

Complexity of Transcriptional and Translational Interference of Laminin-332 Subunits in Junctional Epidermolysis Bullosa with *LAMB3* Mutations

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Epidermolysis bullosa (EB) is a group of rare inherited blistering skin disorders, mostly caused by mutations in genes encoding structural proteins within the dermal-epidermal junction (DEJ) (1). Junctional EB (JEB) is one of the 4 classical subtypes characterized by blister formation within lamina lucida; inheritance is autosomal recessive underscored by bi-allelic mutations in *LAMA3*, *LAMB3*, *LAMC2* (the laminin-332 genes), *COL17A1* (type XVII collagen), *ITGA6*, *ITGB4* or *ITGA3* ($\alpha6$, $\beta4$, $\alpha3$ integrin subunits). Approximately 80% of patients with laminin-332-deficient JEB have mutations in *LAMB3*, encoding the $\beta3$ subunit of laminin-332, a heterotrimeric protein ($\alpha3$, $\beta3$ and $\gamma2$ laminin polypeptides) at DEJ (2).

MATERIALS, METHODS AND RESULTS

Although the laminin-332 molecular pathology in JEB may involve mutations in any of the *LAMA3*, *LAMB3* or *LAMC2* genes, it is unclear whether or how mutations in any of these genes affect the expression of the other 2 genes/proteins, and how this impacts on phenotype. We report 3 JEB patients presenting a variable degree of blistering, nail and teeth abnormalities (Fig. 1a) in whom we also explore the laminin-332 pathology in detail. Patient 1, a 29-year-old female, had recurrent erosions on thighs with persistent bacterial colonization. She also had nail dystrophy, corneal erosions, and dental enamel defects. Patient 2, a 16-year-old boy, had blisters, eczematous plaques on legs, nail dystrophy, but no visible enamel anomaly. Patient 3, an 18-month-old girl, had mild generalized blistering at birth with gradual spontaneous improvement. All her nails were dystrophic, with teeth being difficult to evaluate because only deciduous teeth were present.

Using whole-exome sequencing, compound heterozygous mutations in *LAMB3* (NM_000228.3) were identified in all 3 cases: Patient 1: c.373-9T>A and c.3119G>A (p.Trp1040Ter); Patient 2: c.629-12T>A and c.3512G>A (p.Cys1171Tyr); Patient 3: c.629-12T>A and c.2137+1G>T (Fig. 1b, Fig. S1a¹). Among them, c.3119G>A, c.3512G>A and c.629-12T>A were reported (3). Reverse-transcription PCR and Sanger sequencing confirmed the interference with *LAMB3* mRNA splicing by all 3 splice-site mutations: c.373-9T>A led to exon 6 skipping, causing in-frame deletion; c.629-12T>A caused retention of the first 10 bp in intron 7; and c.2137+1G>T resulted in exon 15 skipping, both of which caused frameshifts and downstream premature termination codons

(Fig. 1c). Microfluidic electrophoresis demonstrated leakiness of all 3 splicing variants: mutant versus wild-type transcript level was 40:1 for c.373-9T>A, 1.3–1.7 for c.629-12T>A, and 3:1 for c.2137+1G>T (Fig. S1b¹). Immunofluorescence microscopy (IFM) demonstrated either absence or decreased staining for $\beta3$ and $\gamma2$ subunits, whereas $\alpha3$ subunit immunoreactivity showed linear labelling (Fig. S2a¹). Quantitative PCR (Q-PCR) revealed significant decrease in *LAMA3*, *LAMB3*, *LAMC2* transcripts (Fig. S2b¹). To investigate whether *LAMA3* and *LAMC2* reduction is reproducible in cultured keratinocytes, siRNA knockdown of *LAMB3* in normal human epidermal keratinocytes was performed. Knockdown efficiency was greater than 90%, and *LAMA3* transcripts were significantly reduced. *LAMC2* was modestly reduced by 1 of the 2 *LAMB3* siRNAs. The interference by *LAMB3* knockdown was exclusive to laminin-332, since the transcription of other laminin genes expressed in keratinocytes and at DEJ, *LAMA5*, *LAMB1*, *LAMC1*, was unaffected (Fig. S2c¹). For further details, please see Appendix S1¹.

DISCUSSION

Gene expression, synthesis and the degradation of polypeptides that form complexes, such as laminins, are strictly regulated to maintain proportionality (4). For example, it was shown that mutations in *LAMA3*, *LAMB3* or *LAMC2* modulate transcription and expression of the other 2 subunits (5, 6). Current study investigated the impact of *LAMB3* mutations on laminin-332 subunits at both protein and mRNA levels, revealing that nonsense-mediated mRNA decay hampers the abundance of *LAMB3* transcripts (7) and further impedes the transcription of the 2 other genes. This implies that transcriptional activity may have mutual inter-dependence for these 3 genes, while further work is warranted to ascertain the significance and the detailed mechanism(s) therein (8).

In theory, protein expression should display some correlation with mRNA levels (5), although discrepancies are present in our study. Notably, despite significant decrease in *LAMA3* transcripts, $\alpha3$ subunit expression was nearly normal in all 3 patients. This may be explained by compensated translational efficiency, protein turnover (4) and $\alpha3$ subunit also being expressed in another laminin isoform at DEJ, laminin-311 (9), which is expressed in both JEB skin and keratinocytes with *LAMB3* mutations (10, 11).

