それを知らせることにより，患者，家族と寄り添いともに病気を「克服」し，幸福が得られるようサポートすることが重要である．

患者家族会は，患者や家族の孤立感を解消するために非常に有用であり，社会的支援を得るためにも声を発しやすい環境を提供する．表皮水疱症では以前より世界各国にDebRA（Dystrophic epidermolysis bullosa research association）という患者支援団体があったが本邦でも2007年に患者友の会が発足し，Debra Japanとして活動を開始している（http://www.ne.jp/asahi/eb-japan/com/）．

さいごに

遺伝情報は患者・家族・クライアントの診療に重要であるのみならず，疾患のメカニズムの解明や治療法の開発などの研究においても重要な位置を占める．遺伝情報はまた，生命保険会社の審査や人材採用などに悪用される危険性もはらんでおり，その取り扱いには十分な配慮が必要である．2001年，文部科学省，厚生労働省，経済産業省の三省により「ヒトゲノム・遺伝子解析研究に関する倫理指針」が出され，倫理委員会の設置，個人情報管理者の設置，検体の匿名化など具体的な指針が示された．遺伝医療および研究に携わるものは高い倫理観をもち，検体と情報の管理には細心の注意を払う必要がある[9]．

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Clinical and Experimental Dermatology

A novel *de novo* splice-site mutation in the *COL7A1* gene in dominant dystrophic epidermolysis bullosa (DDEB): specific exon skipping could be a prognostic factor for DDEB pruriginosa

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Summary

We report a Japanese infant who had a novel *de novo* splice-site mutation in the *COL7A1* gene, which resulted in in-frame exon 87 skipping. Very interestingly, most of the previously reported cases with the same exon skipping presented as dystrophic epidermolysis bullosa (DEB) pruriginosa. The proband in this study showed an extremely mild clinical phenotype, with no nail dystrophy, pruritus or prurigo-like lesions. However, dominant (DDEB) pruriginosa often shows a typical mild DEB phenotype until the onset of pruritus, making it likely that as she gets older the proband will present with features consistent with DDEB pruriginosa. By knowing in advance the anticipated clinical course, it might be possible to reduce or even prevent development of nodular prurigo-like lesions by sufficient control of pruritus. Our study should contribute to further refinement of the genotype–phenotype correlations in DEB, emphasizing the significance of mutation analysis for correct diagnosis and possibly for prediction of prognosis.

Dystrophic epidermolysis bullosa (DEB) is a clinically heterogeneous genodermatosis inherited either in an autosomal dominant (DDEB) or recessive (RDEB) manner, and characterized by blistering and scarring of the skin and mucous membranes in response to minor trauma.[1] All forms of the disease are caused by mutations in the *COL7A1* gene, which encodes type VII collagen,[2] the major component of anchoring fibrils that localize just beneath the lamina densa and maintain dermoepidermal adhesion. In this study, we examined the molecular basis of DDEB in a Japanese infant girl presenting with an extremely mild clinical phenotype, and found a novel *de novo* splice-site mutation in *COL7A1*, which resulted in in-frame exon skipping. We also reviewed the literature reports of mutations leading to the same exon skipping, in order to analyse the genotype–phenotype correlations in the proband.[3–6] Very interestingly, most of the reported cases presented as one particular subtype, DDEB pruriginosa.

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Report

The proband, a 2-month-old Japanese girl, was referred to our clinic for evaluation of recurrent nonscarring blisters on her fingers and toes that had been present since birth. Careful examination revealed a few blisters on the toes, but there was no evidence of nail dystrophy or extracutaneous involvement (Fig. 1a). The patient was the only child of nonconsanguineous parents, and there was no family history of blistering skin diseases.

Histological examination of a skin biopsy showed a subepidermal blister. Electron microscopy examination revealed epidermal detachment beneath the lamina densa, with a reduced number of anchoring fibrils (Fig. 1b). Indirect immunofluorescence using the monoclonal antibody LH7.2 against type VII collagen showed only slightly reduced linear staining along the

