Aberrant splicing as potential modifier of the phenotype of junctional epidermolysis bullosa

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Abstract

Background A lack or dysfunction of the anchoring protein laminin-332 in the basement membrane leads to the skin blistering disorder junctional epidermolysis bullosa (JEB). The mutation c.628G>A in the gene LAMB3 encoding the laminin β3-chain is associated with generalized intermediate JEB; it may introduce an amino acid substitution (p.Glu210Lys) or disrupt splicing.

Objective This retrospective study aimed at determining the effects of aberrant splicing on the JEB phenotype.

Methods LAMB3 transcription was analysed in two siblings compound heterozygous for the LAMB3 mutations p.Glu210Lys and p.Arg635* with a diverging JEB phenotype from late childhood on. Laminin-332 levels in skin sections and in cultured keratinocytes were investigated by immunofluorescence staining. Real-time PCR was used to quantify LAMB3 expression in keratinocytes. RNA splice variants were identified by subcloning of a LAMB3 cDNA fraction and subsequent DNA sequencing. Structural models of laminin-332 helped to assess the impact of certain mutations on laminin-332 folding.

Results Both siblings showed diminished LAMB3 expression. Laminin-332 was equally reduced in skin sections obtained during infancy but differed in keratinocytes isolated during adolescence. Although aberrant LAMB3 splicing with 26 variants was detected in both patients, splicing differed significantly: the full-length LAMB3 transcript harbouring the p.Glu210Lys mutation was found more often in the patient affected less severely (14/108 vs. 5/106 clones; P = 0.03). Structural modelling predicted that several deletions in LAMB3, but not the point mutation p.Glu210Lys, have an effect on laminin-332 folding and secretion.

Conclusions Differential LAMB3 mRNA splicing in the patients may explain the disparate JEB phenotype. By elucidating the regulation of laminin-332 gene expression, these findings may contribute to the development of therapeutic strategies for JEB and might help to understand phenotype modification by splice-site mutations in other hereditary diseases.

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Conflicts of interest

The authors have no conflicts of interest to report.

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caused by missense mutations or by altered splicing, which is frequently found in genetic skin diseases. RNA splicing is a tightly regulated process in eukaryotes relying on specific sequence motifs at the exon-intron borders, and an intronic branch-point sequence.\(^1\) By increasing the number of protein isoforms, alternative splicing contributes significantly to protein diversification. If the regular splicing process is disrupted by mutated splice-site recognition sequences, aberrant splicing may cause disease.\(^2,9\) Splice-site mutations account for some cases of lethal JEB and for up to 20% of generalized intermediate JEB.\(^1,8,10–13\) The LAMB3 point mutation c.628G>A (p.Glu210Lys) is associated with generalized intermediate JEB.\(^3,12–17\) It alters the amino acid at position 210 of the β3-chain and, since affecting the last base of exon 7 of LAMB3, may result in aberrant splicing,\(^1,7–19\) which has also been observed in a LAMB3 c.628G>A knockin mouse model of JEB.\(^20\)

The clinical course of generalized intermediate JEB may differ between affected members of the same family. To determine the effects of aberrant splicing on the JEB phenotype, we analysed LAMB3 expression in two siblings compound heterozygous for the LAMB3 mutations p.Glu210Lys and p.Arg635* but with different severity of JEB symptoms.

### Subjects and methods

#### Subjects and ethics statement

This retrospective study involved two siblings with generalized intermediate JEB who had been referred to the Department of Pediatrics of the University Hospital in Erlangen, Germany, for further clinical workup. The diagnosis of JEB was based on clinical assessment, immunofluorescence staining and molecular genetic analyses. Skin biopsies and peripheral blood samples were obtained for diagnostic purposes after both patients or their legal guardians had given written informed consent to the procedures and to further investigation. Both patients also provided written consent to the publication of their medical data and images. The study was conducted in accordance with the institutional and national regulations and with the principles of the declaration of Helsinki.

#### Isolation and cultivation of epidermal keratinocytes

Primary epidermal keratinocytes of the patients were isolated from a skin punch biopsy from the thigh when the patients were 18 and 15 years old, respectively. Primary keratinocytes from a foreskin sample of a healthy 8-year-old donor served as control. After separation of the epidermis from the dermis by incubation in a protease solution (Dispase II; Merck, Darmstadt, Germany), keratinocytes were released from the skin samples by trypsinization and cultured in defined keratinocyte serum-free medium (K-SFM; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with growth factors, penicillin and streptomycin in tissue culture flasks coated with bovine collagen type I and laminin-111. Keratinocytes of both patients were cultured in parallel. For cDNA synthesis, cells of the fourth passage were harvested at 60–70% of confluency. When used for immunofluorescence analyses, keratinocytes were cultured in a collagen-1-coated imaging dish for 3 days.

