

## Enamel Defects in Carriers of a Novel *LAMA3* Mutation Underlying Epidermolysis Bullosa

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Junctional epidermolysis bullosa, non-Herlitz (JEB-nH) is an autosomal recessive EB subtype caused by mutations in the genes *LAMA3*, *LAMB3*, *LAMC2* or *COL17A1*. The first three genes encode for laminin  $\alpha 3$ , laminin  $\beta 3$ , and laminin  $\gamma 2$ , respectively, which together form the heterotrimer laminin-332 (LM-332), whereas *COL17A1* encodes for the homotrimer type XVII collagen (Col17) (1). LM-332 and Col17 are located in the lamina basale, and are important in epidermal–dermal adhesion. JEB-nH shows blistering after minor trauma, but also extracutaneous symptoms, such as dental abnormalities (1). Both LM-332 and Col17 are crucial in ameloblast differentiation and enamel formation (2, 3), resulting in enamel defects of the entire dentition in all patients with JEB-nH, consisting of hypoplasia, pitting, roughness, thinning or furrowing of enamel (4). In 1996, McGrath et al. (5) reported the first heterozygous carriers of a *COL17A1* mutation with enamel defects. The carriers of the glycine substitution p.G627V had no signs of skin fragility, but did have extensive enamel hypoplasia and pitting, thought to be caused by the dominant-negative effect of the mutant Col17. In 2007, Murrell et al. (6) reported enamel defects in carriers of a *COL17A1* null mutation, which was attributed to haploinsufficiency. Dental abnormalities have not been reported previously in carriers of mutations in the genes encoding LM-332. We report here the first enamel defects in carriers of a *LAMA3* mutation.

### CASE REPORT

The proband was a 23-year-old woman (EB050) diagnosed with JEB-nH. Starting at the age of 2 months she showed generalized blistering and erosions that healed with atrophic scarring. She had dystrophic nails, and, occasionally, oral erosions. There was no loss of primary or secondary hair. Her entire primary and secondary dentition was affected with enamel hypoplasia and enamel pits, which were treated with direct composite (Fig. 1a, b). Her non-consanguineous parents and her brother had no signs of skin fragility. However, her 53-year-old mother and her 25-year-old brother also had enamel defects in their dentition, consisting of roughness and pits, which had led to a higher susceptibility to caries in both (Fig. 1c, d). The mother was treated with maxillary dentures and numerous restorations (Fig. 1c). In contrast, the father's teeth were not affected.

Skin biopsies in the proband and her mother were taken, and immunofluorescence antigen mapping was performed on non-lesional skin. Staining of LM-332 with monoclonal antibody GB3 was slightly reduced in both mother and daughter, while staining for Col17 with monoclonal antibodies NCC-lu-226 and 233 was normal.

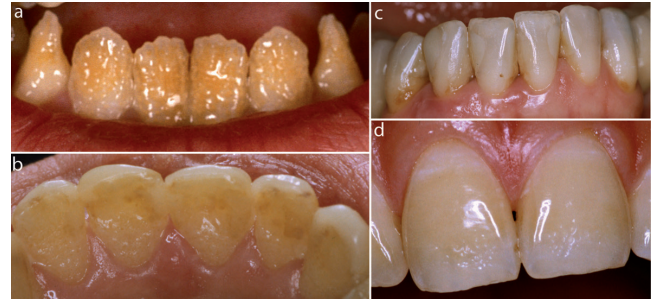


Fig. 1. Features of enamel defects. (a, b) The proband (c) her mother, and (d) her brother had enamel defects in their dentition. (a) Rough pitted enamel is seen on the buccal side of the mandibular teeth, and (b) on the palatal side of the maxillary teeth in the proband. The buccal side of the maxillary teeth are treated with direct composite. (c) The enamel defects on the labial side of the mandibular teeth are no longer visible due to multiple restorations in the mother. (d) In the brother there is typical pitted enamel in the incisal area of the central incisors.