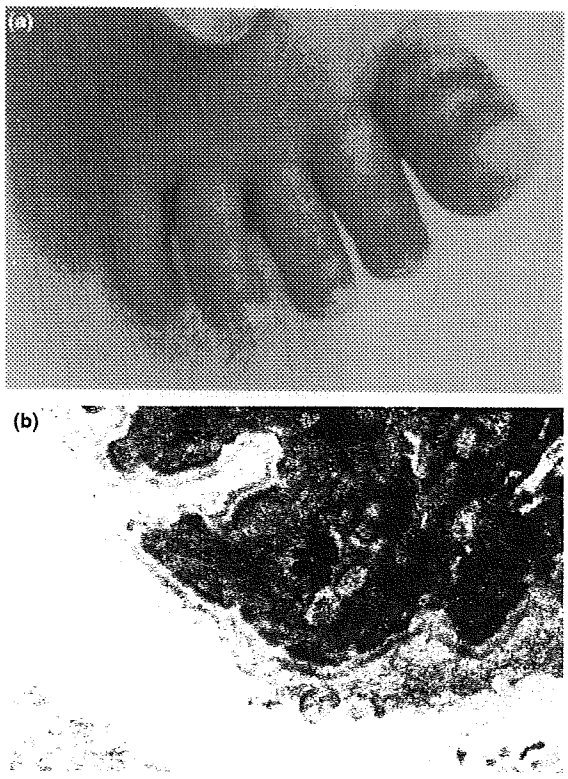

Figure 1 Clinical and ultrastructural features of the proband. (a) A few blisters on the toes, but there was no evidence of scarring or nail dystrophy. (b) Ultrastructural observation showed that a cleft had formed beneath the lamina densa and that anchoring fibrils were reduced in number (original magnification × 4000).

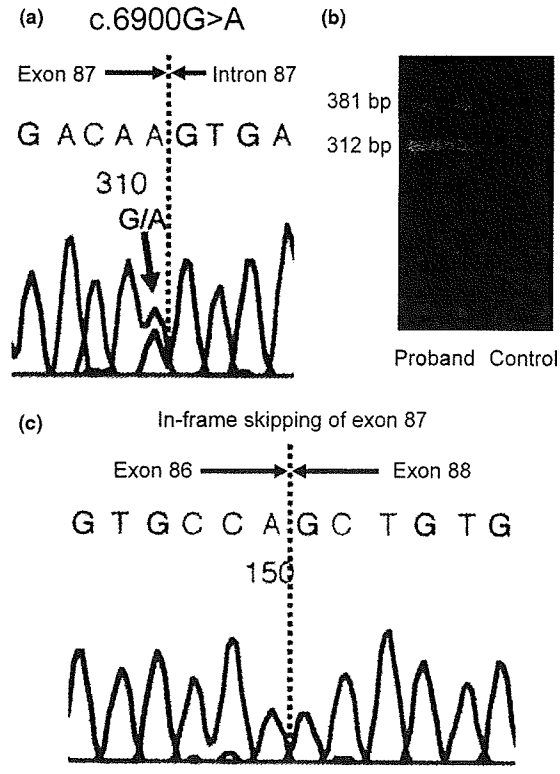

Figure 2 Identification of a novel mutation in exon 87 of *COL7A1*. (a) Sequencing analysis of the proband's genomic DNA showed a heterozygous G → A transition in the last nucleotide of exon 87 (c.6900G → A). (b) Electrophoresis of the RT-PCR product revealed the presence of a band of smaller size (312 bp), in addition to an expected band of normal size (381 bp). (c) Sequencing of the 312-bp RT-PCR product showed deletion of 69 nucleotides corresponding to the entire exon 87 (in-frame skipping of exon 87).

dermoepidermal junction (data not shown). Based on these observations, a diagnosis of DEB was made.

To identify the underlying mutations in the type VII collagen gene, genomic DNA was extracted from peripheral blood leucocytes of the proband and her parents, and amplified by PCR using gene-specific primers covering the entire coding segments of *COL7A1*, as described previously.[7] The PCR products were then subjected to direct automated sequencing. To examine the consequences of the detected mutation, total RNA was isolated from the proband's skin and reverse-transcription PCR (RT-PCR) was carried out using the following primers for exons 84–91 (forward primer 5′-ACTGGACCTACTGGAGCTGTG-3′, and reverse primer 5′-ACGGCCAGCTTCACCCTTCTC-3′). The amplified product was separated by electrophoresis in a nondenaturing polyacrylamide gradient gel. After electrophoresis, the respective bands were extracted from the gel and sequenced directly.

Sequencing analysis of the proband's PCR product corresponding to exon 87 showed a G → A transition in one allele at nucleotide position 6900, which is the last nucleotide of exon 87, and this substitution was designated as c.6900G → A (Fig. 2a). Direct sequencing of the parents' PCR products showed that they had no mutant allele, indicating that the proband was heterozygous for a *de novo* mutation. Although the possibility of nonpaternity could not be excluded in this family, we did not perform haplotype analysis because the parents were not interested in this. No other nucleotide substitution, insertion or deletion leading to an amino acid change, frameshift, or aberrant splicing was detected in sequencing of all other PCR products, and DNA analysis of 50 unrelated healthy Japanese individuals did not detect any nucleotide substitution at position 6900 in the *COL7A1* gene (data not shown).

Table 1 *COL7A1* mutations causing skipping of exon 87.

