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A missense mutation (G1506E) in the adhesion G domain of laminin-5 causes mild junctional epidermolysis bullosa

Maria Scaturro,[a] Patrizia Posteraro,[b] Alessandro Mastrogiacomo,[a] Maria Letizia Zaccaria,[a] Naomi De Luca,[a] Cinzia Mazzanti,[c] Giovanna Zambruno,[a] and Daniele Castiglia[a,*]

[a] *Laboratory of Molecular and Cell Biology, Via dei Monti di Creta 104, 00167 Rome, Italy*
[b] *Department of Clinical and Molecular Pathology, Via dei Monti di Creta 104, 00167 Rome, Italy*
[c] *VIII Dermatology Division, Istituto Dermopatico dell'Immacolata, IRCCS, Via dei Monti di Creta 104, 00167 Rome, Italy*

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Abstract

Laminin-5 is the major adhesion ligand for epithelial cells. Mutations in the genes encoding laminin-5 cause junctional epidermolysis bullosa (JEB), a recessive inherited disease characterized by extensive epithelial–mesenchymal disadhesion. We describe a JEB patient compound heterozygote for two novel mutations in the gene (LAMA3) encoding the laminin α3 chain. The maternal mutation (1644delG) generates mRNA transcripts that undergo nonsense-mediated decay. The paternal mutation results in the Gly1506 → Glu substitution (G1506E) within the C-terminal globular region of the α3 chain (G domain). Mutation G1506E affects the proper folding of the fourth module of the G domain and results in the retention of most of the mutated polypeptide within the endoplasmic reticulum (ER). However, scant amounts of the mutated laminin-5 are secreted, undergo physiologic extracellular maturation, and correctly localize within the cutaneous basement membrane zone in patient's skin. Our findings represent the first demonstration of an ER-retained mutant laminin-5 leading to a mild JEB phenotype.

Keywords: Inherited blistering skin diseases; LAMA3; LG module; Mutation; Protein folding

Laminin-5 is a laminin isoform expressed in the basement membrane of the skin and mucous epithelia and represents the major adhesion ligand of epithelial cells [1]. Laminin-5 is synthesized within the basal keratinocytes as a precursor heterotrimeric molecule composed of an α3 (200 kDa), a β3 (140 kDa), and a γ2 (155 kDa) chain encoded by the genes LAMA3, LAMB3, and LAMC2, respectively. The three chains associate within the endoplasmic reticulum (ER) through their C-terminal domains I and II to form a triple-stranded α-helical coiled-coil rod domain [2,3]. After assembly, laminin-5 molecules are secreted in the extracellular matrix, where the γ2 and α3 chains undergo proteolytic processing. A cleavage at the NH_2-terminus of the γ2 chain generates a 105 kDa mature γ2 polypeptide [1,4]. A distinct extracellular proteolytic processing cleaves the α3 chain within its G domain reducing its size from 200 to 165 kDa [1,5,6]. Like other laminins, the G domain of laminin-5 is composed of five homologous modules (LG1-LG5) of approximately 180–200 residues disposed in a tandem array [7,8], and each of them forms an autonomous folding unit which harbors unique binding sites for integrin receptors and cell surface heparan sulfate proteoglycans [9,10]. Thus, the LG subdomains appear essential for laminin-5-mediated cell adhesion and motility [5,11].

Laminin-5 binds to cells via two integrin receptors, α3β1 and α6β4. The α6β4 integrin is the only integrin component of hemidesmosomes (HD), multiprotein complexes that connect basal keratinocytes to the basement membrane zone (BMZ) [12]. Mutations in

* Corresponding author. Fax: +39-06-6646-4705.
E-mail address: d.castiglia@idi.it (D. Castiglia).