#### cDNA synthesis and quantitative real-time PCR (RT-PCR)

RNA was isolated from keratinocytes using TRizol reagent (Life Technologies, Carlsbad, CA, USA) and was treated with DNase. CDNA was synthesized from a standardized amount of extracted RNA. In a quantitative RT-PCR, nucleotides 2012–2080 of LAMB3 cDNA were amplified with the primers 5'–TGGAGGAG GAGACGTTGTC–3' and 5'–TAAGGAGACCATTGAACTTCT G-3' using the IQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). Glycerinaldehyde-3-phosphat-Dehydrogenase (GAP DH) expression was quantified as internal standard using the primers 5'–GCCATCAATGACCCCTTCATT–3' and 5'–TTGACG GTGCCCCCATGAAATT–3'. The experiment was performed three times; each time the control sample and the patients’ samples were investigated in duplicates. The mean value was used for calculating the relative LAMB3 gene expression in the patients as follows:

\[
2^{\Delta \Delta Ct} = \frac{(Ct\text{ patient} - Ct\text{ GAPDH})}{(Ct\text{ control} - Ct\text{ GAPDH})}
\]

#### Analysis of LAMB3 splicing

To investigate LAMB3 splicing in the patients, nucleotides 431–997 of LAMB3 cDNA were amplified by PCR with the oligonucleotides 5’–GAGTGTTACGATACCTGGCTG–3' and 5’– CAAACACAGGGGTCAAGAG–3' (5 min at 95°C, then 45 cycles of 30 s at 95°C, 30 s at 58°C and 45 s at 72°C, followed by 7 min at 72°C). The PCR products were analysed by subcloning with the pGEM-T Easy Vector Systems (Promega, Fitchburg, MA, USA) according to the manufacturer’s protocol. Ligation was performed with a vector-insert ratio of 4 : 1. The plasmid DNA of single white colonies was extracted using a commercial DNA preparation kit. The insert was amplified by PCR with the LAMB3-specific primers mentioned above or with primers binding to the vector backbone. After subcloning of the PCR product, 124 bacterial colonies were picked from patient #1 and 127 from patient #2. A total of 108 and 106 clones obtained from patient #1 and patient #2, respectively, contained plasmids with DNA inserts in the multiple cloning site; these were used for further analyses.

Fractions of genomic DNA of LAMB3 and COL17A1 were amplified by PCR for sequence analysis using the primers listed in Table S1, Supporting Information.

#### Sequencing of PCR products was carried out by a commercial DNA sequencing service (Eurofins Genomics, Ebersberg, Germany).

#### Immunofluorescence analyses

For antigen mapping of the basement membrane zone, skin punch biopsies were obtained when the patients were 8 and 5 years old, respectively. Preparation of skin sections and
immunofluorescence staining was performed as described previously. For investigation of cultured keratinocytes, cells were fixated with 4% paraformaldehyde and blocked with 10% serum-free protein-block (Agilent, Santa Clara, CA, USA) before incubation with the monoclonal antibody K140 recognizing the human laminin β3-chain. Alexa Fluor 488 (Thermo Fisher Scientific, Waltham, MA, USA) was used as secondary antibody. After staining of the cell nuclei with 4',6-diamidino-2-phenylindole (DAPI), the dishes were investigated under a fluorescence microscope.

Statistical analysis
The chi-square test was used for all statistical analyses.

Structural analysis
The locations and likely structural consequences of the laminin β3-chain mutations were assessed using the crystal structure of the murine laminin β1-chain. The sequence identity of the two laminin chains is 40.6% in their laminin N-terminal (LN) domains, allowing straightforward homology modelling.

Results
Patient #1 is a now 23-year-old male with generalized intermediate JEB who developed widespread skin blistering, especially in areas exposed to mechanical stress, from the neonatal period on. Nail dystrophy, enamel defects and relapsing small corneal erosions also occurred. His now 20-year-old sister, patient #2, suffered from the same symptoms. Molecular genetic analysis confirmed compound heterozygosity for the LAMB3 mutations p.Glu210Lys and p.Arg635* in both siblings.

