

## A Novel Insertion Mutation in COL7A1 Identified in Hallopeau-Siemens Recessive Dystrophic Epidermolysis Bullosa

Kazuko C. Sato-Matsumura<sup>1</sup>, Daisuke Sawamura<sup>1</sup>, Mikio Goto<sup>2</sup>, Maki Goto<sup>1</sup>, Hideki Nakamura<sup>1</sup> and Hiroshi Shimizu<sup>1</sup>

<sup>1</sup>Department of Dermatology Hokkaido University Graduate School of Medicine, N 15 W 7, Kita-ku, Sapporo, 060-8638, Japan, and <sup>2</sup>Department of Pediatrics, Shimada Municipal Hospital, Shimada, Japan. E-mail: kmatsum@med.hokudai.ac.jp

Accepted November 11, 2002.

Sir,

Different forms of dystrophic epidermolysis bullosa (DEB) caused by mutations in the type VII collagen gene (COL7A1, GenBank L23982, L02870) are inherited either in an autosomal dominant or autosomal recessive fashion. Hallopeau-Siemens (HS) type is the most severe form of recessive DEB and is characterized by the total absence of type VII collagen in the epidermal basement membrane. The mutations in DEB vary widely and more than 170 kinds of mutations have been reported so far, most of them representing single nucleotide substitutions. We report on a child with HS-DEB showing compound heterozygous mutations with a novel four base-pair insertion in one allele, and a non-sense point mutation on the other allele of COL7A1.

### CASE REPORT

A male patient was born with bullae and erosions predominantly on the trunk and the inverse sites of the axillaries, hands, feet, elbows, knees and genito-anal regions starting continuously or shortly after birth (Fig. 1a, b). The mucous membranes of the mouth,

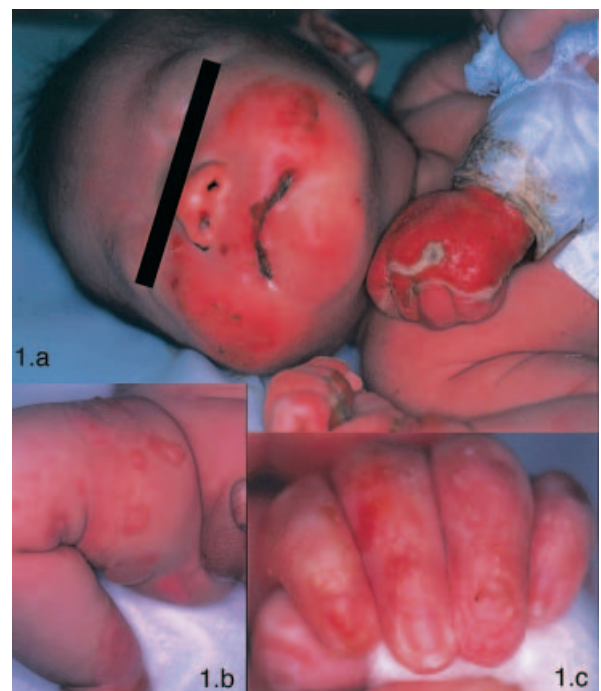
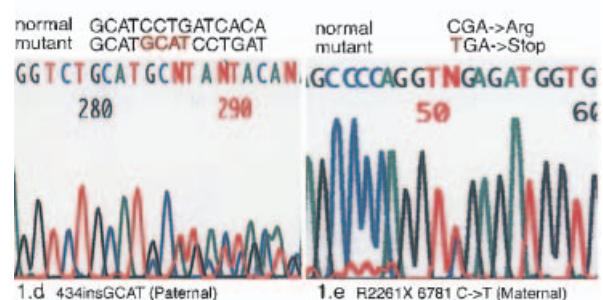


Fig. 1. Photographs taken 3 months after birth. Erosions were present on almost the entire body including lips, oral and nasal mucosa (a). Tense blisters appeared after minor accidental friction (b). The nails of the left ring fingers were lost, and all the remaining nails were deformed. Numerous millia were present on the re-epithelized skin (c). The DNA automated sequence revealed the paternal mutation, a four-base-pair insertion at position 434 resulting in a frame shift and a premature termination codon (d). The maternal mutation, C to T transition at nucleotide 6781, resulted in R2261X (e).



nose and conjunctiva of the eye were also erosive (Fig. 1a). The fingernails and toenails dropped off 3 months after birth (Fig. 1c). The infant's skin was fragile and bullae appeared easily after minor mechanical trauma. He was fed by nasogastric tube because he could not suckle milk from his mother's breast owing to continuous formation of bullae in his mouth. The patient was the parents' first child and there was no family history of any signs of skin fragility or nail dystrophy.