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either laminin-5 or α6β4 integrin genes are associated with junctional epidermolysis bullosa (JEB), a recessive inherited blistering disease of skin and mucous membranes in which tissue separation occurs within the lamina lucida of the BMZ [13]. Specifically, null mutations in the genes encoding the three laminin-5 chains are distinctive of the Herlitz variant of JEB (H JEB; OMIM 226700) in which severe defects in epithelial cell adhesion lead to death early in infancy. Other mutations, usually missense or in frame deletions, result in reduced expression of aberrant laminin-5 molecules that maintain residual biological activity and cause a milder non-lethal variant of JEB, named non-Herlitz JEB (non-H JEB; OMIM 226650) [14]. In the majority of JEB cases with laminin-5 deficiency, the affected gene is LAMB3, due to the recurrence of hotspot mutations [15]. A recent study on a cohort of JEB families has described for the first time a significant proportion of mutations also in the LAMA3 gene. Most of these mutations result in premature termination codon (PTC) formation [14]. Two missense mutations, both affecting conserved residues within the α3 G domain, have been identified but their respective etiopathological role in the disease has not been investigated at the biochemical level [14,16].

In the present study, we report on a compound heterozygous non-H JEB patient harboring novel frameshift (1644delG) and missense (G1506E) mutations in the LAMA3 gene. By structural modeling analysis, we provide evidence that missense mutation G1506E interferes with proper folding of the LG4 module of α3. In addition, analysis of mutation consequences at the protein level demonstrates that most of misfolded α3 polypeptides are retained in the ER. However, scant amounts of heterotrimeric laminin-5 harboring the mutated α3 chain are secreted and undergo physiological proteolytic processing, thus providing partial adhesion functions and explaining the mild phenotype of the proband.

Materials and methods

Case report. The proband, a 43-years-old female, was the second child of healthy, non-consanguineous parents. The patient suffered from cutaneous blistering since birth, but growth was normal and skin condition significantly improved after puberty. Physical examination revealed cutaneous blisters and atrophic scarring strictly localized at trauma sites, i.e., hands, feet, elbows, knees, and legs, as well as nail dystrophy and dental abnormalities. Hair involvement was limited to eyebrow alopecia. The diagnosis of JEB was confirmed by ultrastructural examination of a perilesional skin biopsy that showed tissue separation within the lamina lucida of the dermal–epidermal junction.

Keratinocyte cultures. Human epidermal keratinocytes obtained from skin biopsies of the patient and healthy volunteers were cultivated on a feeder-layer of lethally irradiated 3T3-J2 murine fibroblasts (a gift from Prof. Howard Green), as described [17].

Immunofluorescence studies. Frozen skin sections obtained from a skin biopsy of the proband and healthy controls were processed for immunofluorescence according to a three step biotin–streptavidin–fluorescein procedure previously described [18]. Cultured primary keratinocytes grown on glass coverslips in 6-well tissue culture plates were subjected to an indirect immunofluorescence procedure [19]. Antigen mapping was performed using monoclonal antibody (mAb) GB3 raised against native laminin-5 (gift from G. Meneguzzi, INSERM U385, Faculté de Medicine, Nice, France), mAb K140 specific to the laminin β3 chain (gift from R. Burgeson, Cutaneous Biology Research Center, Charlestown, MA), mAb BM165 specific to the laminin α3 chain (gift from R. Burgeson), and a polyclonal antibody (pAb) raised against the glutathione *S*-transferase fusion protein of the NH_2-terminal region of the processed (105 kDa) human laminin γ2 chain (amino acid residues 488–886). Rabbit immunization was carried out by PRIMM (Milan, Italy). MAb 1A8C specific to type XVII collagen (gift from K. Owaribe, Nagoya University, Nagoya, Japan), mAb G0H3 against the α6 integrin subunit (gift from A. Sonnenberg, the Netherland Cancer Institute, Amsterdam, The Netherland), and the mAb 3E1 directed against the β4 integrin subunit (Telios Pharmaceuticals) were also used.

Northern analysis. Total RNA was extracted from keratinocyte cultures by the guanidinium–thiocyanate method and electrophoresed on formaldehyde agarose gel followed by blotting on nylon membranes (Amersham Biosciences). Membranes were hybridized with ^{32}P-labeled cDNA probes corresponding to the three chains of laminin-5 as previously reported [21]. Quantification of autoradiograms was performed by densitometric scanning with a Gel Doc 1000 (Bio-Rad).

