Hirosaki Med. J. 59 : 15—22, 2007

ORIGINAL ARTICLE

NOVEL *COL7A1* MUTATIONS OF HALLOPEAU-SIEMENS TYPE RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA

Takaya Murai*, Katsuto Tamai, Hajime Nakano, Katsumi Hanada, Isao Hashimoto and Daisuke Sawamura

Abstract Dystrophic epidermolysis bullosa (DEB) is caused by mutations in the *COL7A1* gene encoding type VII collagen. DEB is clinically characterized by mucocutaneous blistering in response to minor trauma, followed by scarring and nail dystrophy. DEB is inherited in either an autosomal dominant (DDEB) or recessive (RDEB) fashion. DDEB basically results from a glycine substitution mutation within the collagenous domain on one *COL7A1* allele, while a combination of mutations such as premature termination codon, missense, splice-site mutations on both alleles causes RDEB. In this study, we examined a Japanese EB boy with generalized blistering and scar formation, and made a diagnosis of the Hallopeau-Siemens type RDEB (HS-RDEB), the most severe form of RDEB. Mutational analysis of the *COL7A1* gene revealed a novel missense mutation A80P and a novel nonsense mutation Q1211X. In general, HS-RDEB is caused by combination of premature termination codon mutations, but 3 HS-RDEB cases have been reported to have combination of premature termination codon and missense mutations one of which was S48P. This study suggests that even missense mutation, which leads to substitution for proline in amino terminal end of type VII collagen, can cause HS-RDEB. Hirosaki Med. J. **59** : 15—22, 2007

Key words: dystrophic epidermolysis bullosa; Hallopeau-Siemens type; type VII collagen; missense mutations.

原 著

Hallopeau-Siemens 型劣性栄養障害型表皮水疱症における新規 *COL7A1* ミスセンス変異

村井孝弥* 玉井克人 中野 創
花田勝美 橋本 功 澤村大輔

抄録 栄養障害型表皮水疱症, dystrophic epidermolysis bullosa (DEB) は, VII 型コラーゲンをコードする *COL7A1* 遺伝子の変異で生ずる. 本症は, 臨床的に軽微な外傷による皮膚粘膜の水疱とその後の瘢痕や爪の変形を特徴とする. DEB は常染色体性優性 (DDEB) と常染色体性劣性 (RDEB) の両型がある. DDEB は通常 *COL7A1* のコラーゲン領域のグリシン置換で起こり, 一方 RDEB は早期終止コドン変異, ミスセンス変異, スプライス部位変異の組み合わせで生ずる. 本研究では, 全身の水疱と瘢痕形成を有し, RDEB のうちでもっとも重症である Hallopeau-Siemens 型 (HS)-RDEB と診断した日本人少年を検索した. *COL7A1* 遺伝子の解析では, 新規ミスセンス変異 A80P と新規ノンセンス変異 Q1211X を検出した. 一般に HS-RDEB は早期終止コドンの組み合わせで生ずるが, 現在までに 3 例で HS-RDEB が早期終コドン変異とミスセンス変異の組み合わせで起こることが報告され, そのなかのひとつが S48P であった. 今回の研究は, ミスセンス変異でも, VII 型コラーゲンのアミノ末端でのプロリンへの変異を誘導するものであれば, 最重症型である RDEB (HS-RDEB) も起こしうることを示唆していた.
弘前医学 **59** : 15—22, 2007

キーワード：栄養障害型表皮水疱症; Hallopeau-Siemens 型; VII 型コラーゲン; ミスセンス変異.

Department of Dermatology, Hirosaki University Graduate School of Medicine
* Present Address: Department of Dermatology, Hachinohe City Hospital
Correspondence: T. Murai
Received for publication, July 4, 2007
Accepted for publication, July 31, 2007

弘前大学大学院医学研究科皮膚科学講座
* 現住所：八戸市立市民病院皮膚科
別刷請求先：村井孝弥
平成19年 7 月 4 日受付
平成19年 7 月31日受理

Introduction

Dystrophic epidermolysis bullosa (DEB) is clinically characterized by mucocutaneous blistering in response to minor trauma, followed by scarring and nail dystrophy, in which patients exhibit tissue separation beneath the lamina densa close to dermal anchoring fibrils. DEB is inherited in either an autosomal recessive (RDEB) or dominant (DDEB) fashion, each form having a different specific clinical presentation and severity[1].

