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SHORT COMMUNICATION

Localised Dominant Dystrophic Epidermolysis Bullosa with a Novel *de Novo* Mutation in *COL7A1* Diagnosed by Next-generation Sequencing

Makoto Nagai[1#], Hiroshi Nagai[2#], Chiharu Tominaga[1], Yoshiko Sakaguchi[1], Orie Jitsukawa[1], Noriko Ohgo[3], Chikako Nishigori[2] and Kiyofumi Yamanishi[1]*

*[1]Department of Dermatology, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo, 663-8501, [2]Division of Dermatology, Department of Internal Related, Kobe University Graduate School of Medicine, Kobe, and [3]Ohgo Clinic, Kobe, Japan. *E-mail: kyamanis@hyo-med.ac.jp*

[#]These authors contributed equally to this study.

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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[1]). Electron microscopy revealed blister formation beneath the lamina densa (Fig. S1B[1]). 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[1]), whereas positive staining was separately visible at both the epidermal and dermal sides in a lesion (Fig. S1D[1]).

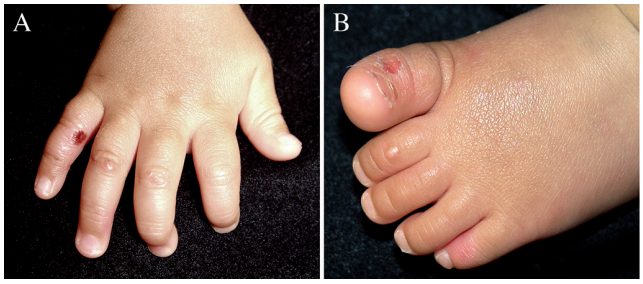

Fig. 1. Clinical findings of the present case. (A) Small crusted ulcers on the right fifth finger and scars of 1 to 2 mm in diameter on the proximal interphalangeal (PIP) joints of the right third and fourth fingers. (B) A small erosion on the left hallux and a blister on the PIP joint of the left second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

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 Avadis 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[1]). 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

[1]http://www.medicaljournals.se/acta/content/?doi=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[2].

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[3].

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.

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REFERENCES

1. Dang N, Murrell DF. Mutation analysis and characterization of COL7A1 mutations in dystrophic epidermolysis bullosa. Exp Dermatol 2008; 17: 553–568.
2. van den Akker PC, Jonkman MF, Rengaw T, Bruckner-Tuderman L, Has C, Bauer JW, et al. The international dystrophic epidermolysis bullosa patient registry: an online database of dystrophic epidermolysis bullosa patients and their COL7A1 mutations. Hum Mutat 2011; 32: 1100–1107.
3. Wertheim-Tysarowska K, Sobczynska-Tomaszewska A, Kowalewski C, Kutkowska-Kazmierczak A, Wozniak K, Niepokoj K, et al. Novel and recurrent COL7A1 mutation in a Polish population. Eur J Dermatol 2012; 22: 23–28.
4. Iwata T, Nakano H, Nakano A, Toyomaki Y, Tamai K, Tomita Y. Dominant dystrophic epidermolysis bullosa caused by a novel G2037R mutation and by a known G2028R mutation in the type VII collagen gene (COL7A1). J Dermatol 2006; 33: 550–556.
5. Escamez MJ, Garcia M, Cuadrado-Corrales N, Llames SG, Charlesworth A, De Luca N, et al. The first COL7A1 mutation survey in a large Spanish dystrophic epidermolysis bullosa cohort: c.6527insC disclosed as an unusually recurrent mutation. Br J Dermatol 2010; 163: 155–161.
6. van den Akker PC, Mellerio JE, Martinez AE, Liu L, Meijer R, Dopping-Hepenstal PJ, et al. The inversa type of recessive dystrophic epidermolysis bullosa is caused by specific arginine and glycine substitutions in type VII collagen. J Med Genet 2011; 48: 160–167.
7. Schumann H, Has C, Kohlhase J, Bruckner-Tuderman L. Dystrophic epidermolysis bullosa pruriginosa is not associated with frequent FLG gene mutations. Br J Dermatol 2008; 159: 464–469.
8. Kern JS, Gruninger G, Imsak R, Muller ML, Schumann H, Kiritsi D, et al. Forty-two novel COL7A1 mutations and the role of a frequent single nucleotide polymorphism in the MMP1 promoter in modulation of disease severity in a large European dystrophic epidermolysis bullosa cohort. Br J Dermatol 2009; 161: 1089–1097.
9. Almaani N, Liu L, Dopping-Hepenstal PJ, Lai-Cheong JE, Wong A, Nanda A, et al. Identical glycine substitution mutations in type VII collagen may underlie both dominant and recessive forms of dystrophic epidermolysis bullosa. Acta Derm Venereol 2011; 91: 262–266.
10. Jiang W, Sun TT, Lei PC, Zhu XJ. Genotype-phenotype correlation in Chinese patients with dystrophic epidermolysis

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[2]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[1]).

[3]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.

bullosa pruriginosa. Acta Derm Venereol 2012; 92: 50–53.
11. Dharma B, Moss C, McGrath JA, Mellerio JE, Ilchyshyn A. Dominant dystrophic epidermolysis bullosa presenting as familial nail dystrophy. Clin Exp Dermatol 2001; 26: 93–96.
12. Bursztejn AC, Pinault AL, Le Louarn Y, Lacour JP, Charlesworth A, Meneguzzi G, et al. Localized de novo dominant dystrophic epidermolysis bullosa. Ann Dermatol Venereol 2008; 135: 195–199.
13. Mellerio JE, Ashton GH, Mohammedi R, Lyon CC, Kirby B, Harman KE, et al. Allelic heterogeneity of dominant and recessive COL7A1 mutations underlying epidermolysis bullosa pruriginosa. J Invest Dermatol 1999; 112: 984–987.
14. Fine JD, Bruckner-Tuderman L, Eady RA, Bauer EA, Bauer JW, Has C, et al. Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. J Am Acad Dermatol 2014;70: 1103–1126.
15. Takeichi T, Liu L, Fong K, Ozoemena L, McMillan JR, Salam A, et al. Whole-exome sequencing improves mutation detection in a diagnostic epidermolysis bullosa laboratory. Br J Dermatol. 2014 Jun 19. [Epub ahead of print].