDEB-gen
<i>DDEB, acral</i>	DDEB-ac
<i>DDEB, pretibial</i>	DDEB-Pt
<i>DDEB, pruriginosa</i>	DDEB-Pr
<i>DDEB, nails only</i>	DDEB-na
<i>DDEB, bullous dermolysis of the newborn</i>	DDEB-BDN
RDEB	
RDEB, severe generalized	RDEB-sev gen
RDEB, generalized other	RDEB-O
<i>RDEB, acral</i>	RDEB-ac
<i>RDEB, inversa</i>	RDEB-I
<i>RDEB, pretibial</i>	RDEB-Pt
<i>RDEB, pruriginosa</i>	RDEB-Pr
<i>RDEB, centripetalis</i>	RDEB-Ce
<i>RDEB, bullous dermolysis of the newborn</i>	RDEB-BDN

^a DDEB, dominant dystrophic epidermolysis bullosa; RDEB, recessive dystrophic epidermolysis bullosa. Rare variants are given in *italics*.

DOMINANT DYSTROPHIC EPIDERMOLYSIS BULLOSA

Hoffmann, in 1926, published the first autosomal dominant DEB pedigree.¹³ In 1928, Pasini and Maschkilleisson independently reported that some patients within DDEB families displayed ivory-white papuloid lesions, referred to as “elevures albo-papuloïdes” [albo-papuloid elevations] by Pasini.^{14,15} Cockayne, in 1933, published a large series of DDEB families,¹⁶ and in 1942 Touraine showed that these patients frequently developed ‘hyperplastic’ features, like dystrophic nails.¹⁷

THE CLASSIFICATION OF DYSTROPHIC EPIDERMOLYSIS BULLOSA

Touraine named his DDEB “hyperplasique” with albo-papuloid lesions the *forme albo-papuloïde de Pasini* (the DDEB, Pasini variant) and Maschkilleisson’s work was overlooked. Later, it was proposed to call DDEB without the albo-papuloid lesions the *DDEB, Cockayne-Touraine* form, ignoring Hoffmann’s publication.¹⁸ Of note, in contrast to Hallopeau who classified DEB as a benign disorder,⁷ Touraine classified RDEB as “maligne ou léthale” [malignant or lethal].¹⁹

In 1966, Schnyder proposed the term *RDEB, Hallopeau-Siemens* to distinguish RDEB from DDEB.²⁰ This term was supposed to include all RDEB variants. Gedde-Dahl was one of the first to further split the group of RDEB into separate subtypes: in 1971, he described the peculiar *inversa* type and milder, non-lethal subtypes of RDEB,²¹ which led him and others to introduce a separate name for the *RDEB, inversa* subtype, in order to distinguish this clinically related, but distinct entity from the generalized Hallopeau-Siemens subtypes.²² In the course of time, the term RDEB, Hallopeau-Siemens became more and more a synonym for the most severe

BOX 1 – The classification of epidermolysis bullosa

Schnyder and Anton-Lamprecht were the first to work on a clinical classification for EB in general,³² although the first international consensus classification, which was mainly drawn up by US researchers and clinicians, was only published in 1991.²³ It has since been revised twice^{10,24} and yet another EB consensus meeting has just taken place (in spring 2013). Initially, eponyms were widely used to describe EB subtypes, usually the names of the doctors accredited with first describing the condition. From the first (1991) to the second (2000) to the current (2007) consensus classifications, the tendency has been to replace more and more of these eponyms. Currently, most subtypes have names based on their primary clinical, molecular or genetic characteristic. For instance, in the latest consensus the 'RDEB, Hallopeau-Siemens' and 'RDEB, non-Hallopeau-Siemens' were replaced by 'RDEB, severe generalized' and 'RDEB, generalized other', respectively, "to allow more immediate visualization of this particular subtype of EB". Only Dowling-Meara EBS, Herlitz and non-Herlitz JEB subtypes, and Kindler syndrome, have been able to retain their eponyms, while the EBS, Onga subtype is still named after the Norwegian village where the *PLEC* founder mutation arose.^{21,33} The reasons for keeping some eponyms while eliminating others are not always clear.