Mutation detection. Genomic DNA was obtained from peripheral blood of the patient and family members by a standard procedure. Mutation analysis of the LAMA3 gene encoding the keratinocyte specific "a" transcript was performed by PCR amplification of individual exons and flanking intronic sequences using primer pairs and conditions already described [14,22]. PCR products were subjected to heteroduplex analysis on MDE gels (FMC) and those presenting with heteroduplexes were directly sequenced (ABI Prism 377, Applied Biosystems).

Allele specific analysis of the transcripts. To distinguish transcripts deriving from each of the two mutant alleles of the patient, total RNA from keratinocyte cultures was reverse-transcribed as previously described [21] and amplified using primers (L) 5′-GCAATGATGGG AAATGGCAC-3′ (nts 4328–4347) and (R) 5′-TCTGTGTCCAGTT CCAGGTG-3′ (nts 4900–4919) (T_{ann}, 57 °C) designed on the LAMA3 cDNA sequence (GenBank Accession No. L34155). The resulting PCR product was restricted with *Bsm*I (Roche Molecular Biochemicals) and fractionated by 2% agarose gel electrophoresis.

Immunoprecipitation analysis. Radioimmunoprecipitation of cell lysates and culture medium was performed as described [20]. Immunoprecipitated samples were analyzed by SDS–PAGE on 6% polyacrylamide gels under reducing conditions, followed by autoradiography. Quantification of autoradiograms was performed as above.

Immunoelectron microscopy. Cultured keratinocytes obtained from patient's and control skin biopsies were fixed and processed for immunoperoxidase-electron microscopy as previously described [23,24]. The primary antibody (BM165 recognizing the α3 chain) and the peroxidase-conjugated goat anti-mouse Fab (Biosys) were diluted 1:100. After the 3-3′-diaminobenzidine reaction, specimens were processed as for standard electron microscopy.

Structural modeling. The three-dimensional model of human laminin α3LG4 subdomain was generated on the basis of the known atomic structure of murine laminin α2 chain LG4–LG5 pair deposited with Protein Data Bank entry 1dyk [8]. By using the Insight II software (Molecular Simulation), the sequence of α3LG4 module was inscribed onto the structural frame provided by the residues of α2LG4.

Results

Laminin-5 expression and identification of genetic defects in LAMA3

Immunofluorescence analysis of a patient's skin biopsy showed markedly reduced expression of laminin-5 chains along the cutaneous BMZ, as compared with normal control skin (not shown). The level of expression of the genes for the laminin-5 β3, γ2, and α3 chains was then assessed by Northern blot analysis of total RNA obtained from cultured keratinocytes of the patient and a healthy control and standardized by GAPDH hybridization. Densitometric analysis of the LAMA3/GAPDH signal ratio revealed an ≅ 40% reduction of the α3 mRNA steady-state level in the patient, indicating LAMA3 as the candidate gene for the disease (Fig. 1A).

Heteroduplex analysis of the PCR products spanning the LAMA3 exons and subsequent sequencing of exons 14 and 34 disclosed compound heterozygosity for mutations 1644delG and 4517G → A (Fig. 1B). The G deletion generates a PTC at amino acid residue 587, within the rod domain, while the G → A transition leads to the missense mutation G1506E within the LG4 module and results in the formation of a restriction site for *Bsm*I. The patient's mother was heterozygous for 1644delG and both patient's sons were healthy heterozygous carriers for G1506E (not shown). The latter mutation was not detected in 75 constitutive DNA samples from healthy unrelated individuals. No additional nucleotide sequence change was identified in the coding region of α3 chain.

Allele specific analysis of the transcripts

Since the two mutations might have a different impact on the stability of the corresponding mRNAs, we evaluated the relative abundance of the transcripts originating from the two LAMA3 alleles by RT-PCR. The cDNA region (nts 4328–4919) encompassing the missense mutation G1506E was amplified from the patient and a normal control and the resulting 592-bp PCR product digested by *Bsm*I (Fig. 1C). In the patient, enzyme digestion resulted in two bands of 397- and 195-bp, representing the cleavage products of cDNA transcripts carrying mutation G1506E and thus attesting at the expression of the corresponding allele. In contrast, the 592-bp band became undetectable following the *Bsm*I digestion, indicating that the 1644delG-containing allele is not expressed at the mRNA level.