Mutation	Phenotype	Reference
c.6847del27	DDEB pretibial	Sakuntabhai *et al*[3]
c.6862del16	DDEB pruriginosa	Mellerio *et al*[4]
c.6899A → G	DDEB pruriginosa	Jiang *et al*[5]
c.6900 + 2delTGAT	DDEB pruriginosa	Drera *et al*[5]
c.6900 + 4A → G	DDEB pruriginosa	Drera *et al*[6]

DDEB, dominant dystrophic epidermolysis bullosa.

Because the nucleotide at position 6900 is highly conserved,[8] RT-PCR was performed to confirm the consequences of the putative splice-site mutation. Electrophoresis of the RT-PCR product revealed not only an expected band of normal size (381 bp), but also an extra band of smaller size (312 bp) (Fig. 2b). Sequencing of each DNA fragment extracted from the gel showed that one had a normal sequence and the other had a sequence that had lost 69 nucleotides, corresponding to the entire exon 87 (Fig. 2c). This deletion would be in frame. The mutation (c.6900G → A) detected in this study is novel, and to our knowledge, this is the first reported case of DDEB due to a *de novo* splice-site mutation.

The majority of the reported mutations in DDEB have involved glycine substitutions in the collagenous domain of type VII collagen, but DDEB cases with mutations causing skipping of exon 87 have also been reported, including c.6847del27, c.6862del16, c.6899 A → G, c.6900 + 2delTGAT and c.6900 + 4 A → G mutations (Table 1).[3–6] Interestingly, all but one of the heterozygous mutations resulting in exon 87 skipping have been reported in cases of DDEB pruriginosa, a rare DEB variant characterized by intense pruritus and nodular prurigo-like lesions, mostly on the limbs.[4–6,9] The exception is c.6847del27, which was found in a case reported as pretibial DDEB; the patient was also described as having frequent pruritus.[3] Accordingly, it seems clear that exon 87 skipping is strongly correlated with the phenotype of DDEB pruriginosa, and no other specific genotypic correlations have been evident to date.

The proband in this study presented with an extremely mild clinical phenotype represented by a lack of nail dystrophy, and without pruritus or prurigo-like lesions. However, a previous report showed that all seven reported patients with dominant or recessive DEB pruriginosa had a typical mild DEB phenotype until the onset of pruritus,[6] making it likely that as she gets older the proband will present with more severe clinical features, consistent with DDEB pruriginosa. It is yet to be clarified whether exon 87 skipping is associated with development of pruritus over time and whether unidentified factors responsible for the pathogenesis of DDEB pruriginosa may also be involved.[5,6] It is important for treatment of the proband to know the anticipated clinical course in advance, as it might be possible to reduce or even prevent development of nodular prurigo-like lesions by sufficient control of pruritus. Finally, to our knowledge, correlation between skipping of a specific exon and a distinct subtype of DEB has rarely been reported, and our study should contribute to further refinement of genotype–phenotype correlations in DEB, emphasizing the significance of mutation analysis for correct diagnosis and possibly for prediction of prognosis.

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RESEARCH ARTICLE

Human Mutation

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Plectin Expression Patterns Determine Two Distinct Subtypes of Epidermolysis Bullosa Simplex

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ABSTRACT: Plectin is a cytoskeletal linker protein that has a dumbbell-like structure with a long central rod and N- and C-terminal globular domains. Mutations in the gene encoding plectin (*PLEC1*) cause two distinct autosomal recessive subtypes of epidermolysis bullosa (EB): EB simplex with muscular dystrophy (EBS-MD), and EB simplex with pyloric atresia (EBS-PA). Here, we demonstrate that normal human fibroblasts express two different plectin isoforms including full-length and rodless forms of plectin. We performed detailed analysis of plectin expression patterns in six EBS-MD and three EBS-PA patients. In EBS-PA, expression of all plectin domains was found to be markedly attenuated or completely lost; in EBS-MD, the expression of the N- and C-terminal domains of plectin remained detectable, although the expression of rod domains was absent or markedly reduced. Our data suggest that loss of the full-length plectin isoform with residual expression of the rodless plectin isoform leads to EBS-MD, and that complete loss or marked attenuation of full-length and rodless plectin expression underlies the more severe EBS-PA phenotype. These results also clearly account for the majority of EBS-MD *PLEC1* mutation restriction within the large exon 31 that encodes the plectin rod domain, whereas EBS-PA *PLEC1* mutations are generally outside exon 31.
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KEY WORDS: PLEC1; basement membrane zone; skeletal muscle; mRNA decay; truncation

Introduction

Plectin is a 500-kDa intermediate filament-binding protein that serves as a crosslinking element of the cytoskeleton to supply mechanical strength to cells and tissues [Wiche, 1998]. Plectin is expressed in a wide variety of tissues, including skin, striated muscle, and gastrointestinal tract epithelia. Within the cutaneous epithelium, plectin is especially concentrated along the basal pole of basal keratinocytes, where it functions as a linker between the keratin intermediate filament cytoskeleton, hemidesmosomes, and the underlying basement membrane zone (BMZ) [Borradori and Sonnenberg, 1999]. Plectin interacts with α6 and β4 integrins [Geerts et al., 1999; Litjens et al., 2003, 2005; Niessen et al., 1997a,b; Rezniczek et al., 1998; Schaapveld et al., 1998], BPAG2 [Koster et al., 2003], and periplakin [Boczonadi et al., 2007].

