Continuing Medical Education

LABORATORY DIAGNOSIS OF EPIDERMOLYSIS BULLOSA

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Epidermolysis bullosa (EB) is a group of inherited disorders that manifest with blistering of the skin and mucous membrane after trivial trauma. On the basis of the level of cleavage, EB has been categorised into 3 major types: epidermolysis bullosa simplex in which an epidermal cleavage develops at the level of basal keratinocytes, junctional epidermolysis bullosa which demonstrates a cleavage within the basement membrane and dystrophic epidermolysis bullosa with a cleavage below the level of the basement membrane.[1] Each major type of EB has several subtypes.[2] Accurate classification of EB into types and subtypes is not possible based on clinical examination or on routine histopathology and till recently, electron microscopy was required to establish the diagnosis. Lately, major advances have been made in understanding the molecular basis of EB and this has led to the development of immunoreagents to map antigens in the basement membrane zone and molecular techniques to identify the underlying gene defect. This review focusses on the laboratory methods to diagnose and classify the types and subtypes of EB.

Need for accurate subclassification

In a disease for which no effective treatment is available, classification may appear to be an unavailing academic exercise. In the usual situation, this is probably true. Fortunately, it is now possible to make a prenatal diagnosis with a very high degree of accuracy by looking for the genetic defect in chorionic villus samples. However, defects in a number of different genes are responsible for the different types of EB and it is wasteful and expensive to screen for all the defects in each case. Accurate classification of the affected child by non-molecular methods narrows down the search for the genetic defect in the affected child and during next pregnancy.

Classification is also essential to study the epidemiology and prognosis of the disease and its various subtypes. Valuable clinical lessons have been learnt from the epidermolysis bullosa registry including of clinically differentiating the various types based on signs such as scarring, milia and syndactyly and the reduced life expectancy in dystrophic EB because of an increased risk of squamous cell carcinoma and malignant melanoma.[2]

Collection, preservation and transport of samples

The major techniques used are routine histopathology, immunohistopathology or antigen mapping, electron microscopy and mutation based diagnosis. A skin biopsy is required for the first 3 investigations and blood samples, buccal swabs or skin biopsy for cell cultures are required for mutation based diagnosis.

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Multiple skin biopsies need to be taken for the complete typing and subtyping of EB. Four skin biopsies are required for a complete work up: one each for histopathology, immunohistopathology (indirect immunofluorescence), electron microscopy and cell culture. Ideally, 4 different biopsies should be taken rather than splitting one large biopsy into 4 pieces to prevent crushing and alteration of tissue architecture. It is best to biopsy a fresh blister, preferably less than 12 hours old. Alternatively, a cleavage can be induced by gently rubbing the clinically normal skin with a pencil eraser and biopsy taken from this area.

For routine histopathology, 10% buffered formalin is an adequate fixative. Glutaraldehyde is used as a fixative for electron microscopy. Michel's medium can be used for immunohistopathology. Biopsies for cell culture may be transported in sterile normal saline if they are likely to reach the laboratory in a few hours. If the transit time is longer, a transport cell culture medium should be used. Importantly, biopsies for cell culture should never be placed in formalin.

Samples of peripheral blood for molecular studies should be anticoagulated with EDTA.

Principles of histopathological and electron microscopic examination of the skin are familiar to dermatologists and do not require reiteration.

Antigen mapping or immunohistopathology is based on the ability to stain antigens whose ultrastructural localisation is known.[3] The relation of the cleavage in a given biopsy to these antigens can be studied to identify the type and subtype of EB. In addition, the presence of certain peculiar subtypes or the absence of an antigen that is normally seen aids in the diagnosis.

Mutation based diagnosis is performed by using cell cultures from the skin of the proband.[4] Keratinocyte cultures and fibroblast cultures can provide adequate DNA, mRNA and other biochemical material for the detection of abnormal protein (Western blotting), decreased or absent mRNA levels (Northern blotting) and DNA sequencing. The latter can reveal the mutant allele and the mutation leading to the disease. Alternatively a restriction fragment length polymorphism (RELP) technique on the DNA derived from lymphocytes or buccal swabs can reveal a mutant allele. The detailed methodology for the molecular diagnosis of EB can be found in Ref.3.

