

## SHORT COMMUNICATION

Localised Dominant Dystrophic Epidermolysis Bullosa with a Novel *de Novo* Mutation in *COL7A1* Diagnosed by Next-generation SequencingMakoto Nagai<sup>1#</sup>, Hiroshi Nagai<sup>2#</sup>, Chiharu Tominaga<sup>1</sup>, Yoshiko Sakaguchi<sup>1</sup>, Orié Jitsukawa<sup>1</sup>, Noriko Ohgo<sup>3</sup>, Chikako Nishigori<sup>2</sup> and Kiyofumi Yamanishi<sup>1\*</sup><sup>1</sup>Department of Dermatology, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, <sup>2</sup>Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe, and <sup>3</sup>Ohgo Clinic, Kobe, Japan. \*E-mail: kyamanis@hyo-med.ac.jp<sup>#</sup>These authors contributed equally to this study.

Accepted Nov 24, 2014; Epub ahead of print Nov 26, 2014

Dystrophic epidermolysis bullosa (DEB) is a group of hereditary bullous dermatoses caused by mutations in the *COL7A1* gene (reviewed in (1)).

In autosomal recessive DEB (RDEB) with severe generalised phenotypes, non-sense, frame-shift, indels or splice-site mutations predicting biallelic premature termination codons (PTCs) of *COL7A1* cause deficiencies in anchoring fibrils by non-sense-mediated mRNA decay or due to incomplete type 7 collagens. Allelic PTCs are often detected in generalised but mild RDEB, in which a mutant type 7 collagen can be found. A heterozygous mutation in a Gly residue of a Gly-X-Y repeat is often identified in dominant DEB (DDEB), although some Gly substitutions are linked to recessive or both dominant and recessive inheritance. However, such genotype–phenotype correlations are not fully understood in rare subtypes of DEB (2). The present case is sporadic with blisters on the hands and feet. Multiplex analysis of 16 genes by next-generation sequencing (NGS) revealed that this case is a localised subtype of DDEB with a novel p.Gly1761Asp missense mutation in a Gly-X-Y triplet of type 7 collagen.

## CASE REPORT

A 10-month-old female infant was referred to us for recurrent blisters on her hands and feet. She was born at 37 weeks and 4 days gestation from a healthy mother and had a birth weight of 2,342 g. She had no cutaneous symptoms at birth, but since 4 days after birth small blisters appeared on her fingers. Topical application of nadifloxacin 1% cream was not effective and she was suspected of a congenital blistering disease. Her brother had atopic dermatitis and there was no history of blistering disorders in her family. On examination, small-crusted ulcers and scars of 1 to 3 mm in diameter were seen on the proximal interphalangeal (PIP) joints of both the third to fifth fingers (Fig. 1A) and there was a small erosion on her left hallux and a blister on the PIP joint of her second toe (Fig. 1B). Her palms and soles were intact and nail deformities were absent. She had no complications in her neurological, cardiovascular and digestive systems and no other external malformations. Her growth in height and weight were within normal limits.

H&E staining of a biopsy specimen from a dorsal lesion of her right foot showed subepidermal blisters and some inflammatory infiltrates in the upper dermis (Fig. S1A<sup>1</sup>). Electron

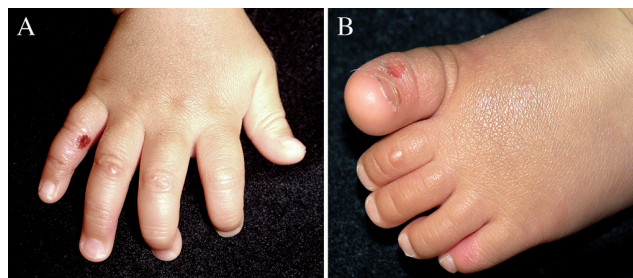


Fig. 1. Clinical findings of the present case. (A) Small crusted ulcers on the right fifth finger and scars of 1 to 2 mm in diameter on the proximal interphalangeal (PIP) joints of the right third and fourth fingers. (B) A small erosion on the left hallux and a blister on the PIP joint of the left second toe; no nail involvement was noted.

microscopy revealed blister formation beneath the lamina densa (Fig. S1B<sup>1</sup>). Type 7 collagen stained with a monoclonal LH7.2 antibody was evident along the basement membrane in the intact skin around a bulla (Fig. S1C<sup>1</sup>), whereas positive staining was separately visible at both the epidermal and dermal sides in a lesion (Fig. S1D<sup>1</sup>).

