ORIGINAL ARTICLE

Epidermolysis bullosa. I. Molecular genetics of the junctional and hemidesmosomal variants

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Introduction: Epidermolysis bullosa (EB), a group of autosomal heritable blistering diseases, is characterised by extensive phenotypic variability with considerable morbidity and mortality. EB is classified into distinct subtypes depending on the location of blistering within the cutaneous dermoepidermal basement membrane zone. Ten genes are known to harbour mutations in the major types of EB, and the level of expression of these genes within the cutaneous basement membrane zone and in extracutaneous tissues, as well as the types and combinations of the mutations, explain in general terms the phenotypic variability.

Methods: The DebRA Molecular Diagnostics Laboratory, established in 1996 and supported in part by the patient advocacy organisation DebRA of America, has analysed over 1000 families with different forms of EB.

Results: In total, 265 cases were submitted with the preliminary diagnosis of junctional or hemidesmosomal forms of EB. We found 393 mutant alleles in seven different genes, with 173 of the mutations being distinct and 71 previously unpublished.

Discussion: These findings attest to the clinical and molecular heterogeneity of the junctional and hemidesmosomal subtypes of EB. The results also reveal exceptions to the general rules on genotype-phenotype correlations, unusual phenotypes, and surprising genetics. Collectively, mutation analysis in different forms of EB provides the basis for improved classification with prognostic implications and for prenatal and preimplantation diagnosis in families at risk for recurrence of EB.

Epidermolysis bullosa (EB) constitutes a group of genodermatoses manifesting with fragility of the skin and mucous membranes and presenting with blisters and erosions at birth or shortly thereafter.^{1, 2} The spectrum of phenotypic manifestations is broad; in the milder forms there is a life long blistering tendency with no impact on the overall longevity of the affected individual, while in the most severe forms children die during the early postnatal period from metabolic perturbations, dehydration, and sepsis. Some forms are characterised by debilitating scarring with the propensity to early death from aggressive squamous cell carcinomas of the skin. In addition to skin and mucous membrane involvement, there are a number of extracutaneous manifestations. In some cases, abnormalities in the hair, nails, and teeth can be described. In other forms of the disease, the gastrointestinal tract is affected either in the form of oesophageal strictures or congenital pyloric atresia. A subset of patients manifests with late onset muscular dystrophy. Adding to the heterogeneity of EB is the fact that inheritance can be either autosomal dominant or autosomal recessive. This heterogeneity, coupled with historical classifications riddled with eponyms, has led to suggestions that there may be as many as 30 different subtypes of EB.³

EB is now known to result from mutations in 10 different genes expressed within the cutaneous basement membrane zone (BMZ) at the dermoepidermal junction (fig 1).² The stratified expression pattern of these genes within the BMZ and in extracutaneous tissues explains the broad phenotypic spectrum of EB. Furthermore, the types and combinations of the mutations and their consequences at the mRNA and protein levels, when superimposed on the individual's genetic background and combined with environmental factors, contribute to the wide range of phenotypes.

Traditionally, EB has been divided into three broad categories based on the level of tissue separation, determined

by diagnostic electron microscopy and/or immunoepitope mapping (fig 1):⁴ (a) the simplex forms of EB (EBS) demonstrate tissue separation within the basal keratinocytes at the bottom layer of epidermis; (b) the junctional forms of EB (JEB) display tissue separation within the dermoepidermal basement membrane, primarily within the lamina lucida; and (c) in the dystrophic forms (DEB), tissue separation occurs below the lamina densa within the upper papillary dermis (fig 1). In addition to this traditional classification, a fourth subtype, the hemidesmosomal variants of EB (HEB), has been proposed (table 1).⁵

Patients with HEB manifest tissue separation at the basal cell/lamina lucida interface at the level of the hemidesmosomes. While there is overlap between the hemidesmosomal variants and the traditional subtypes, particularly the simplex and junctional forms of EB, the incorporation of HEB into the classification scheme has been extremely helpful in guiding initial mutation detection strategies in this subgroup of patients with EB; it should be noted that this addition to the EB classification has not been recognised by the latest International Consensus Meeting.⁴ However, the general classification, consisting of EBS, HEB, JEB, and DEB, combined with information derived from diagnostic immunoepitope mapping of the skin in affected individuals, has provided the platform for successful and streamlined mutation detection in the candidate genes in these families.

Abbreviations: BMZ, basement membrane zone; CV, chorionic villus; DEB, dystrophic epidermolysis bullosa; EB, epidermolysis bullosa; EB-MD, epidermolysis bullosa with muscular dystrophy; EB-PA, epidermolysis bullosa with pyloric atresia; EBS, epidermolysis bullosa simplex; GABEB, generalized atrophic benign epidermolysis bullosa; HEB, hemidesmosomal epidermolysis bullosa; JEB, junctional epidermolysis bullosa; LOC, laryngo-onychocutaneous; PTC, premature termination codon

Figure 1 Complexity of the cutaneous basement membrane zone (BMZ), and classification of epidermolysis bullosa. The figure schematically depicts basal keratinocytes at the lower part of the epidermis, separated from the papillary dermis by a dermoepidermal basement membrane. Ultrastructurally recognisable attachment complexes and structural components of the BMZ are listed on the left, while specific proteins localised within each layer are indicated on the right. The level of tissue separation within each subgroup of epidermolysis bullosa is shown on the right.

Certain subtypes of EB are associated with mutations in specific genes, and examination of the mutation database has revealed general genotype-phenotype correlations. For example, the classic, dominantly inherited, simplex forms are primarily due to mutations in the intermediate keratin filament genes, *KRT5* and *KRT14*, expressed in the basal cells of the epidermis. The dystrophic forms are exclusively due to mutations in the type VII collagen gene, *COL7A1*. The classic junctional forms are associated with mutations in the laminin 5 genes, *LAMA3*, *LAMB3*, and *LAMC2*; in particular, the Herlitz (lethal) JEB variant frequently displays premature termination codon (PTC) mutations in these genes. The non-Herlitz (non-lethal) variant harbours mutations in the same genes, but with milder consequences at the mRNA and protein levels, such as missense or splice junction mutations.

The hemidesmosomal clinical variants have been proposed to include generalised atrophic benign EB (GABEB), which was previously included in the non-Herlitz category of JEB. Most cases of GABEB have been reported to be caused by mutations in the type XVII collagen/180 kDa bullous pemphigoid antigen gene, *COL17A1/BPAG2*,⁵ although this study provides compelling evidence for mutations in the laminin 5 genes in many of these cases. Another hemidesmosomal variant is EB with pyloric atresia (EB-PA). While most of these cases are associated with mutations in the $\alpha 6\beta 4$ integrin genes, *ITGA6* and *ITGB4*,⁵ mutations in the plectin gene (*PLEC1*), which encodes a large, ~500 kDa adhesion protein, have also been identified in some patients with EB-PA.^{6,7} Finally, mutations in the plectin gene have

been identified in patients with EB and late onset muscular dystrophy.⁸ As the tissue separation in the latter cases is intraepidermal within the basal keratinocytes, patients with EB-MD have also been included in the simplex category, although tissue separation occurs at a different level in these patients than in those with classic forms of EBS.