In patient #1, the cutaneous JEB symptoms ameliorated when he was 6 years old; during adolescence and adulthood he was only affected by skin blistering after mechanical trauma. He completed school and professional training. During this phase, there were no hospitalizations related to JEB. At the age of 21 years, he experienced aggravation of skin fragility after a scabies infection with more pronounced blistering in mechanically stressed areas and perturbing pruritus. Two hospitalizations and participation in a dermatological rehabilitation programme were necessary. After local application of permethrin symptoms improved only temporarily, so that the patient was treated systemically with ivermectin which led to amelioration of his skin status and pruritus (Fig. 1). Despite his chronic disease patient #1 was living alone and working in a permanent position at the age of 21 years (Table 1).

In contrast to her brother, patient #2 did not experience an improvement of skin fragility during childhood, but continued to develop skin erosions. At the age of 6 years, she suffered from chronic wounds on her left hand and left foot, which healed only after treatment with an antibacterial phage solution. Extracutaneous JEB manifestations, mucosal erosions, enamel defects and caries were more pronounced than in her brother. Relapsing corneal erosions and keratoconjunctivitis sicca ultimately lead to visual impairment of 50%. Several hospitalizations were necessary during childhood. Skin fragility further aggravated during adolescence. After she had finished school, patient #2 did not find an appropriate position for professional training due to her skin condition. When she was 19 years old, she lived with her mother and had to be hospitalized four times because of acute JEB exacerbation with disseminated skin blistering and erosions (Table 1, Fig. 1). Participation in a dermatological rehabilitation programme was supported with the aim to enable the patient to start professional training (Table 1).

In summary, patient #2 is more severely affected by JEB than her brother, patient #1, who carries the same LAMB3 mutations.

In both siblings, the presence of small amounts of laminin-332 was confirmed by immunofluorescence analyses of skin sections and cultured keratinocytes. Compared with healthy controls, both patients displayed equally diminished amounts of laminin-332 in the basement membrane zone of skin sections obtained during childhood (Fig. 2). At this age, the severity of cutaneous JEB symptoms was similar in the siblings. In immunofluorescence analyses of keratinocytes isolated from skin samples taken during adolescence, the amount of the laminin β3-chain was reduced in patient #1 but almost absent in patient #2, revealing a
The surprising difference between the siblings (Fig. 2) and correlating to the disparate severity of JEB symptoms in the patients.

To quantify \(LAMB3\) transcription in the patients’ keratinocytes, quantitative real-time PCR was performed with cDNA and primers located in exon 15 of \(LAMB3\), downstream of the mutation \(c.1903C>T\) (p.Arg635*) present on one allele in each patient. \(LAMB3\) expression was reduced to approximately 25% in both patients (Fig. 3a).

To analyse \(LAMB3\) transcription and splicing in the patients, a fraction of \(LAMB3\) cDNA was amplified by PCR with primers located in exon 6 and exon 10. Besides the expected PCR product of 567 base pairs, several aberrant amplimers were obtained from the patients’ samples, but not from healthy controls (Fig. 3b). The PCR products were subcloned; 108 clones obtained from patient #1 and 106 clones obtained from patient #2 contained correctly inserted DNA fragments. Sequence analysis of the inserts revealed the presence of 26 different splice variants, including the full-length \(LAMB3\) transcript harbouring the \(628G>A\) missense mutation (#6), a transcript with an additional nucleotide substitution at position \(629\) (#7) and a transcript identical to the reference sequence (#5). The other variants correspond to mutant mRNAs with various deletions (#8–26) and insertions (#1–4).

A total of 18 different splice variants were found in patient #1, 15 in patient #2. The variant most frequently detected in both patients was the previously described mutant mRNA with an in-frame deletion of 165 nucleotides, corresponding to exon 7 and parts of exon 8 (Table 2, variant #10).\textsuperscript{17–19}

The majority of investigated clones contained in-frame splice variants, including the \(LAMB3\) transcript corresponding to the reference sequence, which was detected 15 times in patient #1, and 19 times in patient #2. In both patients, one \(LAMB3\) allele harbours the nonsense mutation p.Arg635* located further downstream than the subcloned cDNA portion, which explains the presence of the splice variant identical to the reference sequence in a small number of analysed clones.