RDEB has wide range of clinical severities. The Hallopeau-Siemens type (HS-RDEB), the most severe form of RDEB, exhibits extracutaneous involvement such as strictures of the esophagus, erosion of the anus, and digital fusions resulting from repeated blister formation. The other end of the clinical spectrum of RDEB shows only localized skin involvement. On the other hand, DDEB is typically less severe than RDEB, and blister formation decreases with age.

An increasing number of DEB mutations have elucidated general genotype-phenotype correlations[2,3]. DDEB patients basically harbor glycine substitution mutations within the collagenous domain on one *COL7A1* allele, leading to disruptions in anchoring fibril assembly and relatively mild clinical features. On the other hand, patients with RDEB in its most severe form, the Hallopeau-Siemens variant (HS-RDEB), have premature termination codon (PTC) mutations on both alleles. These mutations characteristically lead to nonsense-mediated mRNA decay that manifests as a remarked decrease of type VII collagen protein and severe loss of anchoring fibrils. On the other hand, patients with the non-Hallopeau Siemens variant (nHS-RDEB) show milder phenotype and some type VII collagen can be synthesized. This DEB subtype is caused by a combination of mutations such as PTC, missense, splice-site mutations on both alleles.

To understand precise phenotype-genotype correlation in DEB patients, the present study examined *COL7A1* mutations in a Japanese boy with HS-RDEB.

Methods

Clinical feature

The case was a 1 month-old infant and showed bullae and erosions on his trunk, hands, feet, elbows and knees (Fig 1). Epithelization of bullae left scar and milia. Afterwards recurrent blister and scar formation led to fusion of fingers and toes, resulting in a mitten-like deformity of his hands and feet (Fig1). The mucous membranes of his mouth and nose were also eroded. He was the first child of his parents and there was no family history of skin fragility or nail dystrophy. A cicatrical stricture of the preputial orifice was noted when he was 3 years old. To prevent complications, a dorsal incision of the prepuce was performed. At the age of 17 years, severe dysphagia, extensive caries, constipation, and growth retardation were seen.

Ultrastructural analysis

For electron microscopic examination, skin specimens were taken from the abdomen at the age of 2 years. The samples were fixed in 5% glutaraldehyde and postfixed in 1% osmium tetroxide, stained en-block in uranylacetate. They were dehydrated in a graded series of ethanol solutions, then embedded in Araldite 6005. Ultrathin sections were cut, stained with uranyl acetate and lead citrate. The sections were examined with a transmission electron microscope at 75kv.

PCR amplification and heteroduplex analysis

Genomic DNA samples were extracted from peripheral blood lymphocytes of the patient and parents, and used as a template for amplification of genomic sequences of *COL7A1*. We synthesized

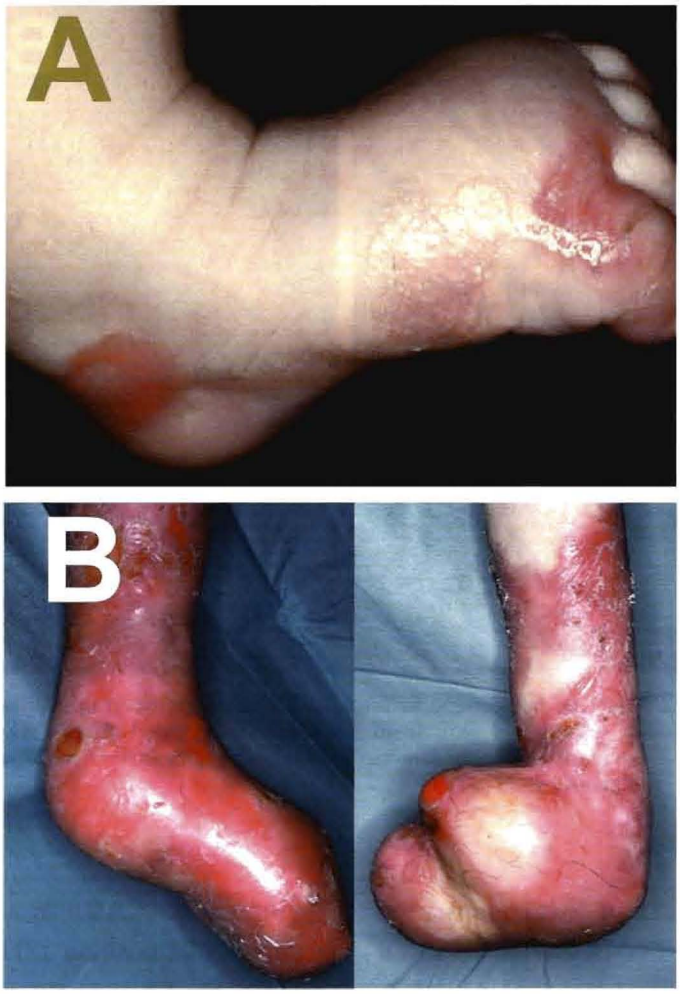

Fig. 1 Clinical feature. A: The proband was a 1 month-year old infant and showed bullae and erosions on his trunk, hands, feet, elbows and knees. B: Recurrence blister and scar formation led to fusion of fingers, resulting in a mitten-like deformity of his hands.