The DebRA Molecular Diagnostics Laboratory, established in 1996 at the Department of Dermatology and Cutaneous Biology at Jefferson Medical College, has collected over 1000 families with different forms of EB for analysis for mutations in the candidate genes. Among these families, 422 distinct mutations in the 10 genes underlying the major forms of EB have been found. This number does not count homozygous mutations twice or recurring mutations multiple times. Thus, there is a large number of private, family specific mutations in EB with general genotype-phenotype correlations and translational implications for prognostics, genetic counseling, and prenatal/preimplantation genetic testing. In this article, we summarise results of mutation analysis in a cohort of 265 patients with the junctional and hemidesmosomal forms of EB.

MATERIALS AND METHODS

Patients

The experiments were approved by the institutional review board at Thomas Jefferson University, and they adhere to the Helsinki Guidelines.

Table 1 Subclassification of EB with associated mutant genes*

EB subtype	Inheritance†	Level of tissue separation	Genes
Simplex	AD, AR	Basal cells	<i>KRT5</i> , <i>KRT14</i> , <i>PLEC1</i>
Hemidesmosomal	AR	Hemidesmosomes (both intracellular and extracellular)	<i>COL17A1</i> , <i>LAMB3</i> , <i>ITGB4</i> , <i>PLEC1</i> , <i>ITGA6</i> , <i>PLEC1</i>
Junctional	AR	Lamina lucida	<i>LAMA3</i> , <i>LAMB3</i> , <i>LAMC2</i> , <i>COL17A1</i>
Dystrophic	AD, AR	Sub-lamina densa	<i>COL7A1</i>

*Different mutations within each gene have been noted to result in a spectrum of phenotypes (see text); for this reason, multiple genes are listed under each subtype. †AD, autosomal dominant; AR, autosomal recessive

Samples from families with affected individuals and their unaffected relatives were submitted for mutation analysis to the DebRA Molecular Diagnostics Laboratory at Jefferson Medical College, following diagnosis and preliminary determination of the EB subtype by means of clinical assessment and skin biopsy studied using transmission electron microscopy or immunopeptide mapping when available. All samples were submitted through outside referral centres where informed consent was obtained. For prenatal diagnosis, chorionic villus (CV) or amniotic fluid specimens were obtained in outside referral centres from families in which mutations had been previously identified. In cases where mutations had not been previously determined, CV or amniotic fluid samples were submitted together with blood samples from the previously affected individuals and from available family members.

Mutation detection

DNA was extracted from blood samples as previously described.⁹⁻¹⁰ Conditions and primers for generating PCR products spanning all exons of the coding regions and flanking intronic sequences of the genes have been described elsewhere: *LAMA3*, *LAMB3*, *LAMC2*, *ITGA6*, *ITGB4*, *COL17A1*, and *PLEC1*.¹¹⁻²⁰ In some cases, primer pairs corresponding to certain exons were redesigned for improved detection; sequences of these primers are available upon request. The PCR products were screened early on by conformation sensitive gel electrophoresis²¹⁻²² and more recently by denaturing high performance liquid chromatography (WAVE; Transgenomic, Gaithersburg, MD, USA). PCR products showing pattern shifts were sequenced in both directions in most cases. DNA sequencing was performed on an ABI Prism 377 or ABI 3100 automated sequencer (Perkin-Elmer-Cetus, Foster City, CA, USA). Putative mutations were confirmed by restriction enzyme digestion followed by agarose gel electrophoresis. In cases where a restriction site was not altered by the mutation, a mismatch primer was used, which, in combination with the mutation, altered a restriction site. In some cases, allele specific oligonucleotide hybridisation analysis with mutation specific and wild type oligonucleotide probes was performed, as previously described.²³ For amino acid substitution mutations, 100 control alleles were studied to rule out the possibility that the putative mutation might be a polymorphism.

PCR was performed using Qiagen *Taq* polymerase and Q buffer (Qiagen Inc., Valencia, CA, USA), according to the manufacturer's instructions. PCR reactions contained 200 ng DNA as template and 100 ng of each primer in a final volume of 50 µl. Cycling conditions for all primer pairs were 94°C for 5 minutes; followed by 41 cycles of 94°C for 1 minute, the appropriate annealing temperature for a particular primer pair (range 55–60°C) for 1 minute; and 72°C for 1 minute, and finally 72°C for 5 minutes.

GenBank reference sequences for the genes examined in this study were as follows. *LAMB3*: NM_001017402.1; *LAMC2*: NM_005562; *LAMA3*: NM_198129; *COL17A1*: NM_000494.1; *ITGB4*: NM_001005731.1; *ITGA6*: NM_000210; *PLEC1*: NM_000445.

The numbering of the mutant and wild type sequences follows these reference sequences. In some cases, mutations have been published previously using different sequence numbering. These have now been changed to be uniform with the reference sequences above. In cases where a base pair was deleted in a repeating sequence of a particular nucleotide (such as CCCC), the numbering of the mutation has been adjusted so that the base pair deleted corresponds to the position of the last nucleotide in the repeating sequence.

RESULTS

Mutation analysis in patients with JEB and HEB

Over 1000 families with different forms of EB have been referred to the DebRA Molecular Diagnostic Laboratory since 1996 and, during this time period, 265 families were submitted for mutation analysis with the initial diagnosis of JEB. This number includes families with GABEB and EB-PA, hemidesmosomal variants of EB, which have previously been included in the non-Herlitz category of JEB. Owing to the lack of critical material or information corroborating the diagnosis, mutation analysis was not initiated in 31 cases. In a limited number of cases in which a patient sample was not available, carrier parents of an affected individual were subjected to screen of four *LAMB3* recurrent mutations (R42X, Q243X, R635X, and 957ins77).⁹ In the remaining cases, mutation analysis was performed by PCR amplification of all exons and flanking intronic sequences in select genes. The particular gene was chosen from the seven candidate genes that have been previously shown to harbour mutations in junctional and/or hemidesmosomal forms of EB based on biopsy and/or phenotypic information (table 1). Of the 234 patients for whom DNA was available and testing was initiated, mutations in at least one allele were found in 209 cases (89.3%). Mutations, either homozygous or compound heterozygous, in both alleles of any of the seven genes were identified in 183 cases (78.2%). A number of novel mutations were discovered (appendix).

Laminin 5 genes

In the cohort of 209 patients with at least one mutation detected, 393 mutant alleles (94.0% of the total 418 alleles studied) were discovered. Examination of the distribution of mutations within the seven genes revealed that the majority of the mutations (277 of 393; 70.4%), resided in the three laminin 5 genes, *LAMA3*, *LAMB3*, and *LAMC2*. Among all the mutant alleles discovered in the laminin 5 genes, 222 of 277 (80.1%) were in *LAMB3*, 28 (10.1%) mutations resided in *LAMC2*, and 27 (9.7%) were found in *LAMA3* (table 2). Although PTC causing mutations were predominant in all three genes, it is noteworthy that splice junction mutations were relatively common in *LAMA3* (table 2).