Epidermolysis bullosa (EB) comprises a group of heterogeneous disorders in which congenital skin fragility leads to dermal–epidermal junction separation. EB is subdivided into the three major groups of EB simplex, junctional EB, dystrophic EB, and the one minor group of Kindler syndrome, based on the level of blister formation [Fine et al., 2008]. So far, mutations in 13 different genes have been identified as underlying EB subtypes [Fine et al., 2000, 2008]. Among them, mutations in the gene encoding plectin, *PLEC1* (MIM# 601282), are responsible for two distinct types of autosomal recessive EBS (EBS with muscular dystrophy [EBS-MD] and EBS with pyloric atresia [EBS-PA]) and one subtype of autosomal dominant EBS (EBS-Ogna) [Fine et al., 2008]. Patients with EBS-Ogna are heterozygous for one amino acid substitution in the rod domain of plectin [Koss-Harnes et al., 2002]. EBS-Ogna is thought to be caused by plectin perturbation that results from dominant negative interference [Pfendner et al., 2005]. In contrast, homozygous or compound heterozygous loss-of-function mutations in *PLEC1* lead to EBS-MD or EBS-PA.

EBS-MD is characterized by generalized blistering and delayed onset of muscular dystrophy. Defective expression of plectin was found in patients with EBS-MD [Gache et al., 1996] and mutations in *PLEC1* were found to be responsible for the EBS-MD phenotype [McLean et al., 1996; Smith et al., 1996]. To date, more than 30 EBS-MD patients have been reported to have *PLEC1* mutations [Bauer et al., 2001; Chavanas et al., 1996; Dang et al., 1998; Koss-Harnes et al., 2004; Kunz et al., 2000; McMillan et al., 2007; Mellerio et al., 1997; Pfendner et al., 2005; Pulkkinen et al., 1996; Rouan et al., 2000; Sawamura et al., 2007; Takahashi et al., 2005; Takizawa et al., 1999]. Most reported *PLEC1* mutations in EBS-MD patients are located within exon 31 encoding the large rod domain of plectin [Pfendner et al., 2005; Sawamura et al., 2007]. In contrast to the phenotype seen in EBS-MD, clinical manifestations of EBS-PA are more severe and are characterized by more generalized blistering and pyloric atresia,

Additional Supporting Information may be found in the online version of this article.

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which frequently leads to early death in affected patients. Similarly, junctional EB with pyloric atresia (JEB-PA) has been known to be caused by the mutation in the gene encoding α6/β4 integrin (*ITGA6*; MIM# 147556; *ITGB4*; MIM# 147557), and about 60 *ITGA6* or *ITGB4* mutations have been described [Fine et al., 2008; Varki et al., 2006]. Recently, our group and others identified *PLEC1* mutations in eight patients with EBS-PA [Nakamura et al., 2005; Pfendner et al., 2005; Pfendner and Uitto, 2005; Sawamura et al., 2007]. EBS-MD and EBS-PA represent distinct clinical phenotypes, although both are caused by *PLEC1* mutations. The exact mechanisms that produce the clinical differences between EBS-MD and EBS-PA subtypes have not been elucidated, although it has been postulated that the severity of EBS patients with *PLEC1* mutations could be associated with alternative splicing of plectin [Sawamura et al., 2007; Sonnenberg and Liem, 2007].

The present study demonstrates that normal human fibroblasts express two different plectin isoforms: full-length plectin, and a shorter rodless plectin. In light of this finding, we collected skin samples and cultured cells from patients with EBS-MD and EBS-PA in which we precisely analyzed their expression levels of plectin using immunoblotting, immunofluorescence, and semiquantitative RT-PCR to determine the different pathogenic mechanisms underlying *PLEC1* mutations. Our data suggest that EBS-MD and EBS-PA exhibit different plectin expression patterns, and this study gives further insight toward improving our understanding of genotype-phenotype correlation in EBS patients with *PLEC1* mutations.