Apart from these, there were 61/108 and 50/106 clones harbouring in-frame mutations in patients #1 and #2, respectively. The proportion of clones with in-frame transcripts did not differ significantly between the siblings (Table 2) and corresponds to the qPCR findings indicating a reduction of \(LAMB3\) expression to 25% in both patients (Fig. 3a).
Aberrant splicing modifies JEB phenotype

Figure 3 (a) Quantitative PCR of a LAMB3 cDNA fragment in exon 15 showed reduced expression in both patients. Results were normalized to GAPDH expression. The graph displays mean ± SD. (b) A fraction of LAMB3 cDNA was amplified by PCR with primers in exon 6 and 10. Besides the expected 567 bp band aberrant PCR products were detected in the patients’ samples. (c) Schematic view of LAMB3 cDNA displaying that the coding nucleotides for Glu210 are located in two different exons. (d) Orthogonal view of the laminin β1-chain structure22 with the residue corresponding to laminin β3 Glu210 (laminin β1 Asn226) highlighted in magenta. Laminin β3 Glu210 maps to a short helix at the back of the LN domain, well away from the surface region implicated in laminin polymerization (left image). Another view of the laminin β1-chain structure, with the residues deleted in variant #10 highlighted in magenta (right image).

With one exception, a mutant transcript with a deletion reaching further upstream than the cDNA portion investigated here,19 all aberrant mRNAs described previously were found in our patients (Table 2).17–19

Correct splicing was not completely disrupted in the patients: The full-length LAMB3 transcript harbouring the 628G>A missense mutation was detected 14 times in clones obtained from patient #1, the sibling affected less severely, but only five times in clones obtained from patient #2 (P = 0.03). One splice variant found in patient #1 had an additional nucleotide substitution in position 629 resulting in a predicted amino acid replacement in position 210 of the laminin β3-chain (p.Glu210Lys) highlighted in magenta. Laminin β3 Glu210 maps to a short helix at the back of the LN domain, well away from the surface region implicated in laminin polymerization (left image). Another view of the laminin β1-chain structure, with the residues deleted in variant #10 highlighted in magenta (right image).

To exclude differential intrinsic mutations as a cause of aberrant splicing, and to exclude allelic variants in the collagen XVII ectodomain as a genetic modifier of the JEB phenotype, sequencing of the patients’ genomic DNA was performed. The intrinsic sequences neighbouring exons 5–10 were identical in both patients and revealed the presence of the following previously described single nucleotide polymorphisms: c.823-15C>T and c.943 + 52C>G (https://www.ncbi.nlm.nih.gov/snp). Exons 42–56 of the COL17A1 gene did not contain allelic variants.

Structural modelling of the mutated laminin β3-chain carrying the p.Glu210Lys missense mutation showed that the mutated residue is located on the surface of the globular LN domain in an area not expected to be important for laminin polymerization. The mutation is not predicted to impact protein folding or secretion (Fig. 3d).

The frequently detected mutant LAMB3 mRNA with an in-frame deletion of 165 nucleotides (variant #10, Table 2) results in a laminin β3 protein lacking residues 189–243. The deleted residues map to several central β-strands of the globular LN domain and the deletion, therefore, is predicted to result in misfolding of the N-terminal part of the laminin β3-chain (Fig. 3d).

Discussion

We have shown differences in LAMB3 expression and splicing in two siblings compound heterozygous for LAMB3 mutations with a disparate severity of generalized intermediate JEB: In the patient less severely affected by JEB, more full-length transcripts carrying the p.Glu210Lys missense mutation could be detected. Laminin-332 levels in keratinocytes isolated during adolescence differed between the siblings.

Two mechanisms potentially underlie pathogenicity of the LAMB3 628G>A variant. First, it leads to an amino acid substitution which has been speculated to affect heterotrimer formation and adherence of laminin-332 to basement membrane components.19,23–25 Our structural analysis of the mutated laminin β-chain, however, predicts minor if any effects of the mutation on polymerization, protein folding and secretion.