In total, 92 distinct mutations were detected in the laminin 5 genes; of these, 32 (34.8%) were nonsense mutations and 37 (40.2%) were insertions or deletions that resulted in a premature termination codon (PTC) downstream of the site of the mutation. Thus, 75.0% of all distinct laminin 5 gene mutations discovered were PTC causing ones. Examination of the published literature to date reveals that 22 of these PTC causing mutations have not been previously described (appendix). Furthermore, 13 mutations (14.1%) resided at the intron/exon junctions, predicting aberrant splicing and potentially leading to downstream PTC; however, the consequences of these mutations were not routinely examined at the mRNA and/or protein levels. Of these distinct splice site mutations, four were novel. An additional 10 mutations (10.9%) were missense mutations, six of which were previously unreported.

Hemidesmosomal genes

Mutations in the genes *ITGA6* and *ITGB4*, which encode the $\alpha 6\beta 4$ integrin subunit polypeptides, have been found previously in patients with EB-PA.³ In this study, these two genes harboured mutations in 35 cases with EB-PA, and the majority of the mutations (60 out of 70 mutant alleles; 85.7%), were in the *ITGB4* gene, while only four (5.7%) were found in *ITGA6* (table 3).

Of the 64 mutations detected in *ITGA6* and *ITGB4*, 29 (45.3%) were PTC causing nonsense or insertion/deletion mutations, 25 (39.1%) were missense mutations, and 10

Table 2 Characteristics of laminin 5 mutations found in this study in patients with junctional forms of EB

Gene	Types of mutations*			Missense	Splice junction†	Total no. of mutant alleles	% of total laminin 5 mutations
	PTC causing						
	Nonsense	Ins/del	Total				
LAMB3	144	51	195 (87.8%)	13 (5.9%)	14 (6.3%)	222	80.1
LAMC2	15	12	27 (96.4%)	0	1 (3.6%)	258	10.1
LAMA3	7	12	19 (70.4%)	2 (7.4%)	6 (22.2%)	27	9.7
All genes						277	100

*PTC, premature termination codon; Ins/Del, insertion or deletion mutation resulting in frameshift and PTC. †the consequences of the splice junction mutations were not determined at mRNA or protein level in most cases.

(15.6%) resided at splice junctions. There were 13 PTC causing mutations, 5 missense mutations, and 4 splice site mutations discovered that were previously unpublished (appendix). It should be noted that of the total of 42 patients referred for analysis with the diagnosis of EB-PA, seven (16.7%) harboured mutations in the plectin gene (*PLEC1*).

The *COL17A1* gene harboured mutations in 21 (10.0%) of the total cohort of 209 patients, and mutations were found in 38 out of 40 alleles (95.0%). Of these mutant alleles, 27 (71.0%) harboured PTC causing nonsense and insertion/deletion mutations, nine mutations (23.7%) affected the intron/exon junctions, and only two (5.3%) were missense mutations (table 3). Of the novel mutations detected, eleven were PTC causing mutations and five were splice site mutations (appendix). It should be noted that of the 13 patients referred initially with the diagnosis of GABEB, only nine (37.5%) of 24 detected mutant alleles were found in the *COL17A1* gene, while the majority of mutant alleles (62.5%) resided in *LAMB3* (table 4).

Recurrent mutations

During this study, it became evident that some mutations occurred at a relatively high frequency in certain ancestral backgrounds. These recurrent mutations are listed in table 5. Of these mutations, R635X in *LAMB3* has been noted with highest frequency in persons of white ethnicity in several studies.^{24–25} It has been suggested that this mutation is a mutation hot spot, reflecting putative hypermutability of 5-methylcytosine in the CpG dinucleotide sequence. However, it is currently not known if the other recurrent mutations noted in this study represent founder effects or are hot spots. In our study, R635X represented 90 of the 222 mutant alleles discovered in *LAMB3* (40.5%).

Analysis of the mutations found in this study revealed two other recurrent mutations with high frequency in people of certain ancestral backgrounds. One of these mutations, 957ins77 in *LAMB3*, has been described previously in the literature and is highly prevalent in affected families of

African origin.²⁶ Another mutation, also previously reported, is C61Y in *ITGB4*.¹⁷ This mutation was observed to reside frequently in families of Hispanic origin with EB-PA; on examination of the five Hispanic patients with EB-PA in this study, five of 10 mutant alleles detected harboured this mutation.

In addition to those recurrent mutations found in this study, a number of recurrent mutations have been reported in the literature. Examples of such recurrent mutations in *LAMB3* include: Q1083X, detected in patients of Middle Eastern origin; Q166X and W610X, seen in patients of Japanese origin; 31insC (previously reported as 29insC); and W143X, noted in patients of Italian origin.^{26–31} One recurrent mutation, R650X, has been observed in the *LAMA3* gene in Pakistani patients.³² Reports of recurrent mutations in the *LAMC2* gene include R95X, observed in patients of Italian origin, and Q46X, seen in patients in the Middle East region.^{28–31}

DISCUSSION

Genetic heterogeneity of JEB

The junctional forms of EB display a remarkable degree of both clinical and genetic heterogeneity. The clinical heterogeneity is attested by the spectrum of severity of these conditions, varying from lethal outcome during the early postnatal period in Herlitz JEB to life long blistering tendency in non-Herlitz JEB, which does not impact the overall longevity of the affected individual. There is also clinical overlap between the hemidesmosomal and junctional variants of EB; in particular, the generalised atrophic benign EB has been traditionally classified as non-Herlitz JEB. The genetic heterogeneity is reflected by the fact that seven different genes are implicated in the pathogenesis of the junctional and hemidesmosomal variants of EB. In JEB, tissue cleavage occurs within the dermoepidermal basement membrane at the level of the lamina lucida, while the HEB variants display cleavage at the level of hemidesmosomes. Ultrastructurally, abnormalities in the region of the

Table 3 Characteristics of mutations identified in this study within the hemidesmosomal genes

Gene	Types of mutations*					Total no. of mutant alleles	% of total mutant alleles
	PTC causing			Missense	Splice junction†		
	Nonsense	Ins/del	Total				
COL17A1	9	18	27 (71.1%)	2 (5.3%)	9 (23.7%)	38	32.8
ITGB4	9	18	27 (45.0%)	25 (41.2%)	8 (13.3%)	60	51.7
ITGA6	0	2	2 (50.0%)	0	2 (50.0%)	4	3.4
PLEC1	5	8	13 (92.9%)	0	1 (7.1%)	14	12.1
All genes						116	100

*PTC, premature termination codon; †the consequences of the splice junction mutations were not determined at mRNA or protein level in most cases. Ins/Del, insertion or deletion mutation resulting in frameshift and PTC.