Materials and Methods

Patients and Mutation Detection

Nine EBS patients in whom *PLEC1* mutations had been confirmed were analyzed: six EBS-MD and three EBS-PA unrelated individuals (Table 1). *PLEC1* mutations in four EBS-MD and three EBS-PA cases were previously described in the literature [Kunz et al., 2000; Nakamura et al., 2005; Pulkkinen et al., 1996; Sawamura et al., 2007; Takizawa et al., 1999]. Patients EBS-MD1 and EBS-MD5 were newly identified cases in the present study.

EBS-MD1 was a 24-year-old Japanese female. She was the first child of nonconsanguineous, healthy parents. Generalized blistering and erosions of the skin were noted at birth, together with nail dystrophy. She had no history of pyloric atresia. At the age of 10, she developed muscular dystrophy. EBS-MD5 was a 7-year-old Croatian male. He was the second child of nonconsanguineous, healthy parents. His elder brother was healthy. He developed generalized blistering, including of the oral mucosal, and laryngeal stridor, immediately after birth. Pyloric atresia was not observed. To date, he has not developed muscular dystrophy.

Genomic DNA (gDNA) was isolated from peripheral blood leukocytes (EBS-MD1 and her parents) or cultured fibroblasts (EBS-MD5). The mutation detection was performed after polymerase chain reaction (PCR) amplification of all *PLEC1* exons and intron–exon borders, followed by direct automated sequencing using an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA). Oligonucleotide primers and PCR conditions used in this study were derived from a previous report [Nakamura et al., 2005]. The gDNA nucleotides, the complementary DNA (cDNA) nucleotides, and the amino acids of the protein, were numbered based on the previous sequence information (GenBank accession no. AH003623) [McLean et al., 1996].

The medical ethical committees of Hokkaido University, Keio University, and University Medical Center Freiburg approved all described studies. The study was conducted according to The Declaration of Helsinki Principles. Participants gave their written informed consent.

A schematic of plectin structure and *PLEC1* mutations detected in EBS patients in this study is shown in Figure 1A.

Antibodies

The plectin domains where the antibodies used in this study react are summarized in Figure 1B. Mouse monoclonal antibodies (mAbs), PN643 against the actin-binding domain of plectin and PC815 against the C-terminal plectin repeats were prepared by immunizing mice with recombinant His-tagged fusion proteins. To produce recombinant proteins, the cDNAs that encode the actin-binding domain of plectin and C-terminal plectin repeats comprising amino acids 171–595 and 2,930–3,153 (GenBank accession no. AAB05428.1), respectively, were cloned into a pET32c vector. The resultant recombinant proteins were expressed in the *Escherichia coli* expression host BL21(DE3)pLysS and purified using a His-Bind column (Novagen, Madison, WI). Spleen cells derived from immunized mice were fused with mouse myeloma cells. Hybridomas producing antibodies against plectin were selected by immunofluorescent microscopy screening using normal human skin. Immunoblotting using cytoplasmic extracts from DJM-1 cells confirmed that both of the antibodies reacted with a 500-kDa protein.

In addition to PN643 and PC815, the following mAbs against BMZ components were used: mAbs HD1-121 [Hieda et al., 1992; Okumura et al., 1999], 10F6 [Foisner et al., 1991], and 5B3 [Foisner et al., 1994] against the rod domain of plectin; mAb LH7.2 (Sigma, St. Louis, MO) against type VII collagen. mAb 10F6 and 5B3 were kind gifts from Dr. G. Wiche of the University of Vienna. C20, a goat polyclonal antibody against the C-terminus

Table 1. EBS Patients and *PLEC1* Mutations

Patient	Mutation 1 (predicted consequence)	Exon	Mutation 2 (predicted consequence)	Exon	Reference
EBS-MD1	**c.6549_6582del (p. Ala2183fs)**	31	**c.13040dup (p.Gly4347fs)**	32	Present case
EBS-MD2	c.4348C>T (p.Gln1450X)	31	c.4348C>T (p.Gln1450X)	31	Sawamura et al., 2007
EBS-MD3	c.3157C>T (p.Gln1053X)	24	c.5806C>T (p.Gln1936X)	31	Takizawa et al., 1999
EBS-MD4	c.5866del (p.Leu1956fs)	31	c.5866del (p.Leu1956fs)	31	Pulkkinen et al., 1996
EBS-MD5	**c.4643_4667dup (p.Arg1556fs)**	31	**c.7120C>T (p.Gln2374X)**	31	Present case
EBS-MD6	c.5188C>T (p.Gln1713X)	31	c.7102C>T (p.Arg2351X)	31	Kunz et al., 2000
EBS-PA1	c.7396C>T (p.Gln2466X)	31	c.7633C>T (p.Gln2545X)	32	Sawamura et al., 2007
EBS-PA2	c.3565C>T (p.Arg1189X)	27	c.7612C>T (p.Gln2538X)	32	Nakamura et al., 2005
EBS-PA3	c.913C>T (p.Gln305X)	9	c.1344G>A (p.Gln447fs; =)[a]	12	Nakamura et al., 2005

The novel *PLEC1* mutations detected in this study are in bold. MD, Muscular dystrophy; del, deletion; dup, duplication; fs, frameshift.
[a]The mutation c.1344G>A is at the 3′ end of exon12. Predicted consequences resulting from this mutation are discussed in the Results.