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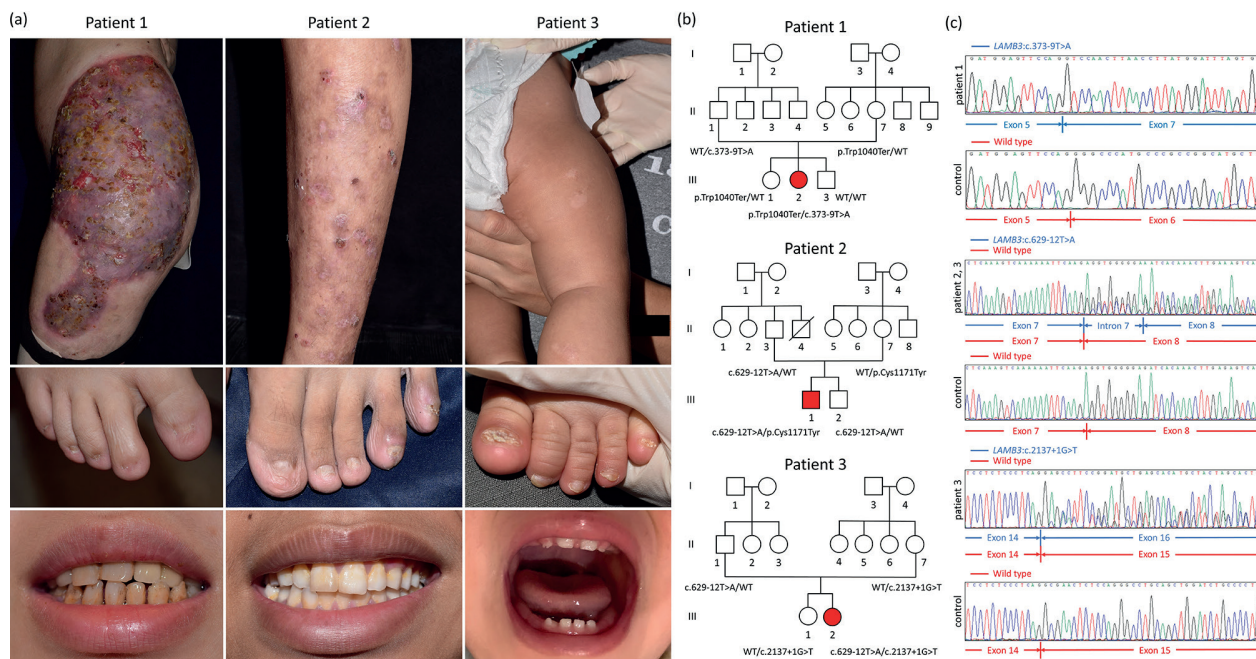


Fig. 1. Clinical presentations and mutational analyses. (a) Diverse clinical manifestations of blisters, nail, and teeth abnormalities in the 3 patients with junctional epidermolysis bullosa. (b) All 3 cases carried compound heterozygous mutations in *LAMB3* (NM_000228.3). Patient 1 carried c.373-9T>A (IVS6-9T>A) and c.3119G>A (p.Trp1040Ter); Patient 2 had c.629-12T>A (IVS8-12T>A) and c.3512G>A (p.Cys1171Tyr); Patient 3 harboured c.629-12T>A (IVS8-12T>A) and c.2137+1G>T (IVS15+1G>T). WT: wild type. Sanger sequencing results are shown in Fig. S1a¹. (c) Reverse-transcription PCR and Sanger sequencing confirmed the interference with *LAMB3* mRNA splicing by all 3 splice-site mutations: c.373-9T>A caused the skipping of the entire exon 6; c.629-12T>A led to the retention of the first 10 bp in intron 7; c.2137+1G>T resulted in the skipping of exon 15.

Besides, protein expression levels are presumed to be predictive of clinical severity and are related to the identified mutations (5). Indeed, immunoreactivity of laminin-332 antibody staining is often used for clinical diagnostics and prognostics (12). Here, however, further disparities were evident. Patient 2 has more severe phenotype than Patient 3, despite IFM showing greater intensity of GB3 staining, which was reported to be reflective of clinical severity in JEB (13). Both individuals have the same *LAMB3* mutation, c.629-12T>A, but differ in their other mutations: Patient 2 has a missense mutation, p.Cys1171Tyr and Patient 3 has a splice-site mutation, c.2137+1G>T. This discordance between IFM and phenotypic severity is likely to relate to the leakiness of c.2137+1G>T (14) and the functional impact of the cysteine substitution: p.Cys1171Tyr in the β 3 chain forms an interchain disulphide bond with the γ 2 subunit and terminates the coiled-coil region of the heterotrimer (15), and thus p.Cys1171Tyr probably has a greater overall deleterious impact on functional laminin-332 levels. Collectively, current IFM studies reiterate published clinical experience that GB3, a primary antibody targeting a conformational epitope of γ 2 subunit, is probably the best discriminator in the majority of JEB cases carrying *LAMB3* mutations (13). GB3 immunolabelling was substantially reduced in Patient 1, but partially reduced in Patients 2 and 3, congruent with their degree of clinical severity. Notably, the relatively leaky splicing variants, c.629-12T>A and c.2137+1G>T, harboured by Patients 2 and 3 may, in part, explain the residual protein expression and milder phenotype (14).

In summary, current study demonstrates that *LAMB3* mutations downregulate the other 2 laminin-332 encoding genes, *LAMA3* and *LAMC2*. The discordances between transcription and protein expression alterations in the associated subunits of laminin-332 illustrate the complexity of the heterotrimer pathobiology and the clinical challenge in delineating accurate genotype-phenotype correlation in this group of patients.

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The authors have no conflicts of interest to declare.

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