Table 4 Correlation of the types of mutations found in this study and the clinical diagnosis in patients with Herlitz, non-Herlitz, and hemidesmosomal variants of EB

Diagnosis*	Gene	Mutations			Total no. (%) of mutant alleles	% of total alleles detected
		PTC causing	Missense	Splice junction		
H-JEB (n = 107)	LAMA3	15 (75.0%)	0	5 (25.0%)	20	9.8
	LAMB3	140 (95.2%)	2 (1.4%)	5 (3.4%)	147	71.7
	LAMC2	21 (100.0%)	0	0	21	10.2
	COL17A1	13 (76.5%)	0	4 (23.5%)	17	8.3
NH-JEB (n = 23)	LAMA3	2 (40.0%)	2 (40.0%)	1 (20.0%)	5	12.5
	LAMB3	17 (68.0%)	5 (20.0%)	3 (12.0%)	25	62.5
	LAMC2	2 (100.0%)	0	0	2	5.0
	COL17A1	4 (50.0%)	1 (12.5%)	3 (37.5%)	8	20.0
GABEB (n = 13)	COL17A1	6 (66.7%)	1 (11.1%)	2 (22.2%)	9	37.5
	LAMA3	0	0	0	0	0
	LAMB3	10 (66.7%)	4 (26.7%)	1 (6.7%)	15	62.5
	LAMC2	0	0	0	0	0
EB-PA (n = 42)	ITGA6	2 (50.0%)	0	2 (50.0%)	4	5.1
	ITGB4	27 (45.0%)	25 (41.7%)	8 (13.3%)	60	76.9
	PLEC1	13 (92.9%)	0	1 (7.1%)	14	17.9

*This table includes those patients in whom the initial diagnosis was specified by referring physician at the time of sample submission based on clinical observations and confirmed by ultrastructural analysis using electron microscopy and/or immunofluorescence microscopy, when available. In addition, those patients for whom an initial diagnosis was not specified, but who were described as having a severe/lethal phenotype and/or who demonstrated the absence of laminin 5 expression on immunoprecipitation mapping, were classified as having Herlitz phenotype and were included in this table. Numbers in parentheses indicates the number of patients examined in this subtype. GABEB, generalised atrophic benign EB; EB-PA, EB with pyloric atresia; PTC, premature termination codon; H-JEB, Herlitz junctional EB (lethal); NH-JEB, non-Herlitz junctional EB (non-lethal).

hemidesmosome/anchoring filament complexes are observed in both forms of EB.¹⁻⁴

In JEB, the majority of the mutations, both in lethal and non-lethal forms, has been identified in the three genes, *LAMA3*, *LAMB3*, and *LAMC2*, encoding the constituent polypeptides, $\alpha 3$, $\beta 3$, and $\gamma 2$, of laminin 5. A large number of mutations has been encountered in *LAMB3*, which also harbours a number of recurrent mutations, particularly R635X; the reason why the majority of these mutations are

found in *LAMB3* is unclear.¹²⁻²⁵ The majority of the mutations identified in the Herlitz form of JEB are PTC causing mutations that predict synthesis of truncated, non-functional polypeptides, accompanied by markedly reduced levels of the corresponding mRNA transcript through a nonsense mediated mRNA decay mechanism.³³ This results in the absence of laminin 5, as evidenced by negative immunofluorescence with anti-laminin 5 antibodies, the presence of hypoplastic hemidesmosome/anchoring filament complexes viewed by transmission electron microscopy, and the extreme fragility of the skin with the propensity for granulation tissue formation. In the milder, non-Herlitz forms of JEB, laminin 5 gene mutations have also been found frequently. In some cases, one of the mutations is a PTC causing mutation, while the other genetic lesion in *trans* consists of a missense mutation or an in frame exon splicing mutation. These observations suggest that shortened polypeptides with an intact carboxyterminal end are able to assemble into laminin 5 trimer molecules, which then serve a partial function in the anchoring filaments. This interpretation is consistent with the observation that immunofluorescence staining with anti-laminin 5 antibodies is positive, yet frequently attenuated, in these patients with non-lethal JEB.

The molecular basis of the hemidesmosomal variants: generalised atrophic benign EB

Whereas the molecular basis of the classic forms of Herlitz and non-Herlitz JEB are relatively well understood on the basis of laminin 5 mutations, the hemidesmosomal variants of EB demonstrate additional clinical and genetic heterogeneity. Clinically, the hemidesmosomal variants include GABEB, EB-PA, and EB-MD, which demonstrate tissue separation at the hemidesmosomal level, either within the intracellular, transmembrane, or extracellular segments of these attachment structures, depending on the specific type of mutation. GABEB was originally identified as a specific subset of non-lethal JEB.³⁴⁻³⁵ Clinically these patients demonstrate a moderately severe blistering tendency associated with characteristic extracutaneous involvement, including dystrophy of the nails, focal scarring alopecia of the scalp, loss of eyelashes, dental abnormalities, and patchy

Table 5 Recurrent mutations observed in this study

Gene*	Mutation	Allelic frequency†		Ref‡
		n	%	
<i>LAMB3</i> (n = 222)	R635X	90	40.5	12-68
	957ins77	16	7.2	9-13
	Q243X	10	4.5	9-25
	R42X	7	3.2	12-13
	R660X	6	2.7	69
	R144X	5	2.3	9-70
	R569X	5	2.3	9-69
	565-2A→G	4	1.8	9-69
	R972X	4	1.8	9-25
	E210K	4	1.8	31-71
	Q373X	4	1.8	72-73
	2842delG	4	1.8	This study
	Q864X	3	10.7	This study
	1405delAG	2	7.4	72-§
<i>LAMA3</i> (n = 27)	C61Y	5	8.3	17-74
	3793+1G→A	4	6.7	74-75
	658delC	3	5.0	74
	R1281W	3	5.0	17-74
	R252C	3	5.0	17-74
	2944del5	3	7.9	19
	R1226X	2	5.3	18-76
<i>COL17A1</i> (n = 38)				

*n refers to the total number of mutant alleles found in this study within the gene specified; †n indicates the total number of alleles harbouring the recurrent mutation; % refers to the corresponding percentage; ‡selected references of the first report and/or comprehensive review of this mutation; §the position of this mutation reflects the change in the numbering of the gene; this same mutation has been reported in the reference cited as 1372delAG.

macular hyperpigmentation. In a subset of GABEB patients, mutations in the *COL17A1* gene have been identified, many of the mutations being PTC causing nonsense mutations or small insertions or deletions.^{19–36} Of interest is a GABEB family in which a dominantly inherited glycine substitution, G627V, was identified in the *COL17A1* gene; the proband, who demonstrated skin manifestations, also inherited a PTC, 3514ins25, in *trans*. Interestingly, carriers in this family of the glycine substitution alone had markedly abnormal enamel pitting but no skin manifestations. In our study, carriers of a deletion mutation, 717delA in *COL17A1*, had severely pitted tooth enamel in the absence of skin manifestations. Thus, the presence of mutations in *COL17A1* appears to affect the basement membrane of the developing tooth, a function that was previously undisclosed for this transmembrane collagen.³⁷

In the present study, among the 13 patients who were initially referred with the diagnosis of GABEB, only nine of 24 mutant alleles detected (37.5%) were found to have *COL17A1* mutations, while the majority of mutations were discovered in the *LAMB3* gene. At the same time, eight of 40 mutant alleles (20.0%) detected in the patients with non-Herlitz JEB were in *COL17A1*. These findings emphasise the overlap between non-Herlitz JEB and GABEB. Furthermore, it is clear that the presence of mutations in the *COL17A1* gene does not necessarily imply a mild phenotype. This conclusion is emphasised by the fact that 17 (8.3%) of 205 mutated alleles detected in patients with Herlitz (lethal) JEB resided in *COL17A1*, the majority (76.5%) being PTC causing mutations.