Classifying EB

EB has 3 major types: EB simplex, junctional EB and dystrophic EB and each major type has many subtypes. The major types can be identified by electron microscopy and immunohistopathology.

Histopathology

On light microscopic examination of H & E stained skin biopsies, the appearance of all other 3 types is similar with a split that usually appears to be subepidermal. In EB simplex, the vacuoles seen in tha basal cells adjacent to the blister cavity gives a clue that the split is at the level of basal keratinocytes.[3] In sections stained with PAS technique, the PAS positive basement membrane is found on the floor of the blister in EB simplex while it is found on the roof in dystrophic EB. Thus routine histopathology may identify EB simplex but does not differentiate between junctional and dystrophic types. Of the subtypes of EB, EB simplex superficialis is the only one which can be identified confidently on H & E because of the distinctive location of the split just beneath the stratum corneum.[5]

Electron microscopy

Electron microscopy can localize the site of the split and continues to be the gold standard for the diagnosis of EB.[3] In EB simplex, the split occurs through basal keratinocytes. Degenerative cytolytic changes lead to a cleavage between the nucleus of the basal cells and the basement membrane. Early

cytolysis is indicated by the presence of cytoplasmic vacuoles. Junctional EB shows a split in the lamina lucida. In Herlitz variants the hemidesmosomes usually appear dysplastic and reduced in number and may lack subbasal cell dense plaque. Dystrophic EB shows a split below the lamina densa in the superficial papillary dermis.

Table I. Immunohistopathological typing of EB

Type	Localization	Collagen IV/Pankeratin	Collagen IV/BP antigen
EB simplex	Roof	Pankeratin	-
	Floor	Collagen IV (Continuous /segmental)	Collagen IV, 3P antigen
Junctional	Roof	Pankeratin	BP
	Floor	Collagen IV	Collagen IV, BP antigen (Spotty)
Dystrophic EB	Roof	Pankeratin, Collagen IV	Collagen IV, BP antigen
	Floor	-	-

Immunohistopathology

A combination of antibodies against components of the basement membrane zone can also be used to identify the type of EB. Two such combination are commonly used: antibody to collagen IV in conjunction with a pankeratin antibody[6] and antibodies to bullous pemphigoid antigen (BP) and collagen IV[7] (Table I). With the first combination in EB simplex the immunoperoxidase staining for collagen IV is seen linearly along the floor of the blister while the roof is stained with pankeratin antibody. In addition, there is a continuous or segmented staining for keratins on the floor along the basement membrane. In junctional EB too, the staining for collagen IV is present at the floor, while pankeratin antibody stains the roof. However, there is no band of keratin along the floor. In dystrophic EB, the staining for both antigens are on the roof of the blister.

With a combination of collagen IV and BP antigen, both antigens are demonstrated on the floor in EB simplex. In junctional EB, collagen IV localises to the floor while BP antigen is mainly detected on the roof but also has a spotty distribution on the floor of blister. In dystrophic EB, both antigens are seen on the roof.

Subtyping EB

Each type of EB has many major and minor subtypes (Table II). The classification of inherited EB and comprehensive algorithmic approaches using clinical and laboratory criteria to subtype EB are reviewed in detail in Ref 2. The laboratory methods for subtyping are discussed below.

Epidermolysis bullosa simplex

EB simplex manifests trauma induced blisters which heal without scarring. The revised classification system for inherited EB has subclassified EB simplex into 4 common and 3 rare variants, most of which have a dominant inheritance.[2] The 4 common variants are the Weber - Cockayne subtype, the Kobner subtype, Dowling - Meara subtype with grouped blisters and EB simplex with mottled pigmentation, autosomal recessive EB simplex without muscular dystrophy and EB simplex superficialis.

Electron microscopy

Three subtypes of EB simplex can be recognised by electronmicroscopy. Characteristic intracytoplasmic clumping of tonofilaments in the basal cells is noted in the Dowling - Meara variant.[8] In EB simplex with muscular dystrophy, the keratin filaments are dissociated from the hemidesmosomes. In EB simplex superficialis, the blister is found beneath the stratum corneum.