Since the 2007 consensus meeting, ongoing clinical studies and novel gene discovery methods, like next-generation sequencing,³⁴⁻³⁶ have further expanded the spectrum of EB subtypes and the plethora of EB-associated genes and pathogenic mechanisms. Since 2007, nine EB subtypes have been reported as novel or refined, and five new genes have been implicated in EB, bringing the total number of EB genes to 21 (Table 2). These findings can be summarized in the nine following points:

- 1) A novel, lethal syndrome of EB, interstitial lung disease, and congenital nephrotic syndrome (MIM 614748) due to biallelic mutations in the gene for the integrin- $\alpha 3$ chain, *ITGA3*, was reported by Has *et al.*³⁷ This focal adhesionopathy was reported to have multiple levels of skin blistering.
- 2) By applying whole-exome sequencing, McGrath *et al.* identified homozygous frame-shift mutations in *EXPH5*, encoding Slac-2b (also known as exophilin-5), a Rab27B GTPase effector protein presumed to be involved in intracellular vesicle trafficking. The mutations were found in three affected siblings with mild skin blistering and generalized scale-crusts resulting from trauma, and diffuse pigmentary mottling over the trunk.³⁸ There was widening of the intercellular spaces and intracellular intermediate filament aggregation in the lower epidermis, which suggested this form of skin fragility can be regarded as a novel subtype of recessive basal EBS.
- 3) Pigors *et al.* reported a novel 'lethal congenital EB' type (LCEB) due to mutations in the desmosomal protein plakoglobin encoding gene *JUP* that resulted in complete absence of plakoglobin.³⁹ LCEB can be considered a novel form of suprabasal EBS, with resemblance to the lethal acantholytic EB (MIM 609638) form of suprabasal EBS, which is caused by specific mutations in another desmosomal protein gene, *DSP* encoding desmoplakin.⁴⁰

4) In two unrelated families with autosomal recessive EBS (AR-EBS), homozygous nonsense mutations were identified in the coiled-coil rod domain of the *DST* gene, encoding BP230/BPAG-1e.^{41,42}

5) Yuen *et al.* identified the cause of 'junctional EB of late-onset' (JEB-lo), which turned out to be biallelic mutations in the *COL17A1* gene encoding type XVII collagen, the same gene involved in several other JEB subtypes.⁴³

6) Kiritsi *et al.* showed that acral peeling skin syndrome (APSS, MIM 609796) due to biallelic mutations in the transglutaminase-5 gene *TGM5* can mimic EBS in young patients, raising the question whether APSS should be considered to be a form of suprabasal EBS and thus an EB subtype.⁴⁴

7) In five patients with skin fragility, woolly hair, and palmoplantar keratoderma from two unrelated families, Cabral *et al.* identified novel homozygous mutations in *JUP* leading to the presence of a structurally altered protein.⁴⁵ The patients' phenotypes resembled Naxos disease (MIM 601214) due to homozygous *JUP* mutations leading to a truncated protein,⁴⁶ but without the cardiac phenotype. It should, however, be noted that the oldest patient was only 14 years at latest follow-up and the development of cardiomyopathy later in life cannot be excluded. The phenotype had many similarities to that caused by biallelic mutations in other desmosomal genes: compound heterozygous missense/PTC mutations in *DSP* (Carvajal-like without cardiomyopathy, MIM 607655)^{47,48} and loss-of-function mutations in the plakophilin-1 encoding gene *PKP1*.^{49,50} As the phenotype caused by *PKP1* mutations, called ectodermal dysplasia/skin fragility or McGrath syndrome (MIM 604536), is classified as a form of suprabasal EBS ('plakophilin-1 deficiency'), the similar phenotypes caused by *DSP* and *JUP* mutations could be regarded as novel forms of suprabasal EBS too.