Hemidesmosomal gene mutations in EB-PA

Another variant of hemidesmosomal EB is EB-PA, characterised by congenital pyloric atresia and neonatal blistering of the skin as primary manifestations. The initial genetic analyses demonstrated distinct mutations in the $\alpha 6\beta 4$ integrin genes, *ITGA6* and *ITGB4*, the majority of the mutations residing in *ITGB4*.^{5–16} It was noted that premature termination codons in both alleles of either gene were accompanied by severe skin fragility leading to early demise of the affected individuals. Subsequent DNA analyses revealed that more subtle missense mutations could be associated with milder disease, and in some cases the patients, upon surgical correction of the pyloric atresia, experienced a very mild blistering tendency.¹⁷ These observations suggested that the $\alpha 6\beta 4$ integrin plays a tissue specific role in the development of both the skin and the gastrointestinal tract, and that the types of mutation determine the severity of the clinical manifestations. One patient in this study exhibited sigmoid colon obstruction in addition to pyloric atresia and skin blistering in association with a PTC causing mutation on one allele of *ITGB4* (1874delTCTinsC) and a missense mutation on the other (V325D). Recently, *PLEC1* mutations have also been identified in patients with EB-PA with a lethal phenotype.^{6–7} This finding was surprising considering the fact that a number of *PLEC1* mutations have been previously identified in patients with EB-MD, who display relatively mild neonatal skin blistering and late onset muscular dystrophy, but no evidence of pyloric atresia.⁸ The question remains whether these individuals would have developed muscular dystrophy later in life, had they survived. Furthermore, careful examination of the *PLEC1* mutation database does not provide any obvious explanation as to why some patients with *PLEC1* mutations develop congenital pyloric atresia and others do not.

The diverse consequences of plectin gene mutations

EB-MD has been shown to result from mutations in *PLEC1*, which encodes a ~500 kDa adhesion molecule with versatile

binding affinities. Although plectin is expressed in multiple tissues and nearly all cell types, its precise cytoplasmic localisation appears to depend on the specific cell type, and it can appear diffused throughout the cell within the matrix component or reside in a restricted distribution as a focal adhesion protein.³⁸ Nevertheless, besides being present in hemidesmosomes, plectin is also expressed in the sarcolemma and the Z lines of the skeletal muscle, encoded by the same gene (*PLEC1*). Thus, tissue specific expression of *PLEC1* in the skin and muscle explains the pleiotropic consequences of the mutations noted in EB-MD.⁶

As indicated above, plectin is an intracellular component of the hemidesmosomes, and mutations in *PLEC1* are accompanied by intracellular cleavage plane within the basal cells, visualised by transmission electron microscopy. Consequently, these patients have frequently been referred with a diagnosis of EB simplex, prompting mutation analysis in the basal keratin genes.³⁹ Compounding the diagnostic and genetic challenge in EB-MD is the fact that *PLEC1* mutations resulting in neonatal blistering and late onset muscular dystrophy are autosomal recessive, while keratin mutations in classic EB simplex are autosomal dominant, with some cases being de novo “sporadic.” Careful electron microscopy examination revealing the cleavage plane at the lower level of hemidesmosomes, together with thorough inquiry about family history, has been extremely helpful in identifying mutations in *PLEC1* in these patients. These observations have implications for genetic counselling regarding the prospect of developing late onset muscular dystrophy.^{6–39} Finally, it should be noted that *PLEC1* has been shown to harbour mutations in an autosomal dominant form of EBS, the Ogna variant, a rare condition originally identified in Norway, displaying haemorrhagic blistering of the extremities.⁴⁰ Thus far, two families, the original Norwegian pedigree and a large family in northern Germany, have been shown to display a heterozygous R2110W missense mutation in multiple generations. This variant of EB appears to be extremely rare, and no cases with this diagnosis were referred to the DebRA Molecular Diagnostics Laboratory for DNA analysis over the past decade.

Other skin fragility syndromes

A few rare cases with skin fragility at the epidermal level have also been reported with mutations in the desmosomal genes including desmoplakin and plakophilin.^{41–42} Quite recently, Jonkman *et al*⁴³ reported a patient with compound heterozygosity for two desmoplakin mutations, resulting in deletion of the carboxyterminal tail of desmoplakin and superficial, suprabasal blistering; the authors suggested the term “lethal acantholytic EB”.⁴³ Considering the apparent rarity of these conditions, it remains to be seen whether they will be incorporated into the general EB classification. Finally, Kindler syndrome, a genodermatosis with skin fragility and photosensitivity, has features of EB. This condition is caused by mutations in the *KIND1* gene, encoding a novel keratinocyte focal contact protein.^{44–45} Our laboratory has not received any cases of these skin fragility syndromes for DNA analysis.

Exceptions to the rules on genotype-phenotype correlation in JEB and HEB

Examination of the mutation database has revealed that there are generally accepted “rules” regarding phenotype-genotype correlations. In general, patients harbouring PTC causing mutations on both alleles tend to suffer from relatively severe disease, whereas patients exhibiting missense or splice junction mutations on one or both alleles tend to display less severe, non-lethal disease. Exceptions to these rules have been noted in the literature and also observed in

this study. One such study reported a 27 year old proband with blisters, milia, and nail abnormalities at birth and a history of corneal erosions and scars; she was noted at the time of study to have widespread blistering of the torso and extremities without scarring alopecia or oral blisters. Unexpectedly, mutation analysis revealed the patient to be a compound heterozygote for PTC causing mutations in *LAMB3* (R635X/1438del5), a combination frequently associated with the Herlitz JEB phenotype.⁴⁶ Another study described six Italian patients representing four different, apparently unrelated, families with generalised cutaneous and occasional oral blistering from birth with significant improvement after puberty to a relatively mild phenotype. Examination revealed skin blistering and atrophic scarring localised to sites of trauma, nail dystrophy, and dental abnormalities, with some patients exhibiting minimal alopecia. All were found to be homozygous for PTC causing mutations in *COL17A1* on both alleles (R795X/R795X).⁴⁷ A third report described two unrelated children, aged 6 and 3 years, with EB-PA diagnosed shortly after birth. Both patients manifested mild blistering and nail dystrophy, and both underwent corrective surgery for gastrointestinal malformations within a week of birth. One patient went on to develop bladder wall haemorrhaging and blistering, bilateral ureteral reflux, unstable detrusor contraction and recurrent urinary tract infection, and ultimately required vesicostomy. This patient also demonstrated enamel hypoplasia. Surprisingly, in spite of mild skin involvement, molecular analysis of ITGB4 revealed both patients to be compound heterozygotes for one PTC causing mutation and either a missense mutation or a splice site mutation (W1478X/3793+1G→A; 4776delG/C38R).⁴⁸