Molecular pathology

EB simplex subtypes are all associated with mutations of genes encoding keratins 5 (KRT 5) and 14 (KRT 14).[9,10] Keratins have a central α-helical domain flanked by globular domains at either ends. Evolutionary analysis has shown that the region between the - helical and globular domains contain highly conserved sequences. Mutations that alter

these highly conserved regions of keratin 5 or 14 produce the most severe phenotypic effects, such as that seen in Dowling - Meara subtype,[11,12] whereas mutations that involve other areas of keratin 5 and 14 produce a milder disease. This report suggests that the severity of the disease in EBS is determined by the location of the mutation in the keratin gene. However, Sorenson et al[10] state that the phenotype correlates with the type of amino acid substitution rather than with the location of the mutation. The genetic defect in EB simplex with mottled pigmentation involves the globular head domains of keratin.[13,14]

Table II. Types and subtypes of EB

Major EB type	Major EB subtype	Minor EB subtype
EB simplex	EB simplex Weber-Cockayne EB simplex Kobner EB simplex Dowling-Meara EB simplex Muscular dystrophy	EB simplex Mottled pigmentation EB simplex Autosomal recessive EB simplex Superficialis
Junctional EB	Junctional EB-Herlitz Junctional EB-Non-Herlitz Junctional EB-Pyloric atresia	Junctional EB-Inversa Junctional EB-Late onset
Dystrophic EB	Dominant dystrophic EB Recessive dystrophic EB-Hallopeau - Siemens Recessive dystrophic EB-non-Hallopeau - Siemens	Dominant dystrophic EB-Pre-tibial Dystrophic EB-Transient bullous dermolysis of new born Dominant dystrophic EB-pruriginosa Recessive dystrophic EB- Inversa Recessive dystrophic EB - centripetalis Dystrophic EB, autosomal dominant/autosomal recessive heterozygote

The genetic defect in EB simplex with muscular dystrophy, a recessive subtype, has been found to be in the gene for plectin, a protein that links intermediate filaments to keratinocyte plasma membrane and is expressed in hemidesmosomal plaque and Z lines of striated muscles. The mutations of plectin gene are usually premature termination codon mutations which result in absence of plectin expression.[15,16]

The molecular basis of other uncommon EB simplex vaiants are as yet unknown.

Immunohistopathology

Immunofluorescent staining with monoclonal antibodies to various epitopes of plectin and its closely related HD1 molecule on frozen sections demonstrates a complete absence or marked reduction of plectin along the basement membrane zone in EB simplex-muscular dystrophy.[16,17] At present this is the only subtype of EB simplex in which immunostaining is helpful in making an accurate diagnosis.

Junctional epidermolysis bullosa

All the subtypes of junctional EB have autosomal recessive inheritance. There are 3 major and 2 minor subtypes of junctional EB.[2] The major subtypes are junctional EB - Herlitz, junctional EB-non Herlitz and junctional EB with pyloric atresia. The minor subtypes are junctional EB-inversa and junctional EB - late onset.

The Herlitz subtype is the most severe of the junctional EB subtypes. It is frequently lethal and is characterised by generalised blistering of the skin and mucous membranes associated with exuberant periorificial granulation tissue. There is widespread blistering of respiratory, gastrointestinal and genitourinary mucosae. Tracheolaryngeal blistering is common and may lead to stenosis and hoarseness of voice; poor prognostic signs in infancy. Teeth are dysplastic due to enamel defects and nails are usually lost. The non Herlitz variant is characterized by blistering and erosion of skin and mucous membranes which is much less severe than Herlitz variant.

Junctional EB with pyloric atresia is a variant which is characterised by the presence of pyloric atresia in association with chronic blistering and erosions, lack of prominent granulation tissue and

frequent involvement of the genitourinary tract. It is usually lethal in infancy.[18]

Generalised atrophic benign epidermolysis bullosa (GABEB) is a non lethal variant which presents with generalised cutaneous involvement at birth. The revised classification system of EB has eliminated the term GABEB and incorporates it into non-Herlitz variant of junctional EB.[2]

Electron microscopy

In the Herlitz and EB-pyloric atresia variants, there are very few, small hemidesmosomes, indistinct sub-basal dense plaques and reduced association between the hemidesmosomes and the keratinocyte filament network in the basal cells.[3]

There are no characteristic ultrastructural abnormalities in the other subtypes of junctional EB.

