Supplementary Materials and Methods

Transmission electron microscopy

Skin samples were fixed in glutaraldehyde solution, post-fixed in 1% OsO_4, dehydrated, and embedded in Epon 812. The embedded samples were sectioned at 1 μm thickness for light microscopy and thin-sectioned for electron microscopy (70 nm thick). The thin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy (H-7100; Hitachi, Tokyo, Japan).

Immunofluorescence

Skin specimens were frozen on dry ice in an optimal cutting temperature (OCT) compound. Sections were incubated with primary antibodies, followed by FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, USA). The nuclei were stained with propidium iodide (PI). The stained immunofluorescent samples were then observed using a confocal laser scanning microscope FV-1000 (Olympus, Tokyo, Japan). The following primary mouse monoclonal antibodies were used: anti-laminin 332 (L332) (GB3; Sera-lab, Cambridge, UK), anti-type IV collagen (COL4) (PHM-12+CIV22;

NeoMarkers, Fremont, CA, USA), and anti-type VII collagen (COL7) (LH7.2; Sigma-Aldrich, St. Louis, MO, USA).

Whole exome sequencing

gDNA was extracted from peripheral blood using QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA) and sent for whole-exome sequencing to screen EB-related genes (Has et al., 2020; Uitto et al., 2018). 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 an in-house pipeline (Hou et al., 2021). The variants were filtered to retain those with a frequency of less than 0.05% in the 1,000 Genomes Project (http://www.internationalgenome.org/) and in the ExAC Browser (http://exac.broadinstitute.org/).

Sanger sequencing

PCR amplification was performed on *COL7A1* exons that correspond to the proband's variants, followed by direct automated sequencing using an ABI

PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA). The sequencing data were visualized by SnapGene Viewer (snapgene.com).

Guide RNAs (gRNAs)

The gRNAs were assembled using CRISPR RNAs (crRNAs) and trans-activating crRNAs (IDT, Coralville, IA, USA). The crRNA probes were designed by the Benchling software (https://benchling.com/academic) (**Supplementary Table S5)**.

nCATS

Cas9 enrichment and adapter ligation of gDNA samples were performed as described previously (Gilpatrick et al., 2020; Natsuga et al., 2022), following the protocol for “Cas9 targeted sequencing” (ENR_9084_v109_revP_04Dec2018). Briefly, gDNA was dephosphorylated by Quick Calf Intestinal Phosphatase (New England Biolabs). 1–3 μg of gDNA was applied to each reaction. Cleavage and dA-tailing of dephosphorylated DNA samples were performed using Cas9 ribonucleoprotein complexes, dATP (New England Biolabs), and Taq polymerase (New England Biolabs) at 37 ˚C for 15 min. Cas9

ribonucleoprotein complexes were assembled with annealed all crRNAs/tracrRNA (IDT), CutSmart buffer (New England Biolabs), and S. pyogenes HiFi Cas9 nuclease (IDT). Adapter Mix (AMX) of Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore Technologies) was ligated to DNA ends using NEBNext Quick T4 Ligase (New England Biolabs) with Ligation buffer LNB of the Ligation Sequencing Kit (Oxford Nanopore Technologies). The samples were purified using AMPure XP beads (Beckman Coulter), used for library preparation, and applied to sequencing using MinION. For the analysis of the gDNA of the proband, a single reaction for each nCATS experiment (nCATS-1, nCATS-2, and nCATS-3) was prepared and pooled before purification using AMPure XP beads to apply for MinION sequencing as one library.

Reads were base called using Guppy v5.0.11 with dna_r9.4.1_450bps_sup.cfg as a DNA model and were mapped to the genomic sequence of *COL7A1* (NC_000003.12:c48595302-48564073) using minimap2 2.16-r922 (Li, 2017). Using SAMtools 1.9 (Li et al., 2009), information from the reads was mapped to the loci of the three variants and was extracted to generate a pileup output. For c.1474_1505del, the allele on a read was

determined to be the deletion mutant when more than 80% of the corresponding sequence was deleted or determined to be wild-type if less than 20% was deleted. The threshold was based on the accuracy of MinION (90-99%) (Delahaye & Nicolas, 2021; Natsuga et al., 2022). Graphs were prepared using a GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Haplotypes with unexpected bases or indels at the variant loci were excluded from the graph.

Ethics

The institutional review board of the Hokkaido University Graduate School of Medicine approved all human studies described above (ID: 13-043). The study was carried out according to the Declaration of Helsinki Principles. Participants or their legal guardians provided written informed consent.