Immunodetection of mutant laminin-5

To examine mutation consequences at protein level, the expression of laminin-5 was investigated by immunoprecipitation (IP) and immunofluorescence (IF) analyses on patient and control cultured keratinocytes, using mAbs BM165, K140, and GB3 recognizing the α3 and β3 chains and heterotrimeric laminin-5, respectively. By IP analysis, precursor α3 (200 kDa) and γ2 (155 kDa) laminin-5 chains as well as β3 (140 kDa) polypeptides were detected in higher amount in patient's cultured keratinocyte lysates compared to normal control keratinocytes with all antibodies used. No additional truncated α3 polypeptides encoded by transcripts harboring mutation 1644delG were visualized (Fig. 2A). IP analysis of spent culture medium from patient's keratinocytes showed the presence of mature laminin-5, as demonstrated by detection of a 165 kDa

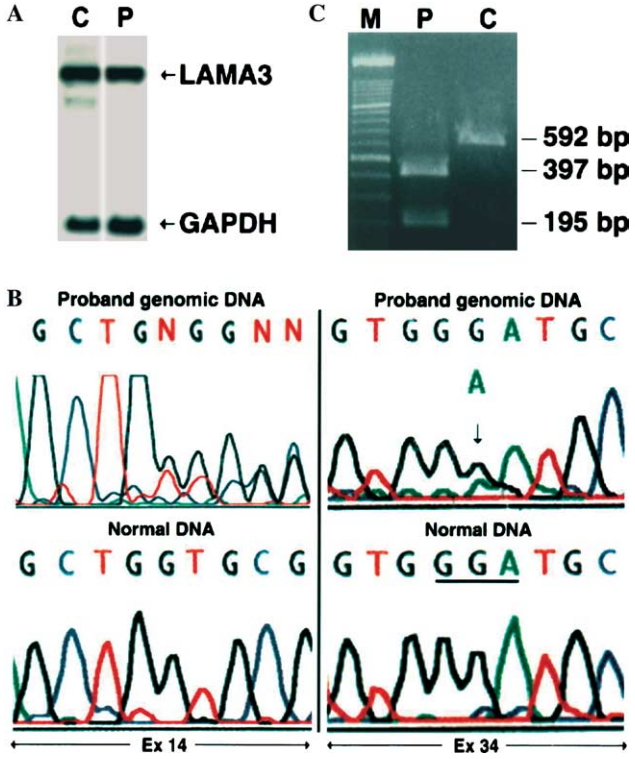

Fig. 1. LAMA3 mRNA expression and mutation detection. (A) Northern blot analysis of total RNA shows a signal of reduced intensity for the LAMA3 probe in the patient (P) compared to control keratinocytes (C); a GAPDH probe was used as loading control. (B) Compared with normal DNA, direct nucleotide sequencing of exon 14 shows a heterozygous frameshift mutation leading to overlapping sequence traces (left panel). Sequence analysis of subcloned DNA fragments revealed a G deletion at nucleotide position 1644 (1644delG) (not shown). Direct DNA sequencing of exon 34 reveals a heterozygous G → A transition at nucleotide position 4517 in the proband genomic DNA (right upper panel, arrow) compared to normal DNA (right lower panel). This mutation converts a glycine residue to glutamic acid at codon 1506 (G1506E) (underlined). (C) Total RNA isolated from proband and healthy control keratinocytes was reverse-transcribed and amplified to generate a 592-bp fragment spanning exons 32–37 of the LAMA3 cDNA. Upon *Bsm*I digestion, this PCR product is completely digested in two bands of 397 and 195 bp in the patient (P), while the same fragment remains unrestricted in the control (C) (M; 100-bp DNA ladder).

