Appendix S1.

SUPPLEMENTARY METHODS

Clinical samples

Peripheral blood samples were collected from the patient and both parents. Skin samples were obtained from the patient only. All laboratory investigations were ordered by the patient's primary neonatologists, after the risks and benefits of testing had been explained to the patient and his family members and written informed consent had been obtained.

Whole exome sequencing and variant confirmation by means of Sanger sequencing

Whole exome sequencing (WES) of genomic DNA extracted from peripheral blood mononuclear cells of the patient and both parents were performed using the SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing Library Protocol Version C0, December 2016 (Agilent Technologies, Santa Clara, CA, USA). Input DNA (200 ng) was fragmented using a Covaris E220 (Covaris Inc., Woburn, MA, USA) to achieve a size of 150-200 bp. End repair, adenylation and adaptor ligation was performed using SureSelectXT Reagent kits (Agilent Technologies). Exons were hybridized to oligonucleotides from SureSelect Human All Exon V6 (Agilent Technologies) in a 24-h reaction at 65°C. Paired-end 125-bp sequencing was performed on HiSeq4000 Illumina platform (Illumina Inc., San Diego, CA, USA). Mean coverage for patient and parents was >100×.

Bioinformatic analysis was performed as per Genome Analysis Tool Kit v3.5 (GATK) guidelines (Broad Institute, Cambridge, MA, USA). Sequencing output files were in the FASTQ format. These were aligned to the reference genome (hg19) using Burrows-Wheeler Aligner (BWA-MEM). Quality control and recalibration performed with GATK resulted in an output file in BAM format. BAM files were inserted into HaplotypeCaller from Broad Institute to find variations from the reference genome generating a variant called file (VCF). Resulting mutations were annotated using Combined Annotation Dependent Depletion (CADD) (http://cadd.gs.washington.edu/contact) and Variant Effect Predictor (http://www.ensembl.org/info/docs/tools/vep/). Variants that were deemed clinically significant in the JEB-related genes ITGA6 and ITGB4 located on chromosome 2 were confirmed by Sanger sequencing in the patient and parental samples.

Ultrastructural analysis of the skin

For transmission electron microscopy, the skin biopsies were fixed with Karnovsky fixative (3% paraformaldehyde, 3.6% glutaraldehyde). Post-fixed samples (1% OsO4 containing 1.5% potassium ferrocyanide in aqua bidest, 2 h) were rinsed with distilled water, block stained with uranyl acetate (2% in distilled water), dehydrated in alcohol (stepwise 50–100%), immersed in propylenoxide, and embedded in glycidyl ether (polymerized 48 h at 60°C; SERVA, Electrophoresis GmbH, Heidelberg, Germany).

Semithin and ultrathin sections were cut with an ultramicrotome (Ultracut, Reichert, Vienna, Austria). Ultra-thin sections (30 nm) were mounted on copper grids and analysed on a Zeiss LIBRA 120 transmission electron microscope (Carl Zeiss, Oberkochen, Germany) operating at 80 kV.

Immunofluorescent antigen mapping

Indirect immunofluorescence staining was performed on 5-µm skin cryosections, which were air-dried and incubated with primary antibodies overnight at 4°C. The primary antibodies used are listed in STable I. The secondary antibodies were Alexa-488 anti-mouse or anti-rabbit IgG (both Invitrogen, Darmstadt, Germany). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (Millipore, Temecula, CA, USA). The stained sections were observed with a confocal laser scanning microscope (LSM510, Carl Zeiss, Jena, Germany).

Dermatopathology

For histopathological examination on light microscopy, skin biopsies were fixed with formalin, embedded in paraffin and stained with haematoxylin & eosin (H&E) using standard protocols. For immunohistochemistry (IHC) for collagen IV, the sections were deparaffinized and rehydrated in graded series: X-TRA-Solv 8 (Medite GmbH , Burgdorf Germany, # 41-5212-00), 15 min at 68°C; xylol, 5 min room temperature (RT), 100% ethanol, 5 min RT; 96% ethanol, 5 min RT; 80% ethanol, 5 min RT; distilled water, 2 min RT. For antigen retrieval, the slides were heated in a Dako Cytomation Pascal Pressure Cooker (DakoCytomation, Glostrup, Denmark) (115°C) and, endogenous peroxidase activity was then blocked using 3% hydrogen peroxide in distilled water (10 min). Normal goat serum was used to block non-specific epitopes (30 min) and the sections were then incubated with the primary antibody against human collagen IV (Dako Cytomation, #CIV 22, dilution 1:50) as well as the corresponding biotinylated anti-goat IgG secondary antibody (1:100 dilution, 30 min). In accordance with the manufacturer's protocol (Dako), visualization was achieved via application of streptavidin conjugated to alkaline phosphatase. Additional Mayer's haematoxylin staining was applied in order to depict the cell nuclei.

STable I. Antibodies used for immunofluorescence mapping

Antigen	Name/clone	Host	Source reference
Keratin 5/6	D5/16 B4	Mouse	Dako
Keratin 14	LL 002	Mouse	Abcam, ab7800
Plectin	10F6	Mouse	Santa Cruz
Plectin	31	Mouse	BD Biosciences
BPAG 1	279	Mouse	Cosmo Bio
Plakophilin 1	PP1-5C2	Mouse	Progen
Plakoglobin	PG5.1	Mouse	Progen
Laminin Γ2 chain	D4B5	Mouse	Millipore
Integrin B4	3E1	Mouse	Millipore
Integrin A6	GOH3	Rat	Progen
Integrin A3	P1B5	Mouse	Chemicon
Collagen type IV	CIV-22	Mouse	Abcam
Collagen type VII	LH 7,2	Mouse	Millipore
Collagen type XVII	NC16A-3	Mouse	Abcam
Laminin B3 chain	6F12	Mouse	Santa Cruz