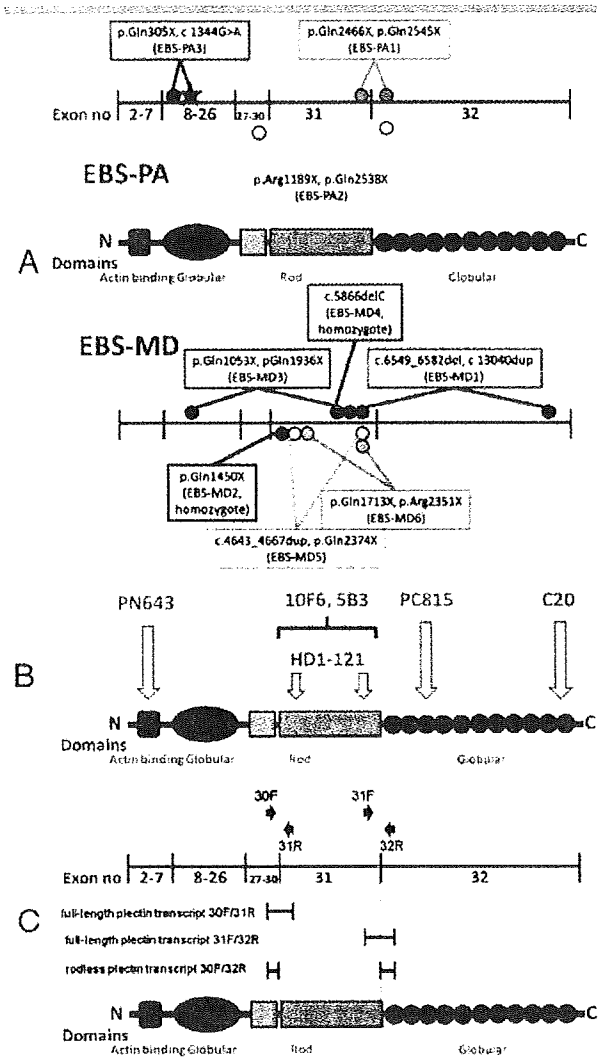

Figure 1. Scheme of plectin structure, PLEC1 mutations, antibodies against plectin and specific primers to detect the rodless transcript form of plectin. **A:** Plectin molecules consist of a central rod flanked by amino-terminal and carboxy-terminal globular domains. PLEC1 mutations observed in EBS patients of this study are indicated. Dots represent truncated mutations. The star indicates a splice-site mutation (c.1344G>A). **B:** PN643 is a monoclonal antibody (mAb) against the N-terminal actin-binding domain of plectin. HD1-121, 10F6, and 5B3 are mAbs against the rod domain of plectin. PC815 is a mAb and C20 is a polyclonal antibody against the C-terminal globular domain of plectin. **C:** Specific primers used to detect the presence of transcripts for full-length (30F/31R and 31F/32R) and rodless plectin (30F/32R) on cDNA synthesized from the mRNA of normal human, EBS-MD5 and EBS-PA3F cells.

of plectin, was purchased from Santa Cruz (Santa Cruz, CA). Anti-beta-actin mAb (AC15, Sigma) was used to confirm equal protein loading.

Immunofluorescence Studies

Immunofluorescence analysis was performed using skin specimens from the patients (Table 1). Fresh-frozen skin specimens were embedded in optimal cutting temperature (OCT) compound and quickly frozen in isopentane cooled over liquid nitrogen. 5-μm cryostat-cut sections were incubated overnight at 4°C with primary antibodies including the following mAbs: PN643 (working dilution of 1:160), HD1-121 (1:100), 10F6 (1:10), 5B3 (1:20), PC815 (1:20), and LH7.2 (1:10). After washing in phosphate-buffered saline, the sections were incubated with secondary antibodies conjugated with fluorescein-isothiocyanate.