8) Besides EBS with muscular dystrophy, EBS with pyloric atresia, and EBS-Ogna, Bolling *et al.* showed that non-syndromic localized EBS can also be caused by heterozygous *PLEC* missense mutations.⁵¹

9) Bolling *et al.* reported a patient with EBS with muscular dystrophy who developed dilated cardiomyopathy, diagnosed at age 30 years, expanding the phenotypic spectrum of EBS associated with *PLEC* mutations.⁵² The patient was compound heterozygous for a missense mutation in the first α -helical spectrin repeat segment and a nonsense mutation in the coiled-coil rod domain of the *PLEC* gene.

subtype of RDEB, whereas the term *RDEB, mitis* [Latin: mild] was introduced to cover the milder RDEB subtypes, a term that was later replaced by 'non-Hallopeau-Siemens'.^{23,24} As can be seen from the summaries of their work, neither Hallopeau nor Siemens described patients with the most severe RDEB phenotype, at least not in their 1898 and 1921 papers. The decision of the latest EB consensus meeting in 2007 to replace the eponym Hallopeau-Siemens by RDEB, severe generalized has finally corrected this misnomer.

The current 2007 consensus classification for DEB is shown in Table 1. In addition to the common DEB variants, the 2007 classification recognizes several rare subtypes, one of which is the RDEB, inversa subtype. Another rare one is the *pruriginosa* subtype that was described first by McGrath *et al.* in 1994.²⁵ It was later shown that the *pruriginosa* subtype occurs mainly on a DDEB background, but can occasionally also occur in RDEB patients.²⁶ In 1966, Bart *et al.* reported on the congenital localized absence of skin (CLAS) in what later appeared to be a DDEB family;^{27,28} this entity became known as *Bart's syndrome*.²⁹ As it was later shown that

Table 2. Updated EB consensus classification (modified after ref. 10).

Major EB type	Minor EB types	Level of skin split	Genes involved	Targeted proteins	References ^a
EB simplex (EBS)	Suprabasal EBS	Corneal cell layer	<i>TGM5</i>	Transglutaminase-5	53
			<i>CSTA</i>	Cystatin A	54
			<i>CDNS</i>	Corneodesmosin	55
		Spinous cell layer	<i>PKP1</i>	Plakophilin-1	
			<i>DSP</i>	Desmoplakin	
	Basal EBS	Basal cell layer	<i>JUP</i>	Plakoglobin	39
			<i>KRT5</i>	Keratin 5	
			<i>KRT14</i>	Keratin 14	
			<i>PLEC</i>	Plectin	
			<i>ITGA6</i>	$\alpha 6$ -chain of $\alpha 6\beta 4$ integrin	
		<i>ITGB4</i>	$\beta 4$ -chain of $\alpha 6\beta 4$ integrin		
		<i>DST</i>	Bullous pemphigoid 230	41	
		<i>EXPH5</i>	Exophilin-5	38	
Junctional EB (JEB)	JEB-Herlitz	Intra-lamina lucida (‘lamina lucidolytic’)	<i>LAMA3</i>	$\alpha 3$ -chain of laminin-332	
			<i>LAMB3</i>	$\beta 3$ -chain of laminin-332	
			<i>LAMC2</i>	$\gamma 2$ -chain of laminin-332	
	JEB-other		<i>LAMA3</i>	$\alpha 3$ -chain of laminin-332	
			<i>LAMB3</i>	$\beta 3$ -chain of laminin-332	
			<i>LAMC2</i>	$\gamma 2$ -chain of laminin-332	
			<i>COL17A1</i>	Type XVII collagen (Bullous pemphigoid 180)	
			<i>ITGA6</i>	$\alpha 6$ -chain of $\alpha 6\beta 4$ integrin	
			<i>ITGB4</i>	$\beta 4$ -chain of $\alpha 6\beta 4$ integrin	
			<i>CD151</i>	Tetraspanin CD151	56
Dystrophic EB (DEB)	Recessive DEB	Sub-lamina densa (‘dermolytic’)	<i>COL7A1</i>	Type VII collagen	
	Dominant DEB		<i>COL7A1</i>	Type VII collagen	
Kindler syndrome (KS)	–	Mixed	<i>FERMT1 (KIND1)</i>	FFH-1 (Kindlin-1)	
EB, interstitial lung disease, congenital nephrotic syndrome	–		<i>ITGA3</i>	$\alpha 3$ -chain of $\alpha 3\beta 1$ integrin	37

^a References given only for subtypes or genes not included in the 2007 consensus classification.