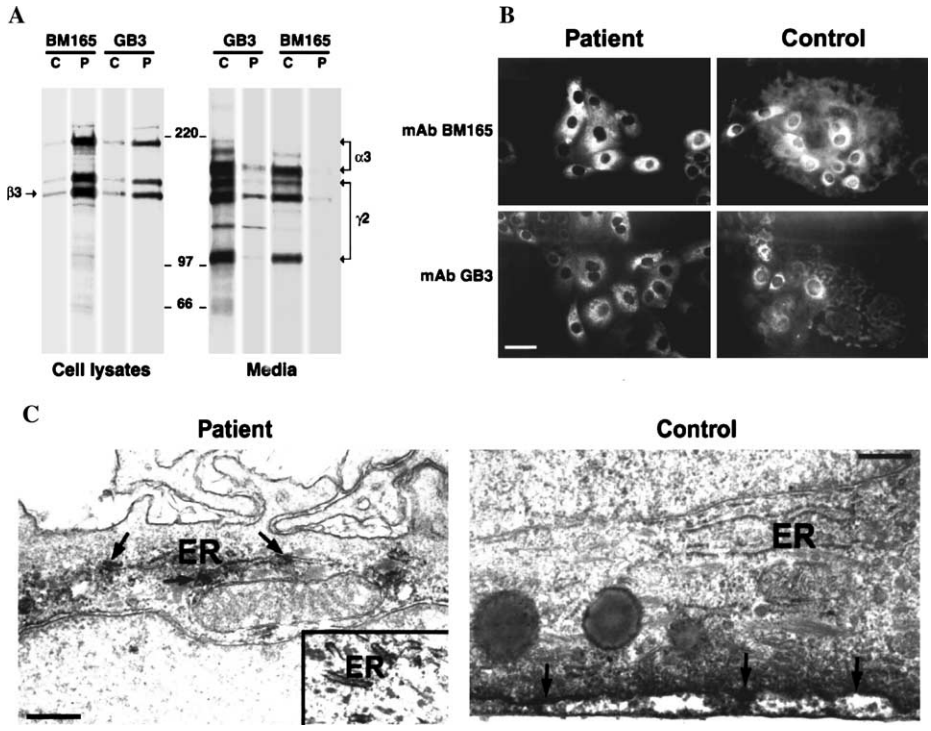

Fig. 2. Laminin-5 expression and its subcellular localization in patient's keratinocytes. (A) Immunoprecipitation of laminin-5 from cell lysates and media of ^{35}S-labeled keratinocytes from the patient (P) and a normal control (C), using monoclonal antibodies (mAbs) BM165 and GB3. Equal amounts of protein-bound radioactivity were immunoprecipitated and loaded on the gels under reducing conditions. Increased levels of unprocessed laminin-5 are observed in lysates from patient's cells in comparison with normal control cells. Conversely, markedly reduced levels of processed laminin-5 are detected in the media from patient's keratinocytes as compared to normal cells. Similar results were obtained using mAb K140 against the β3 chain (not shown). Arrows indicate the position of the three chains of laminin-5. Numbers refer to the position of marker proteins in kilodaltons. (B) Indirect immunofluorescence of patient's and control cultured keratinocytes using mAbs BM165 and GB3. An intense intracellular staining is detected in both patient's and control keratinocytes. In contrast, only control cells exhibit a strong extracellular reactivity with both antibodies. Scale bars, 20 μm. (C) Cultured keratinocytes from the patient and a normal control were fixed and immunolabeled for laminin-5 α3 chain using the BM165 antibody and an anti-mouse Fab fragment conjugated with peroxidase. After labeling, the cells were processed for transmission electron microscopy. In patient's cultured keratinocytes, the immunoreactivity for laminin α3 chain (arrows) is prevalently localized in the ER compartment, as shown at higher magnification in the inset. In the control cells, laminin-5 immunoreactivity concentrates along the plasma membrane (arrows) and outside the cell, within the substratum. In these cells, the ER areas appear mostly immunonegative. Scale bars, 0.13 μm.

band (α3 chain) and a 105 kDa band (γ2 chain). The immunoprecipitated bands were much fainter than in normal keratinocytes, however, and their intensity was estimated to be about 12% of the normal control by densitometric analysis (Fig. 2A). IF studies showed intense staining of the cytoplasm in both patient's and control (Fig. 2B) keratinocytes. However, patient's keratinocytes secreted and deposited laminin-5 at markedly reduced levels compared to control cells, as shown by highly decreased laminin-5 immunoreactivity outside the cells.