Molecular pathology

The Herlitz variant of junctional EB is associated with a mutation in the gene encoding laminis. Laminin 5, a component of anchoring filaments, is a heterodimeric protein consisting of 3 polypeptides 3, 3 and 2, each encoded by a distinct gene, LAMA3, LAMB3, LAMC2, respectively. About 80% of the mutations in the Herlitz variants are in the LAMB3 gene.[19] All the 3 chains of laminin 5 are necessary for the structural assembly of the protein. Premature termination codon mutations on both allels of one of these results in the complete absence of laminin 5, clinically manifestations as the severe Herlitz variant.[20] But when the mutation is of the null type in one allele and of a structural type in another, there is a reduction in the synthesis of laminin 5 protein, resulting in non Herlitz junctional EB.[21]

The previously described entity of GABEB has abnormalities of type XVII collagen. Type XVII collagen, also referred to as bullous pemphigoid antigen 2 (BPAG 2) is a 180 KDa transmembrane protein which is involved in maintaining adhesion between epidermis and BM. Mutations in genes encoding BPAG2 have been detected in cases of GABEB. These are premature termination codon mutations leading to reduced production and expression of BPAG2 protein, which results in the fragility of the adhesion between epidermis and BM.[22] Though the majority of GABEB patients have mutations in type XVII collagen, some patients had a reduced expresson of laminin 5 and a case with a mutation involving LAMB 3 gene has also been identified.[23] The diversity of the genetic defects underlying this clinical variant indicates that it may not be an independent entity and is one of the reasons for including it under non-Herlitz variant in the revised classification of EB.[2]

The expression of $\alpha 6\beta 4$ integrin is abnormal in patients of junctional EB with pyloric atresia. In these cases the $\beta 4$ integrin was absent or markedly reduced. Absence of α 6 integrin has also been reported. Premature termination codon mutations have been detected in genes encoding the integrin $\alpha 6\beta 4$ (ITGA6 and ITGB4).[24-26] The integrin $\alpha 6\beta 4$ is a transmembrane molecule in the hemidesmosome which mediates adhesion by attaching internally to keratin cytoskeleton and externally to laminin. This integrin plays a critical role in hemidesmosomal assembly and in absence of $\beta 4$, the $\alpha 6$, is down regulated, resulting in disruption of hemidesmosomal structure, and the attachment of epidermis to the BM becomes fragile. These integrins are also present in gastrointestinal tract and kidneys, thus leading to the manifestation of pyloric atresia and hydronephrosis.

The molecular pathology of junctional EB late onset is unknown.

Immunohistopathology

Subtyping can be done using various antibodies and an immunofluorescent technique on frozen sections.

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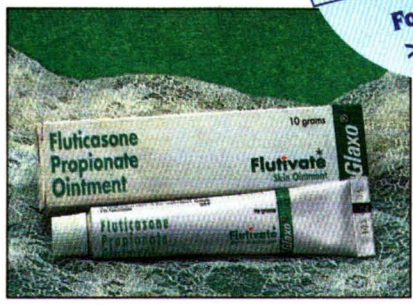

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The Herlitz variant can be recognised by staining with GB3 antibody which reacts poorly or not at all with the basement membrane of patients with Herlitz junctional EB.[27] Though the antibody recognises an epitope on the γ2 polypeptide chain of laminin 5 this epitope is also obscured when there is a defect in the β3 and α3 chains of laminin 5.[28] Consequently, any structural abnormality of laminin 5 can be detected by GB3 antibody.

In junctional EB non-Herlitz and junctional EB-inversa, GB3 staining may be normal or reduced.