CLAS can occur in any of the major EB types, it was recommended to abandon the term Bart’s syndrome in favor of CLAS.^{24,30} Another rare subtype that can occur in both RDEB and DDEB is called *bullous dermolysis of the newborn* (BDN). It was first described by Hashimoto *et al.* in 1985 under the name “transient bullous dermolysis of the newborn” and refers to a transient neonatal blistering episode that gradually improves over several weeks to months.³¹

THE DISCOVERY OF THE ANCHORING FIBRIL AND TYPE VII COLLAGEN

The understanding of blistering levels in the different EB types and the first clues on the pathophysiology increased exponentially with the introduction of electron microscope (EM)

studies.^{57,58} By performing EM on skin sections of different organisms, Palade *et al.* discovered a specific dermal fibrillar structure that they called the “anchoring fibril” (AF) in 1965.⁵⁹ In the following years, AFs were also recognized in human skin as connecting dermal collagen bundles to the lamina densa of the basement membrane zone (BMZ).^{60,61} EM studies performed in the 1970s by Hashimoto, Gedde-Dahl Jr, Anton-Lamprecht, Schnyder, and others demonstrated unequivocally that the level of blistering in DEB was below the BMZ. This subsequently led to the identification of defects of varying degrees in the number and ultrastructural morphology of AFs as the cause of the dermal-epidermal separation in the different subtypes of DEB, first in DDEB⁶²⁻⁶⁴ and soon after also in RDEB.^{22,65} The quantitative differences in AF density along the BMZ were later confirmed by Tidman and Eady using semi-quantitative methods.^{66,67} They demonstrated complete absence of AFs in RDEB, severe generalized skin samples and significantly reduced numbers of hypoplastic AFs in both DDEB and localized RDEB skin samples.

Studies using different enzymes against epidermal and dermal components revealed marked alteration of the BMZ and AFs when skin sections were treated with collagenase, nowadays called matrix metalloproteinase-1 (MMP-1),⁶⁸ which, during the 1960s and 1970s, led several authors to hypothesize that AFs were collagenous structures.^{69,70} In 1983, Bentz *et al.* were the first to isolate the type VII collagen molecule, by then called ‘long-chain collagen’ because of its size, and proposed to call this new collagen ‘type VII collagen’.⁷¹ In the following years, Burgeson’s group was able to confirm the prevailing idea that the AF was a collagenous structure and to prove that type VII collagen was its major, if not sole, component.⁷²⁻⁷⁴ Moreover, this group contributed greatly to dissecting the structure of the pro- α (VII) procollagen peptide, its assembly into trimers and subsequent dimerization, and its function.⁷⁵⁻⁷⁷ Of note, before 1991 the orientation of type VII collagen was considered to be reversed, i.e. the NC1 domain was thought to be the carboxyl-terminal and the NC2 domain the amino-terminal.^{73,78} With the identification of the *COL7A1* mRNA and cDNA sequences in the early 1990s, the correct orientation of both NC domains was revealed.⁷⁹⁻⁸¹