The subcellular localization of the mutated polypeptides was then investigated by immunoelectron microscopy (IEM). In the patient's cells, the laminin α3 chain immunoreactivity was prevalently and abundantly localized in the lumen of the ER, with only rare cisterns of Golgi apparatus being immunostained. Conversely, in the control cells immunoreactivity concentrated mainly along the plasma membrane and in the matrix deposited onto the culture vessel (Fig. 2C).

Homology modeling of G1506E mutant LG4

Collectively, these results indicated that full-sized trimeric laminin-5 was retained intracellularly probably as a consequence of a local folding defect in the mutant LG4 subdomain. To rationalize the effects of mutation G1506E on the folding of this subdomain, we used the known crystal structure of the laminin α2LG4 as a template to model the structure of α3LG4 module in the vicinity of the mutation. Laminin LG modules are β sandwiches of roughly globular shape composed of 14 antiparallel strands (A–N) arranged in two sheets [25]. G1506 resides in a buried position at the beginning of the β-strand L at one edge of the convex β-sheet, surrounded by T1398 of β-strand C and F1504 of the loop connecting β-strands K and L (Fig. 3C). G1506 and T1398 are strictly conserved between α2LG4 and α3LG4, whereas F1504 corresponds to L2902 in α2 (Fig. 3A). However, phenylalanine in the latter position is highly conserved among LG modules from laminins