A subset of junctional EB-non-Herlitz patients who were previously termed as GABEB demonstrate a reduced or absent staining with antibodies to collagen XVII (BPAG2) along the BM.[22,29]

Immunostaining for laminin 5 is normal in junctional EB-pyloric atresia. Antibodies to epitopes of 4 integrin demonstrate reduced or absent staining along the basement membrane zone of these patients.[19,30] Rarely, staining for α6 integrin may also be absent.[24,31]

Dystrophic epidermolysis bullosa

Dystrophic EB is clinically characterized by extensive blistering and erosions of the skin and mucous membranes which heal with scarring, milia and atrophy. The nails may be dystrophic or absent. There are 3 major subtypes of dystrophic EB[2]: dominant dystrophic epidermolysis bullosa, recessive dystrophic EB- Hallopeau - Siemens and recessive dystrophic EB, non-Hallopeau-Siemens.

Electron microscopy

The cleavage is detected below the lamina densa in the superficial papillary dermis. The anchoring fibrils are reduced or entirely absent or may be morphologically abnormal.[32] Anchoring fibrils are usually absent in the univolved skin of generalised recessive dystrophic EB. In localised recessive dystrophic EB and dominant dystrophic EB, the anchoring fibrils are present though reduced in number.[33] Occasionally, intraepidermal granular inclusions have been noted in some subtypes of dystrophic EB and in transient bullous dermolysis of the newborn.[34] Immunoelectron microscopy has shown these inclusions to consist of collagen VII.[35,36]

Molecular pathology

All the variants of dystrophic EB have a mutation in the gene encoding collagen VII (COL7A1), a component of anchoring fibrils. The type of mutation appears to determine the particular phenotype.[37] In the severe recessive dystrophic EB-Hallopeau-Siemens there are premature termination codon mutations on both alleles of COL7A1 gene, which leads to a complete absence of type VII collagen.[38] Milder and localised variants of recessive dystrophic EB have a structural mutation encoding a full length protein in one allele and a premature termination codon mutation in the order COL7A1 gene.[39] The nature of the structural mutation appears to determine the clinical phenotype.

In dominant dystrophic EB, the mutation almost always is a glycine substitution that destabilises the triple helix of type VIII collagen and interferes with the secretion or promotes degradation of the mutant molecule.[40,41]

Immunohistopathology

LH 7.2 is a monoclonal antibody that reacts with N-terminal of collagen VII. On immunostaining of frozen sections using LH 7.2 antibody, there is an absence or reduced staining for collagen VII along the basement membrane in generalised or localised forms of recessive dystrophic EB, respectively.[42,43] A normal linear pattern of staining is noted in dominant dystrophic EB. Staining with a specific lamina densa antigen associated antibody (KF-1) and with other antibodies against collagen VII have also demonstrated reduced or absent stains in recessive dystrophic EB and normal staining in dominant dystrophic EB.[44,45]

In transient bullous dermolysis of newborn, LH 7.2 antibody has demonstrated intraepidermal inclusions of collagen VII.[36] Similar inclusions have also been seen in occasional cases of generalised and localised dystrophic EB.

Prenatal diagnosis of EB

EB can be diagnosed in utero made by obtaining a fetal skin biopsy at 15-18 weeks of gestation. The presence or absence of a split can be identified by light microscopy but electron microscopy is needed to detect the precise level of cleavage. Immunohistopathology using monoclonal antibodies that are of diagnostic value in the affected parent or sibling can also be used for prenatal diagnosis. However fetal skin biopsy has some disadvantages. It can be done only at 15-18 weeks of pregnancy, which is a fairly advanced stage of pregnancy with attendant risks if a termination of pregnancy is necessary. In addition, the procedure carries a small risk of fetal loss.