The next major improvement in clinical diagnostics and understanding of the pathophysiology of DEB came in 1981 with the introduction of the immunofluorescent (IF) antigen mapping technique by Hintner *et al.*⁸² In this indirect IF technique, monoclonal or polyclonal antibodies are used to recognize the different components of the hemidesmosome adhesion complex. In 1983, Goldsmith and Briggaman were the first to develop AF-specific monoclonal antibodies and to demonstrate a complete absence of AFs by IF in RDEB, severe generalized skin.⁸³ The LH7:2 monoclonal antibody against AFs was introduced in disease diagnosis by Heagerty *et al.* in 1986,⁸⁴ and this antibody is still the gold standard in diagnostic IF antigen mapping. In 1987, Leigh *et al.* demonstrated that it is type VII collagen that houses the epitope for LH7:2,⁸⁵ which was further refined in 1994 by Tanaka *et al.*, who showed that the LH7:2 epitope lies within the NC1 domain of type VII collagen.⁸⁶

DISSECTING THE PATHOPHYSIOLOGY OF DEB

In 1962, Pearson, based on his observations of marked degenerative changes of dermal collagens below the BMZ, speculated that the pathophysiologic mechanism underlying DEB involved either a collagenolytic process or the production of abnormal collagens in DEB skin.⁵⁸ For the next two decades, a debate continued between researchers about the precise pathophysiology of DEB. Eisen, in 1969, observed a six-fold increase in collagenase activity in cultured cells from blistered skin of DEB patients, which led him to suggest that over-production of skin collagenases was the cause of the skin blistering and the dermal collagenolysis observed by Pearson.⁸⁷ In 1972, Lazarus also found increased levels of collagenase in non-bullous, erythematous, scarred skin of two DEB patients, which seemed to fully agree with Eisen's conclusions.⁸⁸ However, Lazarus did not detect increased levels of collagenase in apparently normal skin samples from seven DEB patients. Contrary to Pearson and Eisen, Lazarus thus concluded that, although the focal "increased amounts of collagenase might perpetuate the blistering and scarring by degrading the dermal connective tissue and thus weakening the mechanical integrity of the skin [...] the primary pathophysiological event in this disease, however, is probably a genetically determined defect in the structure of the papillary dermis". On the one hand, Briggaman and Wheeler, based on their epidermal/dermal recombinant graft experiments and the observation of absent AFs in the skin of two newborns with RDEB, Hallopeau-Siemens, strongly supported Lazarus' idea of a primary AF formation defect.⁶⁵ On the other hand, based on their EM studies on skin biopsy sections from eight patients with generalized RDEB and nine with localized Hallopeau-Siemens RDEB, as well as another five with RDEB, inversa, Hashimoto *et al.* again favored the AF degradation theory.²² Since they found markedly reduced numbers of AFs (but not totally absent) in all but three patients and normal AFs in three other patients, Hashimoto *et al.* speculated: "It is thought that secondary degradation of anchoring fibrils and/or collagen fibrils plays an important role in blistering mechanism in the Hallopeau-Siemens and inverse types of recessive dystrophic epidermolysis bullosa, whereas a primary aplasia of anchoring fibrils as causative defect has been out ruled." Around the year 1980, the AF degradation theory was further supported by experiments by Bauer and Eisen, Valle and Bauer, and Stricklin *et al.* who all showed that RDEB fibroblasts in culture over-expressed collagenase,^{89,90} although DDEB fibroblasts did not.⁹¹ Moreover, Bauer *et al.* showed that systemic treatment of RDEB patients with phenytoin reduced the level of collagenase and the number of blisters.⁹²

THE DISCOVERY OF COL7A1 AS THE DEB GENE

With the continuous unraveling of the structure of AFs and the identification of type VII collagen as their major constituent from the early 1980s onwards,^{71,72,75,78} combined with the identification of type VII collagen retention in basal keratinocytes of some DEB patients,^{93,94} there was an