67 pairs of oligonucleotide primers, which spanned all 118 exons of the gene[4,5], on the basis of the intronic sequences. The PCR products were screened by heteroduplex analysis utilizing conformation-sensitive gel electrophoresis (CSGE). PCR products with altered mobility (heteroduplexes) detected by CSGE were then directly sequenced. Some of the PCR product was inserted into the TA cloning vector (Invitrogen, San Diego, CA) to isolate sequences derived from each allele. The cloned products also were sequenced. When the mutation led to gain or loss of a restriction enzyme site, the PCR product was subjected to digestion with the appropriate restriction enzyme to verify the mutation (PCR-restriction enzyme fragment length polymorphism (PCR-RFLP).

Genomic DNA samples were obtained from 50 normal individuals, and used for confirmation of the detected mutations.

Informed consent was obtained from the parents when we took genomic DNA samples.

Results

Electron microscopic findings

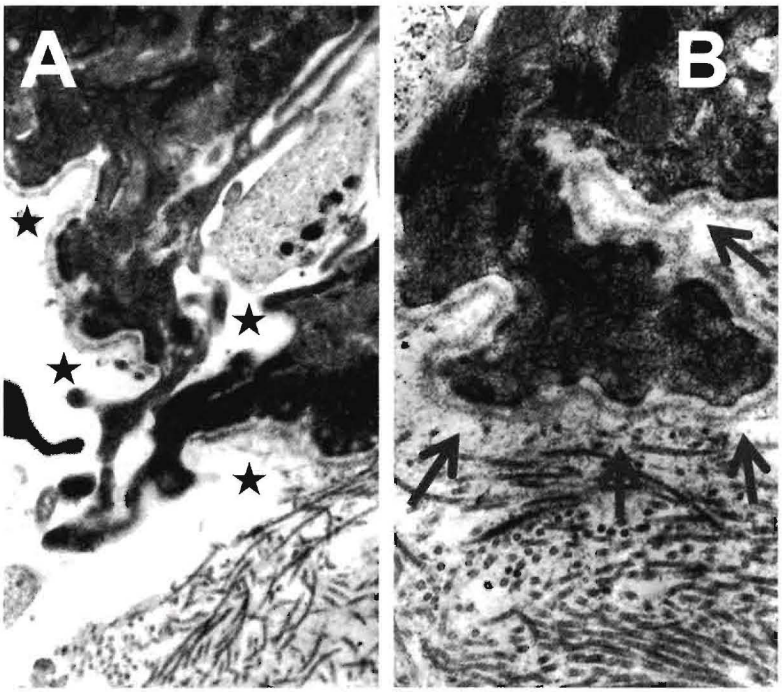

Fig. 2 Electron microscopic findings. A: Transmission electron microscopy of uninvolved skin from his forearm revealed splits (★) just beneath the basal lamina. B: Anchoring fibrils (→) were much diminished.

Transmission electron microscopy of uninvolved skin from his forearm revealed splits just beneath the basal lamina. Although tonofilaments, hemidesmosomes and lamina densa were seen normally, anchoring fibrils were much diminished (Fig. 2B). Thus, the patient was diagnosed as having DEB.

Mutation detection

The heteroduplex analysis of exon 2 revealed heteroduplex bands in the patient and his mother (Fig. 3). Sequence analysis of this PCR product revealed a guanine to a cytosine change at nucleotide position 238 and the substitution converted an alanine (GCC) to a proline (CCC) at amino acid position 80, designated as A80P.

Also CSGE analysis of exon 27 disclosed abnormal bands in the patient and the father. Sequence analysis of those bands demonstrated a cytosine to a thymine transition at nucleotide position 3631 and the transition substituted a glutamine (CAG) to a stop codon (TAG) at amino acid position 1211, designated as Q1211X (Fig 4). This nucleotide change 3631 C to T generated a recognition site of restriction endonuclease MaeI (CTAG). So digestion of the PCR product from the patient and his father showed heterozygous for the Q1211X mutation.