Detection of specific molecular defects and the responsible genes has made the DNA-based prenatal diagnosis of EB a reality. By doing a chronic villus biopsy, specific mutations can be detected as early as the 10th week of gestation.[46] Genetic linkage analysis for dystrophic EB can be performed without the knowledge of the specific mutation in the family, because all forms of dystrophic EB result in mutations in COL 7A1 gene on chromosome 3p21.1.[47] On the other hand, a different approach is required for DNA based prenatal testing in the types of EB where the same clinical and ultrastructural phenotype is produced by mutations in different genes e.g. LAMA3, LAMB3 or LAMC2 in Herlitz junctional EB and ITGA6 or ITGB4 in EB-pyloric atresia). Since these genes are located in different chromosomal regions, knowledge of the specific mutation in a particular family is a prerequisite for prenatal diagnosis.[48]

Conclusion

Depending on the level of cleavage, epidermolysis bullosa is broadly classified into 3 types: simplex, junctional and dystrophic, and more than 20 subtypes are described. Routine histopathology is insufficient to subclassify the disease. Electron micrscopy is helpful in classifying the type of EB and in detecting certain subtypes. Immunostaining with particular monoclonal antibodies is as effective as electron microscopy. Mutation based diagnosis is being increasingly applied to identify the specific genetic defect in a particular patient. Accurate subtyping facilitates genetic counselling and DNA based prenatal diagnosis for subsequent pregnancies.

References

1. Pearson RW. Clinicopathologic types of epidermolysis bullosa and their non-dermatological complications. Arch Dermatol 1998; 124: 718-725.

2. Fine JD, Eady RAJ, Bauer EA et al. Revised classification for inherited epidermolysis bullosa. Report of second international consensus meeting on diagnosis and classification of epidermolysis bullosa. J Am Acad Dermatol 2000; 42: 1051-1066.

3. Bergman R. Immunohistopathologic diagnosis of epidermolysis bullosa. Am J Dermatopathol 1999; 21 : 185 - 189.

4. Marinkovich MP, Herron GS, Khavari PA, et al. Hereditary epidermolysis bullosa. In : Dermatology in General Medicine. Freedberg IM, Eisen AZ, Wolff K et al (editors), V edition, McGraw Hill, New York 1999; 690-702.

5. Fine JD, Johnson L Wright T. Epidermolysis bullosa simplex superficialis. A new variant of epidermolysis bullosa characterized by subcorneal skin cleavage mimicking peeling skin syndrome. Arch Dermatol 1989; 125: 633-638.

6. Bolte C, Gonzalez S. Rapid diagnosis of major variants of epidermolysis bullosa using a monoclonal antibody against collagen IV. Am J Dermatopathol 1995; 17:580-583.

7. Hintner H, Stingl G, Schuler G et al. Immunofluorescence mapping of antigenic determinants within the dermal - epidermal junction in mechano bullous disorders. J Invest Dermatol 1981;76:113-118.

8. Anton - Lamrecht I, Schnyder UW. Epidermolysis bullosa herpetiformis Dowling-Meara. Report of a case and pathomorphogenesis. Dermatologica 1982; 164:221-235.

9. Bonifas JM, Rothman AL Epstein EH. Epidermolysis bullosa simplex: Evidence in two families for keratin gene abnormalities. Science 1991; 254: 1202-1205.

10. Sorenson CB, Ladekjaer - Mikkelsen AS, Andresen BS et al. Identification of novel and known mutations in genes for keratin 5 and 15 in Danish patients with epidermolysis bullosa simplex: Correlation between genotype and phenotype. J Invest Dermatol 1999;112: 184-190.

11. Fuchs E. Genetic skin disorders of keratin and their associated proteins. J Dermatol Sci 1996; 13 : 181 - 192.

12. Ishida - Yamamoto A, McGrath JA, Chapman SJ et al. Epidermolysis bullosa simplex (Dowling - Meara type) is a genetic disease characterised by an abnormal keratin - filament network involving keratins K5 and K14. J Invest Dermatol 1991; 97:959-968.

13. Uttam J, Hutton E, Coulombe PA, et al. The genetic basis of epidermolysis

bullosa simplex with mottled pigmentation. Proc Natl Acad Sci USA 1996; 93 : 9079-9084.

14. Irvine AD, McKenna KE, Jenkinson H, et al. A mutation in the V1 domain of keratin 5 causes epidermolysis bullosa simplex with mottled pigmentation. J Invest Dermatol 1997;108:808-810.

15. Chanavas S, Pulkkinen L, Gache Y, et al. A homozygous nonsense mutation in the PLEC 1 gene in patients with epidermolysis bullosa simplex with muscular dystrophy. J Clin Invest 1996; 98:2196 - 2200.