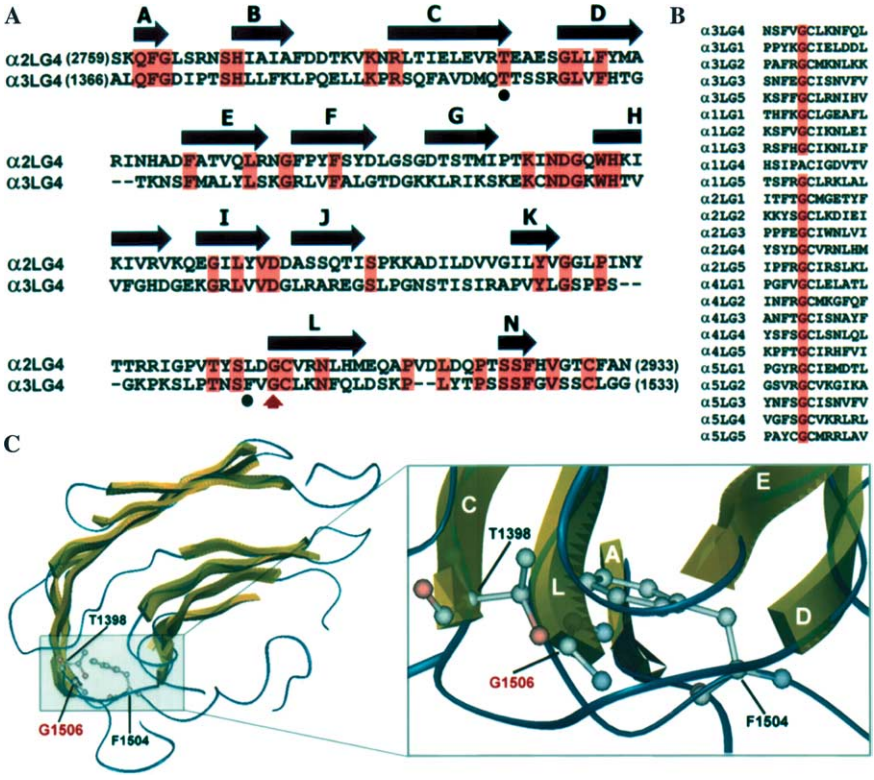

Fig. 3. Structure-based sequence alignment and structural modeling of the LG4 sequence involved in mutation G1506E. (A) Alignment of the LG4 amino acid sequence of human laminin α3 (α3LG4) and mouse α2 (α2LG4) chains (24% sequence identity, 59.4% similarity). Amino acid identity is pink-shaded. G1506 is marked by a red arrow and overlaps with conserved G2904 in the α2 chain. β-Strands of the LG fold are indicated above the alignment. Amino acid residues shown in panel C are marked by a black dot. (B) Alignment of the sequences of human α1–α5 laminin LG1-5 modules encompassing-strand L. Glycine conservation is highlighted in pink. Sequences were taken from SwissProt P25391 (hLAMA1), P24043 (hLAMA2), Q16787 (hLAMA3), Q16363 (hLAMA4), Q60675 (mLAMA2) and from GenBank NP_005551 (hLAMA5). (C) Modeling of the local structure of the α3LG4 subdomain around the Gly residue (G1506) involved in the mutation. Selected residues T1398 and F1504, important for interpreting the effects of mutation G1506E in the α3LG4 module, are shown as ball & stick models (see text). Black lines point to the Cα of residues.

(Fig. 3B) and other related molecules [25]. G1506 is immediately followed by the conserved disulfide bridge typical of the LG fold, formed by Cys1507 and Cys1530 [7]. When mutation G1506E is introduced in this model, the Cγ and the carboxylate group of the bulky E side chain clash against the aromatic ring of F1504 (not shown). Furthermore, the Cβ and the carboxylate oxygen of E bump into the Cβ of T1398, and the carboxylate oxygen of E also bumps into the Cγ of the same threonine. Thereby, G1506E mutation is predicted to cause imperfect local folding.

Discussion

In this report, we describe two novel mutations in the LAMA3 gene in a patient affected with a non-H JEB phenotype. The two mutations, 1644delG and G1506E, differently affect the expression of the LAMA3 alleles. Frameshift 1644delG results in a PTC and is expected to cause rapid nonsense-mediated decay of the corresponding RNA transcripts, thus leading to undetectable gene products [26]. The pathogenic effect of this mechanism has been described for all laminin-5 genes [21,26]. Consistent with this notion, northern and allele-specific RT-PCR analysis of the total RNA isolated from the patient's keratinocytes showed undetectable levels of transcripts carrying mutation 1644delG and a normal steady-state level of mRNA bearing mutation G1506E. Furthermore, no additional truncated α3 polypeptide potentially encoded by the RNA transcripts harboring PTC was detected by immunoprecipitation analysis of patient's keratinocytes. These findings demonstrate the functional hemizygosity of our proband for mutation G1506E at the mRNA and protein level.

Mutation G1506E is localized in the LG4 subdomain. Laminin LG modules display a well-conserved globular structure made of two antiparallel seven-stranded β sheets forming an imperfect jelly roll motif [25] (Fig. 3C, left). In particular, residue G1506 is strictly conserved throughout all the LG modules of the known human α chains, with the exception of the LG4 module of the α1 chain, where it is replaced by an alanine residue, another small aliphatic amino acid (Fig. 3B). Moreover, a glycine residue at this position represents an important structural feature of the LG fold, because it is conserved

in LG modules distantly related to laminin α chain, as those in protein S, Gas6, sex hormone binding globulin [27], neurexins [28], and agrin [25]. The structural model of α3LG4 presented in Fig. 3C clearly shows that, due to the tight packing of residues T1398 and F1504 around G1506, the large charged side chain of a glutamic acid at position 1506 cannot be accomodated without causing structural changes.

The predicted altered folding of the α3LG4 carrying mutation G1506E is likely to be responsible for ER-retention through persistent binding of the mutated domain to resident ER foldases [29]. Indeed, IP studies on cultured patient's keratinocytes demonstrated heterotrimeric laminin-5 intracellular accumulation and IEM analysis confirmed that laminin-5 accumulation occurs within the ER. Conversely, IF and IP studies showed markedly reduced secretion of heterotrimeric laminin-5 molecules. Numerous missense mutations that impair secretion of multi-domain glycoproteins have been shown to affect residues conserved in related sequences and likely involved in determining the structure of single protein modules [30]. Among PROS1 gene mutations that cause hereditary thrombophylia [27], substitution of conserved and structurally important glycines within the LG modules of protein S that resides at the beginning of buried β-strands lead to decreased secretion and low plasma levels of the protein [27,31]. Similarly, a Gly to Glu mutation in the globular domain N2 of the α3 chain of type VI collagen has been shown to cause protein misfolding and reduced secretion of heterotrimeric collagen VI in a family with Bethlem myopathy [32]. More recently, a mouse model of recessive nephrogenic diabetes insipidus due to an ER-retained mutant aquaporin-2 (T126M) was established [33]. Concerning LG modules in laminin isoforms, a single missense mutation (L2564P) in the G domain of the laminin α2 chain has been reported in a compound heterozygous patient with a mild form of congenital muscular dystrophy. Mutation L2564P involves a highly conserved leucine residue within β-strand D of the LG3 subdomain. In this patient, the mild phenotype correlated with residual tissue expression of mutant laminin-2 [34].