Skin biopsy specimens obtained from a fresh blister were snap frozen with liquid nitrogen and 5 µm cryostat sections were prepared. Immunostaining for antigen mapping was performed using S1193 (BPAG1), HDD20 (BPAG2), GoH3 ( $\alpha$ -6 integrin), 3E1 ( $\beta$ -4 integrin), 121 (plectin), 19DEJ1 (uncein), GB3 (laminin 5), LH7.2 (type VII collagen), and type IV collagen as described elsewhere (1). Histologic examination revealed a subepidermal blister. Immunohistochemically, type VII collagen (LH7.2) was completely negative. Other basement membrane zone antigens, including plectin,  $\alpha$ -6  $\beta$ -4 integrins, type XVII collagen (BPAG2), and laminin 5, were expressed normally. Antigen mapping confirmed the occurrence of blister formation below the lamina densa. An electron microscopy examination revealed that there were no normal anchoring fibrils at the basement membrane zone. Genomic DNA extracted from peripheral blood mononuclear cells was examined for mutations in COL7A1, as described elsewhere (1, 2). In brief, the PCR product was screened by conformation sensitive gel electrophoresis. The products that showed hetero-duplex bands were directly sequenced by an automated sequencer (ABI PRISM 3100 Genetic Analyzer, PE Biosystems, Foster City, CA, USA). The mutation was confirmed by restriction enzyme digestion; the 434insGCAT was confirmed with *Sph*I, and R2261X with *Taq*I. Since the present mutations were novel and had not been previously reported, we examined DNA extracted from 50 unrelated healthy Japanese volunteers as a control. All were negative for the 434insGCAT and R2261X mutations. Genetic screening of the COL7A1 gene revealed that both mutations led to premature termination codons (PTC). The paternal mutation (434insGCAT; Fig. 1d) resulted in a frame shift and lead to PTC in exon 5, at amino acid position 180. The maternal mutation (R2261X) (Fig. 1e) was caused by a C to T transition at nucleotide 6781 in exon 86.

## DISCUSSION

We identified a four-base-pair insertion (434insGCAT) in the present case of HS-RDEB. According to The

Human Mutation Database Cardiff (<http://archive.uwcm.ac.uk/uwcm/mg/search/128750.html>), multiple-base insertion is a rare cause of DEB. Among 174 different kinds of mutations registered before July 1, 2002, only 10 were insertion mutations; of 7 cases of single-base-pair insertions (3–6), 2 were two-base-pair insertions (5, 6), and only one was a four-base-pair insertion (6). The maternal mutation R2261X was also novel. In the present case, the severe clinical symptoms could be explained by a total absence of type VII collagen owing to the mutations in both alleles resulting in PTC.

## ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for the Encouragement of Young Scientists, No. 13770424, from the Japan Society for the Promotion of Science to Dr Sato-Matsumura, the 11th Grant for Cosmetology Research from The Cosmetology Research Foundation (Tokyo, Japan) to Dr Sato-Matsumura; Grant-in-Aid For Scientific Research (B) No. 12470175 and Grant-in-Aid for Scientific Research on Priority Areas (2) No. 14031202 from the Ministry of Education, Culture, Sports, Science and Technology of Japan to Dr Shimizu.

## REFERENCES

- Shimizu H, McGrath JA, Christiano AM, Nishikawa T, Uitto J. Molecular basis of recessive dystrophic epidermolysis bullosa: genotype/phenotype correlation in a case of moderate clinical severity. *J Invest Dermatol* 1996; 106: 119–124.
- Christiano A, Anhalt G, Gibbons S, Bauer E, Uitto J. Premature termination codons in the type VII collagen gene (COL7A1) underlie severe, mutilating recessive dystrophic epidermolysis bullosa. *Genomics* 1994; 21: 160–168.
- Christiano A, Anton-Lamprecht I, Amano S, Ebschner U, Burgeson R, Uitto J. Compound heterozygosity for COL7A1 mutations in twins with dystrophic epidermolysis bullosa: a recessive paternal deletion/insertion mutation and a dominant negative maternal glycine substitution result in a severe phenotype. *Am J Hum Genet* 1996; 58: 682–693.
- Christiano A, D'Alessio M, Paradisi M, Angelo C, Mazzanti C, Puddu P, et al. A common insertion mutation in COL7A1 in two Italian families with recessive dystrophic epidermolysis bullosa. *J Invest Dermatol* 1996; 106: 679–684.
- Hovnanian A, Rochat A, Bodemer C, Petit E, Rivers C, Prost C, et al. Characterization of 18 new mutations in COL7A1 in recessive dystrophic epidermolysis bullosa provides evidence for distinct molecular mechanisms underlying defective anchoring fibril formation. *Am J Hum Genet* 1997; 61: 599–610.
- Whitlock N, Ashton G, Mohammedi R, Mellerio J, Mathew C, Abbs S, et al. Comparative mutation detection screening of the type VII collagen gene (COL7A1) using the protein truncation test, fluorescent chemical cleavage of mismatch, and conformation sensitive gel electrophoresis. *J Invest Dermatol* 1999; 113: 673–686.