16. Schimzu H, Masunaga T, Kurihara Y, et al. Expression of plectin and HD1 epitopes in patients with epidermolysis bullosa simplex associated with muscular dystrophy. Arch Dermatol Res 1999;291:531-537.

17. Gache Y, Chavanas S, Lacour JP, et al. Defective expression of plectin/ HD1 in epidermolysis bullosa simplex with muscular dystrophy. J Clin Invest 1996;97:2289-2298.

18. Valari MD, Philips RJ, Lake BD, et al. Junctional epidermolysis bullosa and pyloric atresia: A distinct entity. Clinical and pathological studies in five patiens. Br J Dermatol 1995; 133: 732 - 736.

19. Pulkkinen L, Meneguzzi G, MCGrath JA, et al. Predominance of the recurrent mutation R 635 X in the LAMB3 gene in European patients with Herlitz junctional epidermolysis bullosa has implications for mutation detection strategy. J Invest Dermatol 1997;109:232-237.

20. McGrath JA, Kivirikko S, Ciatti S, et al. A homozygous nonsense mutation in the α 3 chain gene laminin 5 (LAMA 3) in Herlitz junctional epidermolysis bullosa : Prenatal exclusion in a fetus at risk. Genomics 1995; 29: 282-284.

21. McGrath JA, Christiano AM, Pulkkinen L, et al. Compound heterozygosity for nonsense and missense mutations in the LAMB3 gene in nonlethal junctional epidermolysis bullosa. J Invest Dermatol 1996;106:1157-1159.

22. Jonkman MF, deJong MC, Heeres K et al. 180 KD bullous pemphigoid antigen (BP 180) is deficient in generalized atrophic benign epidermolysis bullosa. J Clin Invest 1995; 95 : 1345-1352.

23. Mellerio JE, Eady RAJ, Atheron DJ, et al. E210K mutation in the gene encoding b3 chain of laminin 5 (LAMB3) is predictive of a phenotype of generalised atrophic benign epidermolysis bullosa. Br J Dermatol 1998; 139: 325-331.

24. Ruzzi L, Gagnouz - Palacios L, Pinola M, et al. A homozygous mutation in the integrin alpha 6 gene in junctional epidermolysis bullosa with pyloric atresia. J Clin Invest 1997; 99: 2826-2831.

25. Vidal F, Abendam D,Miquel C, et al. Integrin 4 mutation associated with junctional epidermolysis bullosa with Pyloric atresia.Nat Genet 1995;10:229-234.

26. Mellerio JE,Pulkkinen L,McMillan JR, et al. Pyloric atresia- junctional epidermolysis bullosa syndrome mutation in intergrin 4 gene (ITGB4) in two unrelated patient with mild disease. Br J Dermatol 1998; 139:862-871.

27. Schofield OMV, Fine J.D,Verrando P, et al.GB3 Monoclonal antibody for the diagnosis of junctional epidermolysis bullosa: result of a muliticenter study. J Am Acad Dermatol 1990;23:1078-1083.

28. Matsui C,Nelson CF,Hernandez GT, et al.γ2 chain of laminin-5 is recognised by monoclonal antibody GB30 J Invest Dermatol 1995;105:648-652.

29. Darling TN,McGrath JA,Yee C, et al.Premature termination codons are present on both alleles of the bullous pemphigoid antigen 2/type XVII Collagen gene in five Austrian families with generalised atrophic benign epidermolysis bullosa.J Invest Dermatol 1997;108:463-468.

30. Gil SG,Brown TA,Ryan MC,et al.Junctional epidermolysis bullosa: defects in expression of epilgrin/nicein/kalinin and intergrin 4 that inhibit hemidesmosomal formation. J Invest Dermatol 1994;103 (suppl 5) : 31S - 38S.

31. Shimizu H,Suzumori K,Hatta N, et al. Absence of detectable 6 integrin in pyloric atresia-Junctional epidermolysis bullosa syndrome : Application for prenatal diagnosis in a family at risk for recurrence. Arch Dermatol 1996;132:919-925.