On the other hand, our IP findings on the patient's keratinocyte lysates indicate that mutant α3 polypeptides harboring G1506E at the carboxyl terminus assemble with the γ2 and β3 laminin chains through the coiled-coil domains I and II to form heterotrimeric laminin-5 molecules. Therefore, mutation G1506E within the G domain causes ER-retention without significantly impairing trimerization of the rod domain. These data are in keeping with reports on quality-control mechanisms for protein synthesis indicating that: (i) protein folding defects are recognized at the level of individual domains by the ER quality-control system and (ii) large proteins can have some misfolded domains that continue to re-associate with resident ER chaperones in an attempt to fold properly and other correctly folded domains that do not and are therefore available for oligomerization with other protein subunits [29,35,36].

The residual mutant heterotrimeric laminin-5 secreted by patient's keratinocytes undergoes normal proteolytic processing within the LG3/LG4 linker segment, as shown by the presence of the 165 kDa processed α3 chain form in the culture medium. Thus, mutation G1506E does not appear to interfere with the proteolytic processing of the α3 chain that cleaves off the C-terminal LG4-5 modules after secretion into the extracellular matrix. Indeed, the scant amount of immunoreactive heterotrimeric laminin-5 detected along the cutaneous BMZ of our proband corresponds to mature laminin-5 molecules encoded by the mutant G1506E allele.

The mild JEB phenotype of our patient, presenting only skin lesions limited to trauma-exposed sites, could result either from a reduced synthesis and secretion of a still functional mutated laminin-5 or from the combined effect of the impaired laminin-5 secretion and an altered functionality of the mutant G1506E α3 polypeptide. It has been reported that highly reduced amounts (≅100-fold) of wild type laminin-5 fail to restore normal cell adhesion in vitro and cause a lethal EB phenotype [37]. Similarly, in vitro data have indicated that a threshold level of laminin-5 is required to support efficient cell adhesion ([37] and reference herein). Therefore, the reduced availability of laminin-5 molecules in patient's skin is expected to destabilize the basement membrane structure. On the other hand, the processed form of laminin-5 found in the basement membrane conserves the LG1-3 modules, which carry the primary sites for binding to integrin receptors, and is depleted of the LG4-5 modules which are not essential to cell adhesion [5,9–11]. The mutated laminin-5 secreted by the patient's keratinocytes is therefore expected to maintain its integrin-binding function and major adhesive properties, and its reduced availability is likely to be the primary cause of the skin fragility observed in the patient. However, it has also been demonstrated that the LG4-5 fragment of laminin α3 chain supports cell adhesion by binding heparan sulfate proteoglycans on the cell surface via a major binding site localized in the LG4 module (residues 1412–1423) [38,39]. Consequently, it cannot be formally excluded that the LG4-5 proteolytic fragment of laminin α3 chain carrying mutation G1506E in the LG4 domain may have defective adhesive properties and contribute to some extent to the pathological phenotype.

In conclusion, our data expand the repertoire of LAMA3 mutations and correlate the mild non-H JEB phenotype of our patient to the combination of a PTC mutation, affecting LAMA3 mRNA stability, and a missense mutation which hampers laminin-5 secretion

through local protein misfolding. These findings demonstrate for the first time a role for an ER-retained mutant laminin-5 in the etiology of JEB and contribute to a better understanding of the physiologic functions of the laminin G domain.

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