References to Supplementary Materials and Methods

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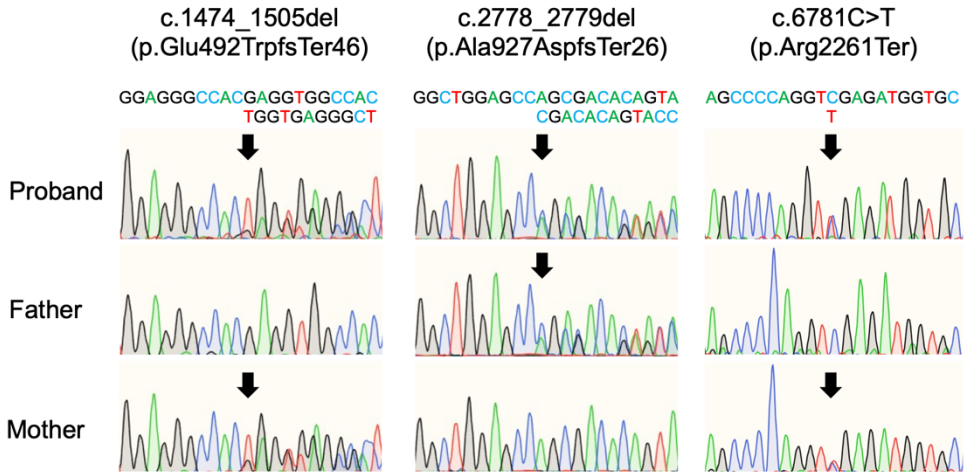

Supplementary Figure S1. *COL7A1* variants detected in the family.

The variant nomenclature is based on NM_000094.4. The presence of variants is indicated by arrows.

Supplementary Table S1. Read counts of *COL7A1* haplotypes by nCATS-1.

Sample	c.1474_1505del	c.2778_2779del	Reads
Proband	Mutant	Wild-type	90
	Wild-type	Mutant	90
	Wild-type	Wild-type	1
	Mutant	Mutant	0
	Others*		29
Total			210

*Haplotypes with unexpected bases or indels at the loci.

Supplementary Table S2. Read counts of *COL7A1* haplotypes by nCATS-2.

Sample	c.2778_2779del	c.6781C>T	Reads
Proband	Mutant	Wild-type	42
	Wild-type	Mutant	38
	Wild-type	Wild-type	1
	Mutant	Mutant	1
	Others*		30
Total			112

*Haplotypes with unexpected bases or indels at the loci.

Supplementary Table S3. Read counts of *COL7A1* haplotypes by nCATS-3 (proband).

Sample	c.1474_1505del	c.2778_2779del	c.6781C>T	Reads
Proband	Mutant	Wild-type	Mutant	9
	Mutant	Mutant	Wild-type	0
	Wild-type	Mutant	Mutant	0
	Mutant	Wild-type	Wild-type	1
	Wild-type	Mutant	Wild-type	16
	Wild-type	Wild-type	Mutant	0
	Mutant	Mutant	Mutant	0
	Wild-type	Wild-type	Wild-type	0
	Others*			17
Total				43

*Haplotypes with unexpected bases or indels at the loci.

Supplementary Table S4. Read counts of *COL7A1* haplotypes by nCATS-3 (mother).

Sample	c.1474_1505del	c.6781C>T	Reads
Mother	Mutant	Wild-type	1
	Wild-type	Mutant	0
	Wild-type	Wild-type	22
	Mutant	Mutant	30
	Others*		4
Total			57

*Haplotypes with unexpected bases or indels at the loci.

Supplementary Table S5. The gRNA sequences and their target sites.

gRNA name	gRNA sequence	Target site (GRCh38/hg38)	nCATS -1	nCATS -2	nCATS -3
COL7A1_c.1474_fwd1	CCACTGGCTACCGTGTGACA	Chr3:(-) 48592386	o		o
COL7A1_c.1474_fwd2	TGTAGAGTCCTGAGTCTGCA	Chr3:(-) 48592301	o		o
COL7A1_c.1474_fwd3	CTTGGAGCCACCGCAGAAGG	Chr3:(-) 48591821	o		o
COL7A1_c.2778_fwd1	GCTTCACGTGGTGCAGCGCG	Chr3:(-) 48588376		o	
COL7A1_c.2778_fwd2	AGGAAGTTAGGGACCATTGG	Chr3:(-) 48588263		o	
COL7A1_c.2778_fwd3	TGGGATCAATGAGTTCATGG	Chr3:(-) 48588128		o	
COL7A1_c.2779_rev1	CATCATGGGAGGTCATGCTG	Chr3:(+) 48587651	o		
COL7A1_c.2779_rev2	AGTTCAATGCTTGGAACACG	Chr3:(+) 48587527	o		
COL7A1_c.2779_ref3	GATCCCTGGAAGTGTCTGCG	Chr3:(+) 48587302	o		
COL7A1_c.6781_rev1	ATGAAAGCTGAGGGTCATGA	Chr3:(+) 48572269		o	o
COL7A1_c.6781_rev2	CAGAAAGATGAGACTCCGGA	Chr3:(+) 48572211		o	o
COL7A1_c.6781_rev3	ATGTGGTGAGAAACAGACCA	Chr3:(+) 48571763		o	o