To make a molecular diagnosis, genomic DNA from the patient's peripheral blood was subjected to NGS for genes including *KRT5*, *KRT14*, *DST*, *EXPH5*, *DSP*, *PLEC*, *LAMA3*, *LAMB3*, *ITGA3*, *LAMC3*, *ITGB4*, *ITGB6*, *COL7A1*, *COL17A1*, *CD151* and *FERMT1*, under approval of the Institutional Review Boards. Informed consent was obtained from her parents. Target sequences of exons and their flanking sequences about 10 bp were captured using SureSelect probes (Agilent Technologies Inc., Santa Clara, CA) and were used for preparation of a DNA library. Base calls and reads from the library were recorded using a NGS sequencer MiSeq (Illumina, Inc., San Diego, CA) and fastq files were analysed using Avasis software (Strand Genomics, San Francisco, CA). Alignment of sequences with human genome sequence hg19 identified a heterozygous single nucleotide variation (SNV) of chr.3: g.48616827C>T (negative strand) (Fig. S2<sup>1</sup>). The SNV corresponded to c.5282G>A in exon 60, which was deduced to cause a missense mutation of p.Gly1761Asp in the triple helix domain of type 7 collagen. No known mutations or novel variations such as SNVs and indels were found in those genes analysed other than *COL7A1*. The SNV was also confirmed using Sanger sequencing. The SNV was not found in her parents and had not been found in 100 healthy Japanese alleles and in dbSNP (release B141). All variations detected in *KRT14*, *KRT5*, *PLEC* and *ITGB4*, which are responsible for epidermolysis bullosa simplex (EBS), were known non-pathogenic variants which were listed in dbSNP and no novel variants were found.

From the clinical manifestations and molecular analysis, localised DDEB due to the *de novo* mutation in *COL7A1* was diagnosed. No exacerbation of blistering has been observed

<sup>1</sup><https://doi.org/10.2340/00015555-2019>

until now. Milia following blisters were evident but scar formation was only mild.

## DISCUSSION

The International DEB Patient Registry enrolled 579 patients in 2011, 79% were RDEB and 21% were DDEB (2). The rates of the generalised and pruriginosa subtypes were 7.8% and 3.3%, respectively, while the localised (acral) subtype was 1.0%. Other subtypes of DDEB, pretibial, nails only and bullous dermolysis of newborns occur less frequently<sup>2</sup>.

All cases with localised DDEB have not necessarily been documented or registered in DEB databases and therefore those mutation reports are limited. Here we report another *de novo* mutation of c.5282G>A (p.Gly1761Asp) in exon 60. A homozygous mutation at the same nucleotide c.5282G>C leading to p.Gly1761Ala has been found in a patient with RDEB inversa (6). RDEB inversa is a subtype characterised by blisters on intertriginous, acral, lumbosacral and axial regions and mucosal involvement of the oral cavity, gastrointestinal and genitourinary tracts, and dystrophic nails. Thus, even if at the same Gly residue, the substitution to Asp or Ala is linked to dominant or recessive disease, respectively, and the heterozygous mutation to Asp causes the phenotype of localised DDEB whereas the homozygous mutation to Ala causes the different phenotype of RDEB inversa. Both Gly and Ala are non-polar amino acids, but Asp is an acidic one with a negative charge. The different charge of the amino acid might destabilise the triple helical structure of type 7 collagens leading to the fragility at the sublamina densa<sup>3</sup>.

Comprehensive multiplex analysis of genes by NGS may be helpful to establish an exact diagnosis for EB, as reported recently by Takeichi et al. (15). The localised subtype of DDEB is often clinically indistinguishable from localised EBS (Weber-Cockayne), EBS Ogna, EBS BP230 (DST) deficiency and localised RDEB. In such

cases, when a skin biopsy specimen is not available, this approach using NGS may be valuable in place of electron microscopy or immunofluorescence staining, although sequence information obtained by the target capture is limited to coding exons and their flanking splice sites.

As discussed by Takeichi et al. (15), to introduce NGS into a diagnostic routine for epidermolysis bullosa, there are some problems with the cost, facilities, staff, and time required.

## ACKNOWLEDGEMENTS

The authors thank members of the Joint-Use Research Facilities of the Hyogo College of Medicine for their technical assistance. This work was supported in-part by a grant from the "Research on Measures for Intractable Diseases" Project: matching fund subsidy (H23-028) from the Ministry of Health, Labour and Welfare, Japan.

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<sup>2</sup>In localised DDEB, Gly-substitutions at a Gly-X-Y repeat, c.5318G>T (p.Gly1773Val) in exon 61 (3), c.6109G>A (p.Gly2037Arg) (4), c.6127G>A (p.Gly2043Arg) in exon 73 (3, 5) and c.8138G>A (p.Gly2713Asp) in exon 110 (unpublished; listed in www.deb-central.org), have been reported (Fig. S3<sup>1</sup>).

<sup>3</sup>Reported mutations in DDEB around p.Gly1761 are c.5264G>T (p.Gly1755Val) (generalised) (7–9), c.5264G>A (p.Gly1755Asp) (generalised; pruriginosa) (7, 8) in exon 59, c.5291G>A (p.Gly1764Asp) in exon 60 (9), c.5308G>A (p.Gly1770Ser) (pretibial; nails only) (9), c.5317G>C (Gly1773Arg) (pruriginosa) (10), c.5327G>A (p.Gly1776Glu) (generalised) (9), c.5326G>A (p.Gly1776Arg) (nail dystrophy, acral blistering and milia) (11), c.5326G>T (p.Gly1776Trp) (lesions localised in knees) (12) and c.5372G>A (p.Gly1791Glu) (pruriginosa) (13) in exon 61. No mutations causing the localised subtype have been documented in exons 59–61. Cases with DDEB, localised DDEB, localised RDEB, pretibial DDEB, pretibial RDEB or the DEB pruriginosa subtype might show blisters on the hands and feet in infancy (14). Lichenoid papules or plaques, or atrophic plaques develop in pretibial subtypes and severe itching occurs in pruriginosa subtypes. The present case has not had such manifestations until now but careful follow-up is necessary.

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