Neither mutation A80P or Q1211X were detected in 50 normal controls.

Discussion

Type VII collagen, a non-fibrillar collagen, is a major component of anchoring fibril loop structures beneath the epidermal basement membrane[6,7]. Cloning of collagen VII cDNA demonstrated a primary sequence of 2944 amino acids and the basic organization of the functional domains[4]. Subsequent genomic cloning has highlighted the structural organization of the collagen VII gene (*COL7A1*)[5]. The central portion of type VII collagen molecule consists

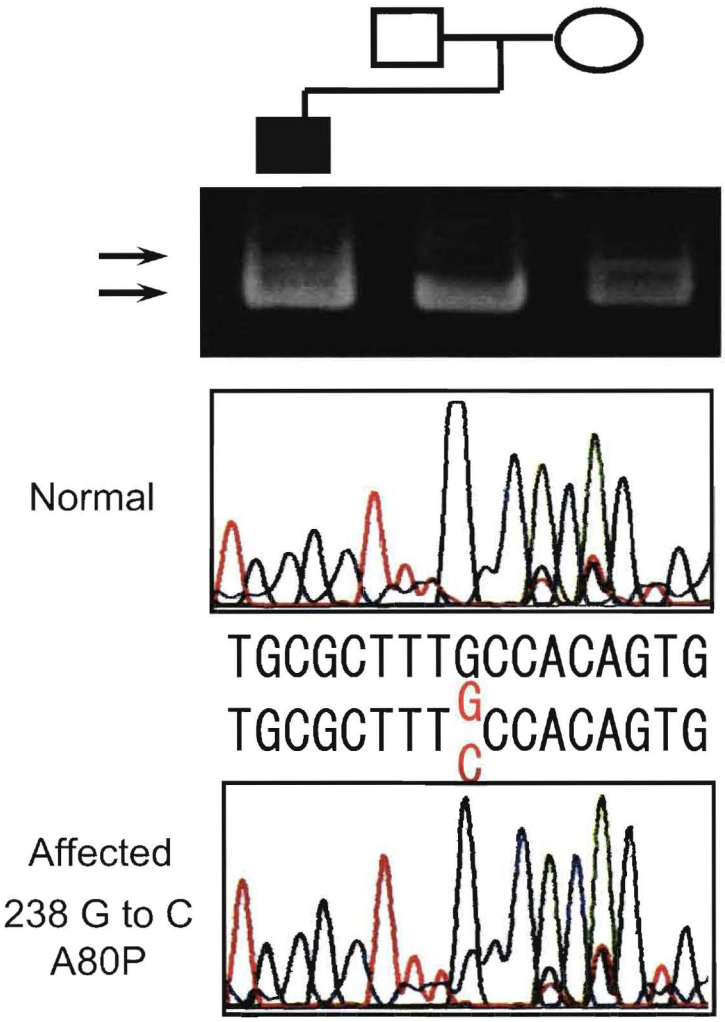

Fig. 3 Identification of maternal mutations. The heteroduplex analysis of exon 2 revealed heteroduplex bands (→) in the patient (■) and his mother (○). Sequence analysis of this PCR product revealed a guanine to a cytosine change at nucleotide position 238 and the substitution converted an alanine (GCC) to a proline (CCC) at amino acid position 80, designated as A80P.

of a collagen segment with Gly-X-Y repeat sequences, which folds into a characteristic triple-helical conformation. Glycine, the smallest amino acid, plays a key role in this conformation. The central collagenous domain is flanked by a large, ~145-kD non-collagenous amino-terminal globular region (NC-1) and a smaller, ~20-kD carboxyl-terminal globular portion (NC-2). Furthermore, elucidation of its genomic structure has led to identification of multiple, specific mutations in *COL7A1* in many DEB cases. To date, almost two hundred pathogenic mutations within the collagenous and noncollagenous domains of type VII collagen gene have been identified in different forms of DEB[8-11], but the detailed phenotype-genotype correlations have not been elucidated yet.