Cell Culture and Immunoblot Analysis

Cultured fibroblasts were obtained from skin biopsies of a normal human volunteer and of patient EBS-MD5. Cultured amniocytes were derived from an aborted fetus who was a sibling of EBS-PA3 (EBS-PA3F). Prenatal diagnosis of EBS-PA3F revealed that the fetus had the same *PLEC1* mutations as were detected in EBS-PA3 (data not shown). Cultured fibroblasts and amniocytes were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum. Cultured oral keratinocytes were obtained from biopsies of a normal human volunteer and were maintained in CnT-57 medium (CELLnTEC). Whole-cell lysates of human skeletal muscle were purchased from Abcam (ab82589). For sample preparation, cultured cells were lysed in Nonidet-40 (NP-40) containing buffer (1% NP-40, 25 mM Tris-HCl [pH 7.6], 4 mM EDTA, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride [PMSF], and proteinase inhibitor cocktail [Sigma]) on ice for 30 min; cell debris was removed by centrifugation at 14,000 rpm for 15 min; and supernatant was collected. Supernatants were solubilized in Laemmli's sample buffer [Laemmli, 1970], applied to SDS-polyacrylamide gels, and transferred to a PVDF membrane. The membrane was incubated with PN643, HD1-121, C20, and AC15 overnight at 4°C followed by incubation with horseradish peroxidase (HRP) conjugated anti-mouse IgG (for PN643, HD1-121, and AC15) and HRP-conjugated antigout IgG (for C20) for 1 hr at room temperature. The blots were detected using ECL Plus Detection Kit (GE Healthcare, Fairfield, CT). The images were obtained with LAS-4000 mini (Fujifilm, Tokyo, Japan). To elucidate the quantitative ratio of full-length/rodless plectin, immunoblotting of lysates from normal human fibroblasts, keratinocytes, and skeletal muscle was performed in triplicate. Band intensities were analyzed by densitometry (ImageJ).

Semiquantitative RT-PCR Analysis

Total RNA was isolated from cultured fibroblasts (normal human volunteers and EBS-MD5) or amniocytes (EBS-PA3F) using RNeasy kit (Qiagen, Valencia, CA), and first-strand cDNA was made using Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA). First-strand cDNA was then amplified by PCR with primers specific for the exon boundaries flanking the rod domain of plectin as described previously [Koster et al., 2004] (Fig. 1C). The following primers were used in this study: 30F, 5′-CATCAGCGAGACTCTGCGGC-3′; 31R, 5′-TGCGCCTGTCG-CTTTTGTGC-3′; 31F, 5′-AGCTGGAGATGAGCGCTGA-3′; 32R, 5′-TGCTGCAGCTCCTCCTGC-3′. PCR conditions were as follows: 94°C for 5 min, followed by 30 cycles (31F/32R, 30F/32R) and 35 cycles (30F/31R) at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min, and extension at 72°C for 7 min. To ensure equal loading, a housekeeping gene (GAPDH) was simultaneously amplified. The PCR products were assessed on a 2% agarose gel. The images were obtained with LAS-4000 mini (Fujifilm). To confirm the skipping of exon 31 in rodless transcript, direct sequencing was performed for the PCR products (30F/32R). To analyze transcripts derived from the c.1344G>A mutant allele,

PCR amplification of synthesized EBS-PA3F cDNA from exon 9 to exon 14 was performed using the following primers: 5′-GATTGAGATCCTGTGGTCTC-3′ and 5′-CTCTGCACACTCT-GCAGAGT-3′. PCR products were cloned in the TA cloning vector pCRII (Invitrogen) and then sequenced.

Results

PLEC1 Mutation Detection

PLEC1 mutational analysis in case EBS-MD1 demonstrated that the affected patient was a compound heterozygote for the maternal c.13040dup mutation in exon 32 and the paternal c.6549_6582del mutation in exon 31 (Supp. Fig. 1A). Both of the mutations resulted in a frameshift that caused 8- and 21-amino-acid missense sequences, respectively, followed by a premature termination codon (PTC). These mutations were novel, and they were confirmed by *MwoI* restriction enzyme digestion and TA-cloning, respectively (data not shown). In addition, the c.10453C>T (p.Arg3485Trp) transition in exon 32 was also detected in one allele of the patient and her mother. This c.10453C>T transition was not found by sequence analysis in 100 normal unrelated Japanese alleles (50 healthy unrelated Japanese individuals), and it was unlikely to be polymorphism, although the contribution of this missense mutation to the EB phenotype remains unclear.

EBS-MD5 was a compound heterozygote for the c.4643_4667dup and c.7120C>T (p.Gln2374X) mutations in exon 31 (Supp. Fig. 1B). The c.4643_4667dup resulted in a frameshift that caused a 90-amino-acid missense sequence, followed by a PTC. These mutations were also novel and were confirmed by TA-cloning and *PstI* restriction enzyme digestion respectively (data not shown).

Differential plectin isoform expression by immunoblotting in normal human fibroblasts

Immunoblot analysis of lysates from normal human cultured fibroblasts revealed that two closely spaced bands, putatively corresponding to two forms of plectin (500 kDa full-length and 390 kDa rodless form) reacted with PN643 and C20, antibodies recognizing the N- and C-termini of plectin (Fig. 2). Using HD1-121, an antibody against the rod domain of plectin, lysates from normal human fibroblasts reacted only with full-length plectin (Fig. 2). These results showed that normal human fibroblasts expressed two different *PLEC1* isoforms: full length and a shorter rodless plectin isoforms.