Second, the LAMB3 628G>A mutation affects a donor splice-site consensus sequence26 and may disrupt LAMB3 mRNA splicing. In previous studies of JEB patients carrying the LAMB3 628G>A mutation, aberrant splicing has not always been observed,14 but in all patients with mutant LAMB3 transcripts, the full-length mRNA harbouring the 628G>A missense mutation was also detectable.17–19 While in a LAMB3 628G>A knockin mouse model of JEB splicing exclusively led to an out-of-frame skipping of exon 7 and to a lethal phenotype,20 the full-length LAMB3 transcript harbouring the 628G>A missense mutation was even more abundant in areas of less fragile skin in JEB patients with revertant mosaicism,17 suggesting that the resulting full-length β3-chain with the predicted p.Glu210Lys amino acid exchange is functional and therefore crucial for the intermediate JEB phenotype. Such revertant mosaicism is a mechanism of natural gene therapy occurring in different types of epidermolysis bullosa17,27–29 and
may be a result of different LAMB3 second-site mutations. No such second-site mutation was present in our patients.

A very high number of LAMB3 transcript variants were detected in our patients. In vivo, keratinocytes expressing out-of-frame splice variants may not be as durable as cells expressing partially functional laminin-332 molecules, which may contribute to the increased skin fragility observed in patient #2. Moreover, mutation screening for genodermatoses is primarily based on analyses of genomic DNA, so that aberrant splicing events may be underestimated.

A substantial percentage of aberrant splicing events observed in our patients led to longer in-frame deletions, some of which are located in the LN domain. In a murine model of congenital muscular dystrophy, an in-frame deletion within the LN domain of the laminin α2-chain led to proteolytic degradation and loss of polymerization. Other in-frame deletions found in our patients reach onto the neighbouring laminin epidermal growth factor-like (LE) motifs. They almost certainly prevent formation of functional laminin-332 or may foster formation of granulation tissue and impaired wound healing.

In mice and potentially in humans, some allelic variants of the collagen XVII ectodomain represent major genetic modifiers of JEB caused by abnormal laminin-332, as they may reduce the strength of dermal-epidermal adhesion in the context of a mutation in laminin-332 genes. Such phenotypic modification was excluded in our patients.

In the patient affected less severely, a scabies infection led to aggravation of skin fragility. Deterioration of epidermolysis bullosa due to scabies or a complicated course of the parasitosis has been described before. No second-site mutations. In mice and potentially in humans, some allelic variants of the collagen XVII ectodomain represent major genetic modifiers of JEB caused by abnormal laminin-332, as they may reduce the strength of dermal-epidermal adhesion in the context of a mutation in laminin-332 genes. Such phenotypic modification was excluded in our patients.

In the patient affected less severely, a scabies infection led to aggravation of skin fragility. Deterioration of epidermolysis bullosa due to scabies or a complicated course of the parasitosis has been described before. Even in patients with intact anchoring proteins, scabies may provoke a bullous phenotype. The parasites can locally manipulate the dermo-epidermal junction, potentially by differential expression of cell adhesion molecules, and thus contribute to increased skin fragility.

Considering the significant reduction of the donor splice-site recognition score predicted in silico for the LAMB3 628G>A

