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Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Whole-exome sequencing

Whole-exome sequencing was used to screen for disease-associated variants in the 20 EB-related genes, including 16 classical EB genes (S1), plus *DSP, PKP1, JUP*, and *TGM5* (S2). Genomic DNA from the patient's peripheral blood was sent for whole-exome sequencing. Exome libraries were generated with SureSelect Human All Exon V6 (Agilent, Santa Clara, CA, USA) and sequenced with 100-bp paired-end sequencing on the NextSeq500 platform (Illumina, San Diego, CA, USA). Variant calling was performed with a previously published in-house pipeline (S3). The variants were filtered to retain those with a frequency less than 0.5% in the 1,000 Genomes Project (http://www.internationalgenome.org/) and in the ExAC Browser, http://exac.broadinstitute.org/).

Extraction of RNA from skin tissue

The RNA was extracted from the skin tissue of the patient and from that of an unrelated healthy control subject using the RNeasy Plus Universal Mini Kit (QIAGEN, Germany). Before extraction of RNA, skin tissue was stored in RNAlater Stabilization Solution (Invitrogen, Carlsbad, CA, USA) at –20°C. The skin tissue was homogenized in lysis buffer. After the addition of genomic DNA eliminator solution and chloroform, the homogenate was separated into aqueous and organic phases by centrifugation. The upper aqueous phase with RNA was collected and mixed with ethanol, and the RNA was purified using RNeasy spin columns. Total RNA bound to the spin column membrane; phenol and other contaminants were efficiently washed away. High-quality RNA was eluted in RNase-free water.

Reverse-transcription PCR (RT-PCR) and microfluidic electrophoresis

Five-hundred nanograms of total RNA were used for reverse transcription and subsequent experiments. The RNA was reverse-transcribed into cDNA by using the RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific, Waltham, MA, USA). For identification of the 3 splice-site mutations, amplification of the regions that span the adjacent exons was performed by Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and designed primer pairs (as shown in **STable I**). The PCR product was electrophoresed in 1.8% agarose gel and visualized by ethidium bromide staining. Sanger sequencing was performed on the PCR product to determine the splicing effect of the mutations. In addition, the amplified PCR products were analysed by microfluidic electrophoresis system LabChip GX Touch (PerkinElmer, Waltham, MA, USA) using DNA high sensitivity assay kit. The fragment size and its corresponding quantity were detected and analysed by smear analysis module using LabChipGX Reviewer software (v.5.4). The ratio of transcripts (mutant to wild-type) was calculated using the quantification results from the smear analysis.

STable I. Primers for three splice-site mutations to detect aberrant splicing effects

Mutation	Forward primer	Reverse primer
c.373-9T>A	5′-CAGAATGATGTGAACCCTGT-3′	5′-CTCCCCCACCTCTTGAATTT-3′
c.629-12T>A*	5′-CCTGTCTCTCTGCAGCTGGA-3′	5′-CCGGTTGTTGTAGAAGGGTG-3′
	5′-CTAAATGGGGGGAAGGTC-3′	5′-GCCTGGTGAAATTGACTCTC-3′
c.2137+1G>T	5′-CCCGGATCCTAGATGCAAAG-3′	5′-ACCTGCCCCGCCATCAAGAA-3′

*The upper primer pair was used to check the resultant splicing event while the PCR product size was too large to determine the percentage of mutant/wild-type transcript in microfluidic electrophoresis. Thus, the lower pair was designed.

Quantification of mRNA of LAMA3, LAMB3, LAMC2 *with quantitative real-time PCR (Q-PCR)*

Q-PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad, Berkeley, CA, USA) and the cDNA. After 40 cycles of PCR amplification using specific primers (as shown in **STable II**), the Ct values (cycle quantification values) were used to evaluate the expression of *LAMA3, LAMB3, LAMC2*. All samples ($n = 1$) were compared with human *18S* rRNA (NR_003286.4) as a housekeeping control and Q-PCR on each subject was repeated 3 times (technical replicates).