Genomic DNA of all family members was extracted from peripheral blood. Previous molecular analysis of *COL17A1*, *LAMB3*, and *LAMC2* yielded no abnormalities in the proband. Screening of *LAMA3* (GenBank accession number NM\_000227.3) showed a compound heterozygosity for the novel missense mutation c.4484C>T, and the novel nonsense mutation c.488delG in the proband (methods available on request). The deletion c.488delG in exon 5 creates a frameshift that results in a premature termination codon (p.G163DfsX30), and the mutation c.4484C>T in exon 33 results in a substitution of alanine to valine at position 1495 (p.A1495V). Proving the pathogenicity of the mutation p.A1495V, are 2 unrelated patients with JEB-nH (EB169-01 and EB173-01) in our EB cohort, who both carry the *LAMA3* mutation p.A1495V. Patient EB169-01 carries it together with the nonsense mutation p.R1126X, and patient 173-01 together with the missense mutation p.R262S (unpublished).

Screening of *LAMA3* in the family members of the proband showed that her father was heterozygous for c.4484C>T. Her mother and brother, showed heterozygosity for c.488delG. To investigate whether the enamel defects in the heterozygous carriers of c.488delG could be caused by a dominant-negative effect of an aberrant laminin  $\alpha 3$  chain by alternatively spliced cDNA, nested PCRs surrounding both mutations were performed with cDNA isolated from skin biopsies from the proband and her mother (methods available on request). There were no alternatively spliced cDNA products visible on agarose gel (Fig. S1a, b; available from <http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1341>). The PCR products were sequenced and cDNA of both alleles of the proband were present (Fig. S1c, d). The allele bearing the mutation c.488delG in the mother was reduced compared with the normal wild-type (Fig. S1c). Together with the absence of alternative spliced products on agarose gel, we hypothesize that this indicates that haploinsufficiency of *LAMA3* is the cause of enamel defects in the mother and brother of the proband.

## DISCUSSION

Enamel is formed in 3 stages: (i) in the presecretory stage the ameloblasts differentiate and then the lamina basale, which supports the ameloblasts, disintegrates; (ii) in the secretory stage ameloblasts secrete enamel proteins that mineralize and form enamel crystals; (iii) in the maturation stage a lamina basale is deposited, adhering ameloblasts to the underlying enamel surface by hemidesmosomes. To harden the enamel, water and organic material are removed by ameloblasts (7). LM-332 is expressed by ameloblasts in all stages of enamel formation (3). In the presecretory and maturation stage LM-332 is a part of the lamina basale. In the secretory stage LM-332 most likely plays a role in the adhesion of ameloblasts to the enamel matrix (3). In patients with JEB-nH the reduction in LM-332 causes detachment from the underlying matrix and degeneration of ameloblasts. This causes enamel defects, such as pitting (4, 8). The enamel that does form has a different chemical composition, as reduction in LM-332 can result in deficiencies in the junctions of ameloblasts that are important in maintaining channels for mineral ions and cell nutrients. This may lead to defective mineral transport or compromised ameloblast metabolism (8). Teeth of patients with JEB have reduced mineral content and presence of serum albumin, which is a known inhibitor of enamel crystal growth (8). It is possible that there is no coping mechanism that can manage the loss of one LAMA3 allele in this complex process of enamel formation, whereas these mechanisms are available in the skin (6). However, it remains peculiar that enamel defects have not been reported previously in carriers of *LAMA3*, *LAMB3*, or *LAMC2* null mutations. Furthermore, none of the carriers of null mutations in LM-332 in the Dutch EB cohort have noted particular dental problems; however, they have not been examined systematically. It is possible that not all carriers are affected; a phenomenon also seen in carriers of *COL17A1* null mutations (6, 9) or the enamel defects are so discrete that they are missed. Furthermore, it is possible that other factors contribute to the enamel defects seen in our carriers, such as the rare inherited enamel defects that occur in the absence of generalized syndromes designated as amelogenesis imperfecta (10), or due to environmental factors such as febrile diseases. However, this is less likely in our patients, considering the clinical dental symptoms (7).

The enamel defects seen in the carriers of *LAMA3* null mutations reported here are comparable to those seen in the carriers of missense *COL17A1* mutations; although the carriers of *COL17A1* null mutations described by Murrell et al. also showed horizontal ridging, a feature that was not present in the carriers we have described (5, 6).

In conclusion, we have reported: (i) two novel *LAMA3* mutations associated with JEB-nH; and (ii) the first carriers of *LAMA3* null mutations to have enamel defects probably caused by haploinsufficiency.

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*The authors declare no conflicts of interest.*

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