Table 2 LAMB3 splice variants detected in the patients’ keratinocytes

| Splice variant # | Effect on LAMB3 splicing | Mutated LAMB3 cdna | Predicted effect on amino acid sequence | Times found in analysed clones |
|------------------|--------------------------|--------------------|----------------------------------------|-------------------------------|
| Patient #1 | Patient #2 | |
| 1 | 628G>A, insertion of 293 bp (intron 7, starting at c.628 + 1) | c.628delins294 | p.Glu210Serfs*48 | 0 | 2 |
| 2 | 628G>A, insertion of 250 bp (intron 7, starting at c.628 + 1) | c.628delins251 | p.Glu210Serfs*48 | 0 | 1 |
| 3 | 628G>A, insertion of 66 bp (intron 7, starting at c.628 + 1) | c.628delins67 | p.Gln209_Glu210del | 0 | 1 |
| 4 | Insertion of 1 bp, 628G>A | c.622_623insA | p.Ile208Asnfs*50 | 2 | 0 |
| 5 | No aberrant splicing, cDNA according to reference sequence | c. - | p. - | 15 | 19 |
| 6 | 628G>A | c.628G>A | p.Glu210Lys | 14 | 5 |
| 7 | 628G>A, 629A>G | c.628_629delinsAG | p.Glu210Arg | 1 | 0 |
| 8 | Deletion of 64 bp (exon 7) | c.565_628del | p.Val189Argfs*8 | 13 | 15 |
| 9 | Deletion of 66 bp (exon 7, 2 bp of exon 8) | c.565_630del | p.Val189_Glu210del | 5 | 12 |
| 10 | Deletion of 165 bp (exon 7, 101 bp of exon 8) | c.565_729del | p.Val189_Gln243del | 30 | 26 |
| 11 | Deletion of 258 bp (exons 7 and 8) | c.565_822del | p.Val189_Gln274del | 3 | 2 |
| 12 | Deletion of 314 bp (exon 7, 189 bp of exon 8) | c.504_817del | p.Trp168Cysfs*19 | 4 | 0 |
| 13 | Deletion of 315 bp (exons 8 and 9) | c.629_943del | p.Glu210_Arg315delinsGly | 0 | 4 |
| 14 | Deletion of 333 bp (exon 7, 8 bp of exon 7, 14 bp of exon 9) | c.504_836del | p.Trp168_Val278del | 1 | 0 |
| 15 | Deletion of 336 bp (exon 7, 8 bp of exon 7, 61 bp of exon 9) | c.548_883del | p.Arg183_Ala294del | 2 | 0 |
| 16 | Deletion of 345 bp (exon 7, 8 bp of exon 7, 185 bp of exon 8) | c.469_813del | p.Phe157_Thr271del | 1 | 0 |
| 17 | Deletion of 355 bp (exon 7, 8 bp of exon 7, 23 bp of exon 10) | c.612_966del | p.Ser205Argfs*73 | 2 | 0 |
| 18 | Deletion of 367 bp (exon 7, 2 bp of exon 8) (exon 8, 29 bp of exon 9) | c.495_861del | p.Ser167Ilefs*107 | 0 | 3 |
| 19 | Deletion of 368 bp (70 bp of exon 6, 2 bp of exon 7, 40 bp of exon 9) | c.495_862del | p.Gln166_Lysfs*3 | 6 | 9 |
| 20 | Deletion of 379 bp (exons 7–9) | c.565_943del | p.Val189_Lysfs*81 | 2 | 0 |
| 21 | Deletion of 379 bp (exon 7, 8 bp of exon 7, 42 bp of exon 9) | c.486_846del | p.Gly163_Lysfs*108 | 0 | 2 |
| 22 | Deletion of 394 bp (exon 7, 117 bp of exon 8, 117 bp of exon 9) | c.548_939del | p.Arg183_Lysfs*82 | 0 | 3 |
| 23 | Deletion of 438 bp (77 bp of exon 6, 2 bp of exon 7, 103 bp of exon 9) | c.488_925del | p.Gly163_Gln308del | 2 | 0 |
| 24 | Deletion of 438 bp (exon 7, 8 bp of exon 7, 89 bp of exon 9) | c.474_911del | p.Arg159_Pro304del | 2 | 0 |
| 25 | Deletion of 443 bp (exon 7, 8 bp of exon 7, 89 bp of exon 9) | c.469_911del | p.Phe157_Glyfs*8 | 0 | 2 |
| 26 | Deletion of 457 bp (exon 7, 8 bp of exon 7, 101 bp of exon 9) | c.467_923del | p.Trp156Argfs*88 | 3 | 0 |

Sum 108 106

*Previous description of a splice variant is indicated.

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variant (from 0.98 to 0.37, https://www.fruitfly.org/seq_tools/splice.html), presence of the mutated full-length LAMB3 mRNA is somewhat surprising. In humans, correct splicing does not only rely on consensus sequences, but also on regulatory elements.9,37 Moreover, sex-specific differences of regulatory structures of gene expression and splicing have been observed in other tissues, including the brain.38,39 Disparate regulation of splicing may sufficiently explain the differences between our patients.

In-frame skipping of exons containing nonsense mutations can rescue laminin-332 production in JEB patients40,41 and may be age-dependent.40 Modification of splicing by induced skipping of exons is being studied as a curative approach for some genetic diseases.42 Genetically corrected autologous keratinocytes represent a promising therapeutic strategy for JEB,43 underlining the importance of profound knowledge about phenotypic effects of mutations in the laminin-332 gene.

This study shows that aberrant LAMB3 mRNA splicing may modify the JEB phenotype. By elucidating the mechanisms underlying the regulation of laminin gene expression, our findings might play an important role in the development of therapeutic approaches for JEB and might help to understand phenotype modification by splice-site mutations in other hereditary diseases.

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**Supporting information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Primers for PCR amplification of genomic DNA