STable II. Primers of laminin-332 genes for Q-PCR on skin specimens

Gene	Forward primer	Reverse primer
LAMA3	5′-ATGAGGGAACTGCGGAACAG-3′	5′-GCTCCCAAAGCTCTCTCGTT-3′
LAMB3	5′-GACTGACCAAGCCTGAGACC-3′	5′-CCGGAGGATGAAGCCACATT-3′
LAMC2	5′-CAATGGGAAGTCCAGGCAGT-3′	5′-GCCATTCTTGCACTTCTCGC-3′
18S rRNA	5′-GCTCGGGCCTGCTTTGAACACTCT-3′	5′-GGCGCCCCCTCGATGCTCTTAG-3′

Immunofluorescence staining

A series of 5-μm thin cryo-cut skin sections from the patient and a healthy subject were used for immunofluorescence studies. The following primary antibodies targeting subunits of laminin-332 were used: P3H9-2 (1:200, Abcam, Cambridge, UK) against α3 subunit; ab97765 (1:100, Abcam, Cambridge, UK; targeting at amino acid 644-930) against β3 subunit; GB3 (1:100, Sera-Lab, Sussex, UK) against γ2 subunit. After washing with PBS, the cells were incubated with the secondary antibodies: goat anti-rabbit IgG H&L (Alexa Fluor 488) (ab150077, 1:1,000, Abcam, Cambridge, UK) and goat anti-mouse IgG H&L (Alexa Fluor 488) (ab150117, 1:1000 Abcam, Cambridge, UK).

siRNA knockdown of LAMB3 *in normal human epidermal keratinocytes*

Normal human epidermal keratinocytes (NHEKs, Lonza, Basel, Switzerland) in passages 3 were seeded with KGM-Gold (Lonza) in a 12-well plastic bottom plate. The cells were transfected with 10 mM of either *LAMB3* siRNAs or the control (Mock) (Silencer Select siRNAs, Thermo Fisher, Waltham, MA, USA) using RNAimax (Thermo Fisher) and Opti-MEM (Thermo Fisher). Forty-eight hours after transfection, RNA was extracted from cultured cells with an RNeasy kit (Qiagen), and cDNA synthesis was performed using the SuperScript III First-Strand Synthesis System (Life Technologies, Carlsbad, CA, USA). The experiments were conducted with 3 biological replicates for each group. Q-PCR was performed using specific primers (*LAMA3, LAMA5, LAMB1, LAMB3, LAMC1* and *LAMC2* as shown in **STable III**) and SYBR

STable III. Primers of laminin genes for Q-PCR on normal human epidermal keratinocytes

Gene	Forward primer	Reverse primer
LAMA3	5′-GTTCACAGCAGCAAAGGGTG-3′	5′-ACACCGTCCGGTATACAAGC-3′
LAMA5	5′-AGCATCACATTCCTGGAGCC-3′	5′-GTCTCCGTATGCCGGAAGTT-3′
LAMB1	5′-AGCACTGGGGCTTAAGCAAT-3′	5′-CGTTGCACTGACGTCCAATC-3′
LAMB3	5′-GATCCGAGCAGTTCTCAGCA-3′	5′-CTCGGAAGGGACAACGTCTC-3′
LAMC1	5′-GTGAGGCCCAGGATGTCAAA-3′	5′-ACGGCTAATTTGGCTGGACA-3′
LAMC2	5′-TCGGGATACTCACAGGCTCA-3′	5′-GCCTCCTGAGCCAGACTTTT-3′
18S rRNA	5′-GCTCGGGCCTGCTTTGAACACTCT-3′	5′-GGCGCCCCCTCGATGCTCTTAG-3′

Select Master Mix (Life Technologies) on StepOnePlus (ABI, Waltham, MA, USA). All samples were compared with human *18S* rRNA (NR_003286.4) as a housekeeping control.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software, La Jolla, CA, USA, RRID:SCR_002798). *p*-values were determined using 1-way analysis of variance (ANOVA) followed by Dunnett's test. *p*-values are indicated as $*0.01<p<0.05$, $**0.001<p<0.01$, $***0.0001<p<0.001$ and $****p<0.0001$. The values are shown as the means ± standard errors (SE).

SUPPLEMENTARY REFERENCES

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