Our study also showed examples of exceptions to the generally accepted genotype-phenotype associations; a subset of three patients with relatively mild JEB disease and minimal blistering tendency was discovered upon mutation analysis to harbour PTC causing mutations on one or both alleles of *LAMB3* or *LAMC2*. Specifically, one patient demonstrated PTC causing mutations on both alleles of *LAMC2* (R245X/R245X), while the other two patients exhibited PTC causing mutations on one allele of *LAMB3* and missense mutations on the other allele (R635X/C293S; W95X/E210K). It should be noted, however, that the nucleotide change (628GAG→AAG) resulting in the E210K mutation occurs in the last nucleotide of exon 7, and this nucleotide change has been shown to result in an alternative mRNA transcript.^{49–50} The mild phenotype in these cases is most likely explained by the in frame splicing of the exon bearing the mutation, leading to synthesis of a shortened, but partially functional polypeptide.

Phenotypic variability of *COL17A1* mutations

Traditionally, mutations in *COL17A1* have been observed to cause a relatively mild phenotype that does not alter the overall lifespan of the affected individual, thus termed generalised atrophic benign EB; however, our study revealed several examples that contradicted this postulate. Of 20 cases in which mutations were discovered in the *COL17A1* gene, seven were reported to display relatively severe disease manifestations and, of these, four exhibited lethal disease. The severe disease manifestations included generalised blistering, haematuria, anaemia, and growth retardation, with one patient demonstrating deformities of the wrists and ankle and pseudosyndactyly, features more frequently associated with recessive DEB. Seven mutant alleles were identified in the four patients with lethal disease; all demonstrated PTC causing mutations (4144del4/4144del4; 3408delC/undetected; 717delA/717delA; R565X/R565X). All four patients with lethal disease were first screened for

mutations in *LAMB3*, *LAMC2*, and *LAMA3*; no abnormalities were found in these genes. Of the remaining three patients with non-lethal phenotype, two exhibited PTC causing mutations on both alleles of *COL17A1* (2690insT/3801insC; 2944del5/Q1023X) and one demonstrated a PTC causing mutation on one allele of *COL17A1* and a splice junction mutation on the other (W465X/2897-2A→C). This study illustrates that the phenotype associated with *COL17A1* gene mutations is more variable than previously recognised and includes the possibility of severe disease manifestations and shortened lifespan in the affected individual. It is conceivable, however, that the quality of the healthcare environment may have an impact on the outcome of these blistering diseases in a global setting.

Further evidence for the variability in phenotype noted with *COL17A1* mutations has been presented in a report describing a patient with clinical features similar to other patients with EBS. Unexpectedly, electron microscopy of the dermoepidermal junction of a skin biopsy from unaffected skin showed both intraepidermal and junctional cleavage. Mutation analysis revealed a paternal nonsense mutation, R1226X, and a large maternal in frame deletion, resulting in elimination of a major portion of the intracellular domain of the collagen XVII (BP180) polypeptide. No abnormalities were found upon search for mutations in the keratin genes, *KRT5* and *KRT14*.⁵¹

Unusual phenotypes

Laryngo-onychocutaneous syndrome

There have been several unusual phenotypes reported in the literature when investigating the genotype-phenotype correlations in JEB and HEB. Firstly, laryngo-onychocutaneous syndrome (LOC syndrome, also known as Shabbir syndrome) represents an autosomal recessive disorder characterised by cutaneous erosions, dystrophic nails, marked dental malformations, and vascular granulation tissue in certain epithelia, especially the conjunctiva and larynx; many affected patients do not survive past childhood. Those patients who do survive the neonatal period and reach adulthood show slow progressive improvement in their condition. This disorder had been seen exclusively in the Punjabi Muslim population and is caused by mutations in the *LAMA3* gene, resulting in loss of gene expression and leading to the characteristic phenotype. Fifteen families from Pakistani Punjabi background were studied in Scotland, England, Northern Ireland, France, and Pakistan; the causative mutation in all except one family was located in exon 39 of *LAMA3A*, designated 151insG. One family demonstrated compound heterozygosity for this mutation and R943X in exon 60 of *LAMA3A*.⁵² The originally described *LAMA3* gene, currently designated as *LAMA3A*, was shown to have an additional 38 exons upstream of the original methionine initiation site and a new upstream methionine initiation codon was found in *LAMA3B*. Thus, the primer location for exon 1 of *LAMA3* was revised during the course of this study and numbering of the mutations was adjusted to reflect the presence of an upstream methionine (+1).⁵² We noted one patient of Pakistani origin in our study who was submitted for mutation analysis with the initial diagnosis of JEB. Electron microscopic examination of skin biopsy showed no definite cleavage plane and focal loss of basal cell hemidesmosomes. Further inquiry revealed a clinical picture of skin erosions on the face and upper extremities, tracheal scarring, gum fibroma-like lesions, and bilateral symplepharon, leading to the suspicion that this patient could have LOC syndrome. Based on the recent publication of the causative mutation for LOC syndrome in families of Pakistani Punjabi background, the patient was subsequently found to be homozygous for the 151insG mutation in exon 39

of *LAMA3A*.⁵² This case serves to further emphasise the phenotypic overlap between JEB and LOC syndrome.

Spontaneous amelioration of the phenotype

Other intriguing cases in the literature have been characterised by spontaneous amelioration of relatively severe EB with age. An early report depicted a male infant born with EB and upper gastrointestinal obstruction diagnosed upon surgical exploration to be pyloric atresia. Shortly after birth, blisters appeared following minimal trauma on the mouth, lower limbs, buttocks, forearms, and hands. A gastroduodenostomy was performed, with numerous blisters developing after surgery. Over the next few months blistering decreased, localising mainly to the fingers, and nail loss was noted. By the age of 1 year, the infant demonstrated only very few blisters on rare occasions.⁵³ Another early report describes a similar clinical course in four different patients who were followed from birth, two of whom were siblings. All four patients were noted to have significant improvement in severity, duration, and rate of blistering occurrence with age when examined at the ages of 17 months, 9 years, and 16 years respectively.⁵⁴ Molecular correlations for these dramatic improvements were lacking in these early cases.