increasing understanding that the gene encoding type VII collagen was the primary candidate gene for DEB. The possible pathophysiologic link between collagenases and DEB, however, continued to be studied into the early 1990s.^{95,96} It took until 1991, with the introduction of molecular linkage techniques, before the debate on the etiology of DEB could be settled in favor of the primary defect being in AF production. Hovnanian *et al.* excluded linkage between the collagenase *MMP1* gene locus and the RDEB phenotype in one family in 1991,⁹⁷ and in the same year, the *COL7A1* gene was identified and located on chromosome 3p21 by Parente *et al.*⁷⁸ At the same time, Ryyananen *et al.* demonstrated genetic linkage of the *COL7A1* gene to the DDEB phenotype in a large pedigree.⁹⁸ In 1992, Colombi *et al.* further excluded linkage of DEB to several *MMP* genes and the fibronectin *FN* gene,⁹⁹ whereas genetic linkage between the *COL7A1* gene and other DDEB families was confirmed by Al-Imara *et al.*, Gruis *et al.*, and again Ryyananen *et al.*¹⁰⁰⁻¹⁰² Moreover, in 1992, Hovnanian *et al.* also demonstrated linkage of the *COL7A1* gene to RDEB.¹⁰³ The ultimate proof that *COL7A1* is the causative gene in DEB followed rapidly with the identification of pathogenic mutations in the gene, first in RDEB families^{104,105} and slightly later in DDEB families.¹⁰⁶ In 1994, Christiano *et al.* dissected the genomic structure of *COL7A1* and published the first complete cDNA sequence of the gene and the deduced amino acid sequence of type VII collagen.^{107,108}

PRENATAL DIAGNOSIS AND PRE-IMPLANTATION GENETIC DIAGNOSIS IN DEB

Because of the possible severity of RDEB, the wish for prenatal diagnosis has been expressed by parents for many years and has been considered ethically justified by the medical society.¹⁰⁹ The introduction of diagnostic EM and IF techniques offered the first possibilities for prenatal diagnosis. In 1980, the first prenatal diagnosis performed for any type of EB was described by Rodeck *et al.*¹¹⁰ using fetoscopy and fetal skin biopsy,^{111,112} followed by EM analysis of fetal skin biopsy sections, Rodeck *et al.* were able to determine separation between epidermis and dermis in the lamina lucida, i.e. the junctional level, indicating that the fetus at risk was actually affected with 'EB letalis' (JEB, Herlitz). Using the same procedure, Anton-Lamprecht *et al.* performed the first prenatal diagnosis for RDEB, severe generalized in 1981.¹¹³ Heagerty *et al.* described the possibility of rapid prenatal diagnosis for RDEB in 1986 by indirect IF using monoclonal antibody LH7:2 instead of the more time-consuming EM.¹¹⁴ These methods required a fetal skin biopsy to be taken between 18-20 weeks gestation, which carried a risk for the fetus.^{112,115}

With the introduction of molecular diagnostic techniques like linkage analysis, restriction enzyme digestion and direct mutation analysis in the early 1990s, the methods for prenatal diagnosis shifted towards DNA-based prenatal diagnostic techniques performed on chorion villi, amniotic fluid cells, or blood samples obtained from the peri-umbilical vein.¹¹⁶⁻¹¹⁸ These methods do not require a risky fetal skin biopsy, but are still invasive procedures that carry an

approximate 0.5-1% risk of inducing labor and fetal loss. An alternative for invasive prenatal diagnosis is pre-implantation genetic diagnosis (PGD). It was first reported in EB in a family with JEB, Herlitz in 2000,¹¹⁹ and in 2006, Fassihi *et al.* demonstrated that PGD is also feasible in RDEB, severe generalized.¹²⁰ As an alternative to mutation-specific PGD protocols, Fassihi *et al.* developed a pre-implantation diagnostic haplotyping strategy that they successfully applied to a JEB, Herlitz family in 2010.¹²¹

CONCLUSION

This chapter has summarized the major historical landmarks and achievements in DEB research, from the very first description of the disease to the discovery of its causative gene. It shows how much work needed to be done to reveal the cause and pathogenesis of DEB, to reach consensus about the classification of its subtypes, and to enable correct disease diagnosis and prenatal testing. And it highlights that there is still much to do! Since the discovery of the *COL7A1* gene, the focus of DEB research has gradually shifted towards investigating potential therapeutic options. This falls outside the scope of this historical chapter, but many of these important scientific achievements are mentioned in the other chapters of this thesis. The rest lies in the future.

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