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

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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 had atopic dermatitis and there was no history of blistering disorders in her family. On examination, small-crusted ulcers were seen on the proximal interphalangeal (PIP) joints of both the third to fifth fingers and scars of 1 to 3 mm in diameter were seen 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.

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

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†). 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. 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.
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 lumbosacral, there are some problems with the cost, facilities, staff, and time required.

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