More recently, one report describes a 14 year old boy who presented at birth with extensive skin blistering, pyloric atresia, and urethrovaginal obstruction.⁵⁵ Over the subsequent years, following surgical correction of the pyloric atresia, his skin condition improved to the point where blisters were induced only upon prolonged rubbing of the skin. Mutation analysis revealed compound heterozygosity for two splice junction mutations in *ITGB4* (3986-19T→A/3802+1G→A). Analysis of mRNA revealed that the intronic location of the 3986-19T→A mutation resulted in aberrant splicing of the integrin $\beta 4$ pre-mRNA. However, RNase protection assays showed that a fraction of the integrin $\beta 4$ pre-mRNA transcribed from the mutated parental allele avoided incorrect splicing and produced wild type mRNA, apparently explaining the improvement in the patient's clinical condition with age.⁵⁵ Another study reported a 7 year old white girl noted shortly after birth to have extensive skin blistering and erosions, corneal ulcerations, pitted tooth enamel, and dystrophic nails. Skin blistering decreased progressively over the first few years of life, and by 4 years of age, the child's skin fragility appeared significantly improved. The patient demonstrated compound heterozygosity in *LAMB3* for the PTC causing mutation R635X and another PTC causing deletion, 1587delAG. Reverse transcriptase PCR amplification of total RNA purified from the child's keratinocytes showed that the altered laminin $\beta 3$ mRNA underwent rapid decay shortly after birth. Skipping of the exon containing the 1587delAG mutation restored the open reading frame, thus permitting expression of a shortened laminin $\beta 3$ polypeptide and partially functional laminin 5 protein.⁵⁶ In our study, we observed one patient who fit the picture of spontaneous amelioration. An 8 month old patient was noted with mild blistering at birth on his legs and feet but, since then, has exhibited no further skin blistering. Mutation analysis revealed compound heterozygosity in *LAMB3* for two missense mutations (R366W/D982G), suggesting that the abnormal proteins were able to function in the postnatal period.

Renal and vesicourinary tract involvement in EB-PA

An association has been drawn in the literature between EB-PA and renal and ureteral anomalies. Early reports of this phenomenon included descriptions of three patients with severe genitourinary disease who died in early infancy. An additional report described a 5 month old patient with EB-PA

who developed macroscopic haematuria and was found to have bilateral hydronephrosis with dilated ureters. Interestingly, cutaneous manifestations remained very mild, and further examination revealed pitted tooth enamel, nail dystrophy, and diffuse partial alopecia, mainly over the occiput. Numerous urological procedures were required to preserve renal function.⁵⁷ Another patient with EB-PA had a history of abdominal mass on prenatal ultrasound and required respiratory support at birth owing to the size of the mass. Ultrasound revealed a severely hydronephrotic right kidney with dilated ureter, which was surgically removed. This patient died at 20 days of age from skin infection leading to staphylococcal sepsis and respiratory failure.⁵⁸ Another patient with skin blistering and pyloric atresia that was corrected shortly after birth had history of growth retardation, multiple urinary tract infections caused by bilateral grade 2 vesiculoureteral reflux, and chronic renal insufficiency by 3 years of age. She underwent bilateral ureteral reimplantation and was found to have severely fibrosed ureters and multiple bullae involving her bladder epithelium. Following surgical intervention, her renal function declined to end stage renal disease.⁵⁹ Molecular correlations were not performed in these cases.

There have been reports of EB-PA and renal and ureteral anomalies with molecular correlation. One patient presented shortly after birth with persistent vomiting and was subsequently diagnosed with pyloric atresia. During a later hospitalisation for aspiration pneumonitis, he was noted to have loss of several fingernails and was confirmed by skin biopsy to have EB-PA. Investigation for nephrotic range proteinuria during this hospitalisation led to a renal biopsy, which showed focal segmental glomerulosclerosis and severe foot process effacement. The child had subsequent hospitalisations for respiratory distress leading to tracheostomy, recurrent haemorrhagic cystitis, and secondary obstruction of the vesicoureteral junction. Mutation analysis revealed him to be homozygous for a missense mutation (R1281W) in *ITGB4*.⁶⁰

A group of five patients with EB-PA in our study demonstrated similar renal and ureteral anomalies, including dysplastic/multicystic kidney, hydronephrosis/hydroureter, acute renal tubular necrosis, obstructive uropathy, ureterocele, duplicated renal collecting system, and absent bladder. Some of these patients were noted on prenatal examination to have oligohydramnios, elevated α -fetoprotein and acetylcholinesterase levels, and echogenic material in the amniotic fluid. Three patients exhibited homozygosity or compound heterozygosity for missense mutations in the *ITGB4* gene (D132Y/G276D; R1225H/L336P; C61Y/C61Y). One patient demonstrated a missense mutation on one allele and a splice junction mutation on the other (R60C/3793+1 G→A) and, at 8 years of age, had clinical features of mild blistering on hands and feet only, dystrophic nails, duplicated renal collecting system, and frequent cavities. Another patient, in whom one mutant allele was detected to be a PTC causing one (5040delC), was delivered prematurely at 34 weeks, was noted to have extensive skin blistering, hydronephrosis/hydroureter, and ureterocele, and did not survive past the first day of life.

Surprising genetics

As all 10 genes shown to harbour mutations in the major variants of EB reside in the autosomes, the mutations display autosomal dominant or autosomal recessive inheritance. In fact, previous suggestions of X linked inheritance in EB have been largely discounted.⁴ The assessment of the mode of inheritance on a clinical basis in the absence of family history is complicated by de novo dominant mutations in EBS and DEB in the keratin and type VII collagen genes, respectively.

As JEB cases are inherited exclusively in an autosomal recessive pattern, the occurrence of de novo mutations with an established phenotype is very rare, although paternal germline mosaicism has been documented in one allele of the *LAMB3* gene.⁶¹

In a number of families, mutation detection has yielded evidence for unusual genetics, often with implications for genetic counselling regarding the risk of recurrence. One such situation revolves around uniparental isodisomy, which has been documented in a number of Herlitz JEB cases involving laminin 5 genes. The first reported case was a classic lethal Herlitz JEB case homozygous for a nonsense mutation, Q238X, in *LAMB3*. The mother was found to be a carrier of this mutation while the father had two normal *LAMB3* alleles. Non-paternity was excluded by the use of informative microsatellite markers on different chromosomes. Scanning of the entire chromosome 1 by microsatellite markers revealed that the patient had maternal uniparental meiosis-disomy of region 1q containing the maternal *LAMB3* mutation, thus reducing the mutation to homozygosity.⁶² Similar Herlitz JEB cases, including complete paternal uniparental isodisomy of chromosome 1, have been reported subsequently.⁶³ These unusual mechanisms for Herlitz JEB have implications for assessment of the risk for recurrence in subsequent pregnancies. Specifically, uniparental isodisomy most probably results from non-disjunction at the first meiotic division, preceded by a recombination event and followed by either gamete complementation at fertilisation or trisomy rescue at postzygotic mitosis.⁶² Advanced maternal age has been shown to be associated with non-disjunction in humans. The carrier risk for Herlitz JEB mutations in the laminin 5 genes is 1 in 770, and, therefore, the risk for recurrence in the case of only one parent being a carrier is expected to be much lower than 1 in 4, which would be expected in an autosomal recessive disease.⁹ At the same time, the frequency of uniparental isodisomy in chromosome 1 has been suggested to be ~1 in 200, again attesting to the extremely low risk of recurrence of Herlitz JEB through this mechanism.⁶⁴