32. McGrath JA,Ishida-Yamamoto A,O'Grady A,et al.Structural variation in anchoring fibrils in dystropic epidermolysis bullosa:correlation with type VII collagen expression.J.Invest Dermatol 1993;100:366-372.

33. Tidman MJ,Eady RAJ.Evaluation of anchoring fibrils and other components of dermal-epidermal junction in dystrophic epidermolysis bullosa: correlation with type VII collagen expression. J Invest Dermatol 1993; 100 : 366 - 372.

34. Hashimoto K, Matsumoto M, Iacobelli D. Transient bullous dermolysis of the new born. Arch Dermatol 1985; 121 : 1429 - 1438.

35. Fine JD, Horiguchi Y, Stein DH, et al. Intraepidermal type VII collagen: evidence for abnormal intracytoplasmic processing of a major basement membrane protein in rare patients with dominant and possible localised recessive forms of dystrophic epidermolysis bullosa. J Am Acad Dermtol 1990; 22 : 188-195.

36. Philips RJ, Harper JI, Lake BD. Intra epidermal collagen type VII in dystrophic epidermolysis bullosa: report of five new cases. Br J Dermatol 1992; 126:222-230.

37. Uitto J, Pulkkinen L, Christiano AM. Molecular basis of dystrophic and junctional form of epidermolysis bullosa: Mutations in type VII collagen and Kalinin (Laminin 5) genes. J Invest Dermatol 1994; 103 (Suppl.5) : 395 - 45S.

38. Christiano AM, Anhalt G, Gibbons S, et al. Premature termination codons in the type VII collagen gene (COL 7A1) underline severe, mutilating, recessive dystrophic epidermolysis bullosa. Genomics 1994; 21 : 160 - 168.

39. Christiano AM, McGrath JA, Uitto J. Influence of the second COL7A1 mutation in determining the phenotypic severity of recessive dystrophic epidermolysis bullosa. J Invest Dermatol 1996; 106: 766-770.

40. Christiano AM, Ryynanen M, Uitto J. Dominant dystrophic epidermolysis bullosa : Identification of a Gly S sea substitution in the triple - helical domain of type VII collagen. Proc Natl Acad Sci USA 1994; 91 : 3553.

41. Kon A, McGrath JA, Pulkkinen L et al. Glycine substitution mutations in the type VII collagen gene (COL7A1) in dystrophic epidermolysis bullosa: implications for genetic counselling. J Invest Dermatol 1997; 108: 224 - 228.

42. Bruckner - Tuderman L, Mitsuhashi Y, Schnyder UW, et al. Anchoring fibrils and type VII collagen are absent from skin in severe recessive dystrophic epidermolysis bullosa. J Invest Dermatol 1989;93:3-9.

43. Heagerty AHM, Kennedy AR, Leigh IM, et al. Identification of an epidermal basement membrane defect in recessive forms of dystrophic epidermolysis bullosa by LH 7.2 monoclonal antibody : use in diagnosis. Br J Dermatol 1986; 115 : 125 - 131.

44. Fine JD, Breathnach SM, Hintner H, et al. KF -1 monoclonal antibody defines a specific basement membrane antigenic defect in dystrophic forms of epidermolysis bullosa. J Invest Dermatol 1984; 82 : 35-38.

45. Goldsmith LA, Briggaman RA. Monoclonal antibodies to anchoring fibrils for diagnosis of epidermolysis bullosa. J Invest Dermatol 1983; 81:464-466.

46. Uitto J. Molecular diagnosis of epidermolysis bullosa: Novel pathomechanisms and surprising genetics. Exp Dermatol 1999; 8 : 92-95.

47. Chrisiano AM, LaForgia S, Paller AS, et al. Prenatal diagnosis for recessive dystrophic epidermolysis bullosa in ten families by mutation and haplotype analysis in the type VII collagen gene (COL7A1). Mol Med 1996; 2 : 69-76.

48. Christiano AM, Pulkkinen L, McGrath JA, et al. Mutation based prenatal diagnosis of Herlitz junctional epidermolysis bullosa. Prenatal Diag 1997; 17: 343-354.