In this study, the proband showed blistering with scar, severe dysphagia, extensive caries, constipation, and growth retardation, and these clinical features suggested the most severe

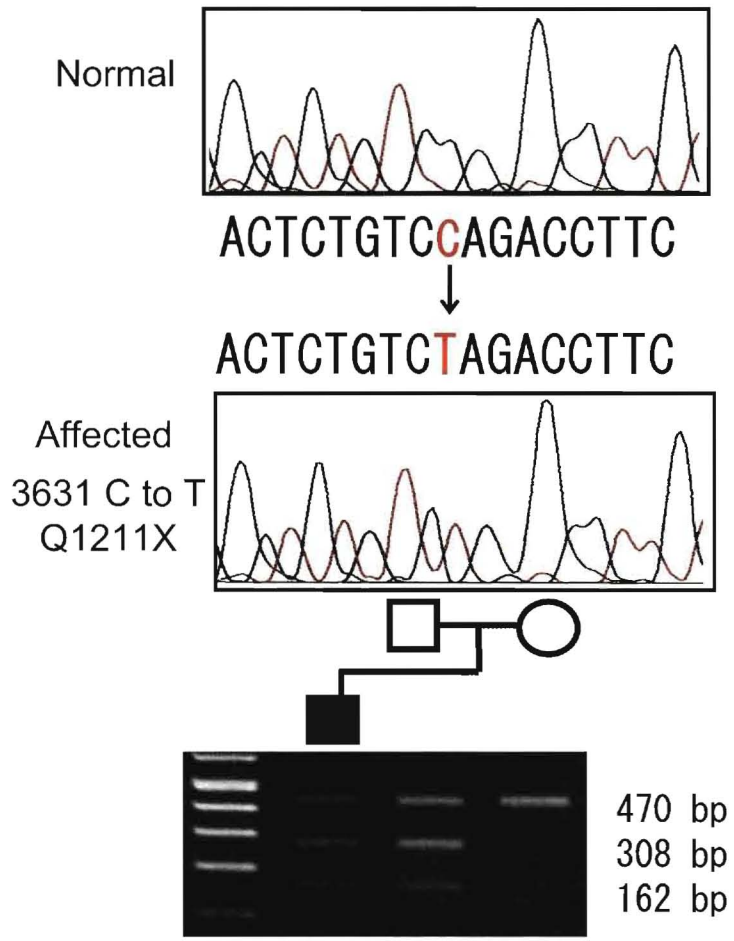

Fig. 4 Identification of paternal mutations. Sequence analysis of the PCR bands from exon 27 demonstrated a cytosine to a thymine transition at nucleotide position 3631 and the transition substituted a glutamine (CAG) to a stop codon (TAG) at amino acid position 1211, designated as Q1211X. Verification of the mutation by digestion with MaeI indicated that the 470-bp PCR product with the mutation was cleaved into 308-bp and 162-bp fragments. The proband (■) and his father (□) were heterozygous for the Q1211X mutation.

form of RDEB, the Hallopeau-Siemens variant (HS-RDEB). In fact, transmission electron microscopy of uninvolved skin from his forearm revealed that separation occurred just beneath the basal lamina and anchoring fibrils were much diminished in number. The mutation analysis revealed maternal missense mutation A80P and paternal nonsense mutation Q1211X. Since we could not detect those changes in 50 normal controls, we thought that both A80P and Q1211X were pathogenic. A80P is the second most amino terminal missense mutation among pathogenic mutations within the collagenous and noncollagenous domains of type VII collagen gene in different forms of DEB[8-11].

The data base of *COL7A1* mutations has indicated that HS-RDEB has PTC mutations on both alleles. The paternal mutation Q1211X is nonsense mutation resulting in nonsense-mediated mRNA decay that manifests as a complete absence of type VII collagen protein. Thus, no or little type VII collagen is synthesized

from the paternal *COL7A1* allele. On the other hand, the maternal mutation A80P is missense mutation and type VII collagen is synthesized from maternal allele. As far as we know, 3 HS-RDEB cases with missense mutation have been reported and the missense mutations were S48P, R2063W and G2351R [12,13]. The missense mutation S48P from Chinese HS-RDEB patient was the most amino terminal missense mutation of all previous missense mutations. Furthermore, both S48P and A80P of our HS-RDEB case interestingly generated a proline residue. The amino terminal end of type VII collagen plays an important role in association of the carboxyl terminal end of laminin 5 in basal lamina[14]. Since a proline residue is a strong α-helix and β-sheet breaker, those missense mutations may destroy attachment of type VII collagen and laminin5[15]. Thus, we suggest that substitution for proline in amino terminal end of type VII collagen can contributes1 to the most severe form, HS-RDEB. The study furthers our understanding of both the clinical and allelic heterogeneity displayed in DEB patients.

Acknowledgment

We are grateful to Komaki Hanada for excellent technical assistance. This study was supported by grants from the Ministry of Health, Welfare and Labor of Japan, the Ministry of Education, Science, Sports and Culture of Japan and Karouji Memorial Foundation.

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