Another unique genetic mechanism was shown to result in an unusual phenotype in a patient with a mosaic pattern of skin fragility. Specifically, the patient presented with generalised blistering as a result of minor trauma, leading to cutaneous atrophy, mild mucous membrane involvement, universal alopecia, pigmentary changes, dental anomalies, and nail dystrophy, findings characteristic of GABEB.⁶⁵ However, there were patches of clinically unaffected skin in a symmetrical, leaf-like pattern on the hands and upper arms. The patient was found to be a compound heterozygote for the paternal R1226X and the maternal 1706delA nonsense mutation in *COL17A1*. Immunofluorescence of the affected skin was negative for type XVII collagen epitopes, while the normal appearing skin showed positive, yet attenuated, staining. Careful analysis of keratinocytes isolated from clinically affected and unaffected skin revealed that there was a loss of heterozygosity and correction of the maternal mutation in unaffected skin, most likely caused by mitotic gene conversion. Because mosaicism, as demonstrated in this case with the GABEB phenotype, represents a way of natural gene correction, this rare patient attests to the feasibility of gene therapy for this group of autosomal recessive disorders.⁶⁵ These observations have been recently extended to two unrelated probands with revertant mosaicism in non-Herlitz JEB with multiple correcting *COL17A1* mutations.⁶⁶

An interesting case impacting on genotype-phenotype correlations in JEB has been suggested to involve digenic mutations in the *LAMB3* and *COL17A1* genes. Specifically, a patient with features of both GABEB and Herlitz JEB was

found to be compound heterozygous for nonsense mutations in *COL17A1* (L855X and R1226X) and also to harbour the recurrent R635X mutation in one allele of the *LAMB3* gene.⁶⁷ The authors, on the basis of the clinical phenotype, the absent expression of type XVII collagen and reduced immunofluorescence of laminin 5, suggested that digenic interactions between these two unlinked genes extend the spectrum of genetic heterogeneity of JEB. It should be noted that the proband's mother, a double heterozygote for a null allele in both the *COL17A1* and *LAMB3* genes, was clinically unaffected, and the ultrastructural features of hemidesmosome anchoring filament complexes were normal. These observations confirm that the nonsense mutations in *COL17A1* and *LAMB3* are recessive, and further suggest that this case with unusual genetics is not truly digenic, as previously described for a number of diseases including retinitis pigmentosa.⁶⁸

Clinical implications

Information derived from mutation analysis has translational implications in terms of improved molecular diagnostics and classification with prognostic implications, as well as accurate genetic counselling for risk of recurrence.²⁻⁶⁹ Furthermore, identification of specific mutations has formed the basis for DNA based prenatal testing and for preimplantation genetic diagnosis through blastomer analysis.⁷⁰⁻⁷¹ These issues are dealt with in detail in a subsequent article in this series.

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ELECTRONIC DATABASE INFORMATION

- OMIM numbers of diseases discussed in this study: JEB:226700; GABEB:226650; DEB:131750, 226600; EBS:131760,131800, 131900, 131950; EB-MD:226670; EB-PA:226730; LOC syndrome:245660; Kindler syndrome:173650.

APPENDIX

Table A Novel mutations discovered in this study

Novel mutation/second allelic mutation and the corresponding phenotype*								
PTC causing								
Gene	Nonsense		Insertion/deletion		Missense		Splice site	
LAMB3	E446X/Q373X	H-JEB	54insA/R635X	H-JEB	R366W/D982G	NH-JEB	183+1G→T/183+1G→T	NH-JEB
	C500X/31insC	H-JEB	786delG/786delG	H-JEB	R433W/R635X	H-JEB	299-7C→A/unknown	JEB
	R532X/unknown	H-JEB	976delC/R635X	H-JEB	R569C/R635X	JEB		
	Q586X/167insT	H-JEB	1188del11/1188del11	H-JEB				
	Q995X/2379delG	H-JEB	1588delG/R635X	GABEB				
			2379delG/Q995X	H-JEB				
			2842delG/R635X	H-JEB				
			2842delG/R660X	H-JEB				
			2842delG/2842delG	H-JEB				
			744del4/744del4	H-JEB				
LAMC2	Q261X/3356delG	H-JEB	1730delG/1730delG	H-JEB				
	C592X/C592X	H-JEB	2006del7/2006del7	H-JEB				
LAMA3			3356delG/Q261X	H-JEB				
	R1001X/719delG	H-JEB	719delG/R1001X	H-JEB	I17N/4684+1G→A	LOC	1646+2T→A/1646+2T→A	H-JEB
	R1159X/719delG	H-JEB	719delG/R1159X	H-JEB	M878L/unknown	H-JEB	3350+2T→G/R765X	H-JEB
ITGB4			2830delTA/2830delTA	H-JEB				
	R357X/unknown	EB-PA lethal	125del2/C245G	EB-PA lethal	R60C/3793+1G→A	EB-PA mild	264A→G/3112-1G→A	EB-PA mild
	C706X/2254+4A→G	EB-PA lethal	600delC/unknown	EB-PA	D132Y/G276D	EB-PA lethal	2250+1G→A/3793+1G→A	EB-PA
	Q866X/Q866X	EB-PA	884del2/957del4	EB-PA lethal	C245Y/C61Y	EB-PA lethal	2254+4A→G/C706X	EB-PA lethal
			953del3/953del3	EB-PA	G1307W/G1307W	EB-PA lethal		
			957del4/884del2	EB-PA lethal				
			4576del2/C738X	EB-PA lethal				
			4580del2/4580del2	EB-PA mild				
			5040delC/unknown	EB-PA lethal				
			5046delC/5046delC	EB-PA				
ITGA6			2778+1delG/2778+1delG	EB-PA lethal				
			717delA/717delA	GABEB lethal			464-2A→G/2363-2A→G	NH-JEB
COL17A1	W465X/2897-2A→C	H-JEB	2690insT/3801insC	JEB			1372+1G→T/2948-1G→C	JEB
	R565X/R565X	H-JEB					2897-2A→C/W465X	H-JEB
							3766+1G→A/3766+1G→A	GABEB
	R1169X/R1169X	NH-JEB	3408delC/unknown	H-JEB				
			3569insT/4237insC	GABEB				
			3672del7/unknown	JEB				
			3827insC/R1226X	JEB				
			4144del4/4144delA	H-JEB				

*PTC, premature termination codon; H-JEB, Herlitz JEB; GABEB, generalized atrophic benign EB; NH-JEB, non-Herlitz JEB; LOC, laryngo-onycho-cutaneous syndrome; EB-PA, EB with pyloric atresia. The mutations in **bold** are previously unpublished.

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