The Quantitative Ratio of Full-length/Rodless Plectin in Normal Human Fibroblasts, Keratinocytes, and Skeletal Muscle

To elucidate the relative amount of full-length and rodless plectin in normal human fibroblasts, keratinocytes, and skeletal muscle, we performed immunoblot analysis of lysates from each sample using PN643, an antibody against the N-terminus of plectin. Both full-length and rodless plectin were detected in each sample (Fig. 3). Band intensities were measured in triplicate ±SD. The quantitative ratio of full-length/rodless plectin was 14.2±4.2 in fibroblasts, 21.3±6.4 in keratinocytes, and 1.37±0.23 in skeletal muscle.

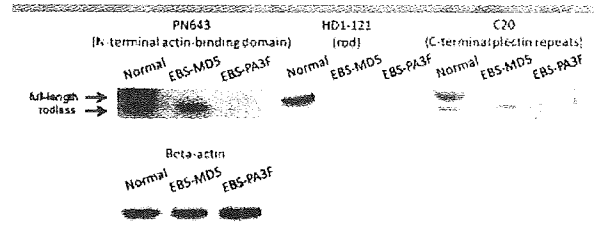

Figure 2. Immunoblot analysis of cultured fibroblasts from normal human control and EBS-MD and amniocytes from EBS-PA. Immunoblot analysis of extracts from cells of normal control, EBS-MD5, and an aborted sibling of EBS-PA3 (EBS-PA3F). Analysis used PN643 against the N-terminal actin-binding domain, HD1-121 against the rod domain and C20 against the C-terminal plectin repeats. Rodless plectin, detected with PN643 and C20, migrates just below full-length plectin in normal human fibroblasts. Using HD1-121, only full-length plectin was demonstrated in the normal control. In contrast, EBS-MD5 fibroblasts contained only rodless plectin, which was detected with PN643 and C20. Full-length plectin did not appear in EBS-MD5 using any antibody. EBS-PA3F amniocytes contained a greatly reduced amount of full-length plectin. Equal protein loading was confirmed by reprobing with AC15 (anti-beta-actin antibody). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

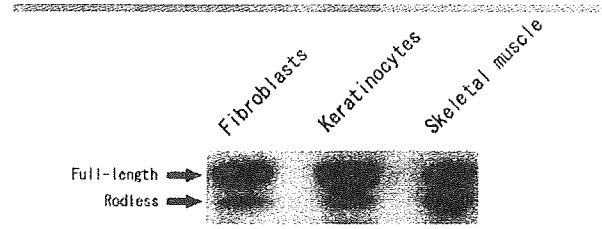

Figure 3. Relative amounts of full-length and rodless plectin in normal human fibroblasts, keratinocytes and skeletal muscle. Immunoblot analysis of lysates from normal human fibroblasts, oral keratinocytes, and skeletal muscle using PN643 against the N-terminal actin-binding domain of plectin was performed. Both full-length and rodless plectin were detected in each sample. The quantitative ratio of the two isoforms was calculated, using Image J software, as follows: 14.2±4.2 in fibroblasts, 21.3±6.4 in keratinocytes, and 1.37±0.23 in skeletal muscle. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Characterization of cutaneous plectin expression patterns in EBS-MD and EBS-PA patients by immunofluorescence analysis

To assess whether expression patterns of plectin in the skin differ between EBS-MD and EBS-PA, we performed immunofluorescence analysis using five different antibodies (Fig. 1B). PN643 weakly reacted with skin specimens from all EBS-MD patients and two out of three EBS-PA patients (EBS-PA1, 3), but failed to react with specimens from EBS-PA2 (Fig. 4A–J). HD1-121 showed weakly reactivity in three EBS-MD patients (EBS-MD1, 4, 6) and one EBS-PA patient (EBS-PA3), but was negative in the other patients (EBS-MD2, 3, 5, EBS-PA1, 2) (data not shown). 5B3, the mAb against the rod domain of plectin, was faint but identifiable in two EBS-MD patients (EBS-MD1, 6) and one EBS-PA patient (EBS-PA3), but was negative in the other patients (EBS-MD2-5, EBS-PA1, 2) (Fig. 4L–T). No skin specimens reacted with 10F6, a monoclonal antibody against the rod domain, except EBS-PA3 (data not shown). PC815 recognized the C-terminus of plectin weakly but detectably in all EBS-MD patients and EBS-PA3, but not in EBS-PA patients 1 and 2 (Fig. 4V–AD). These results

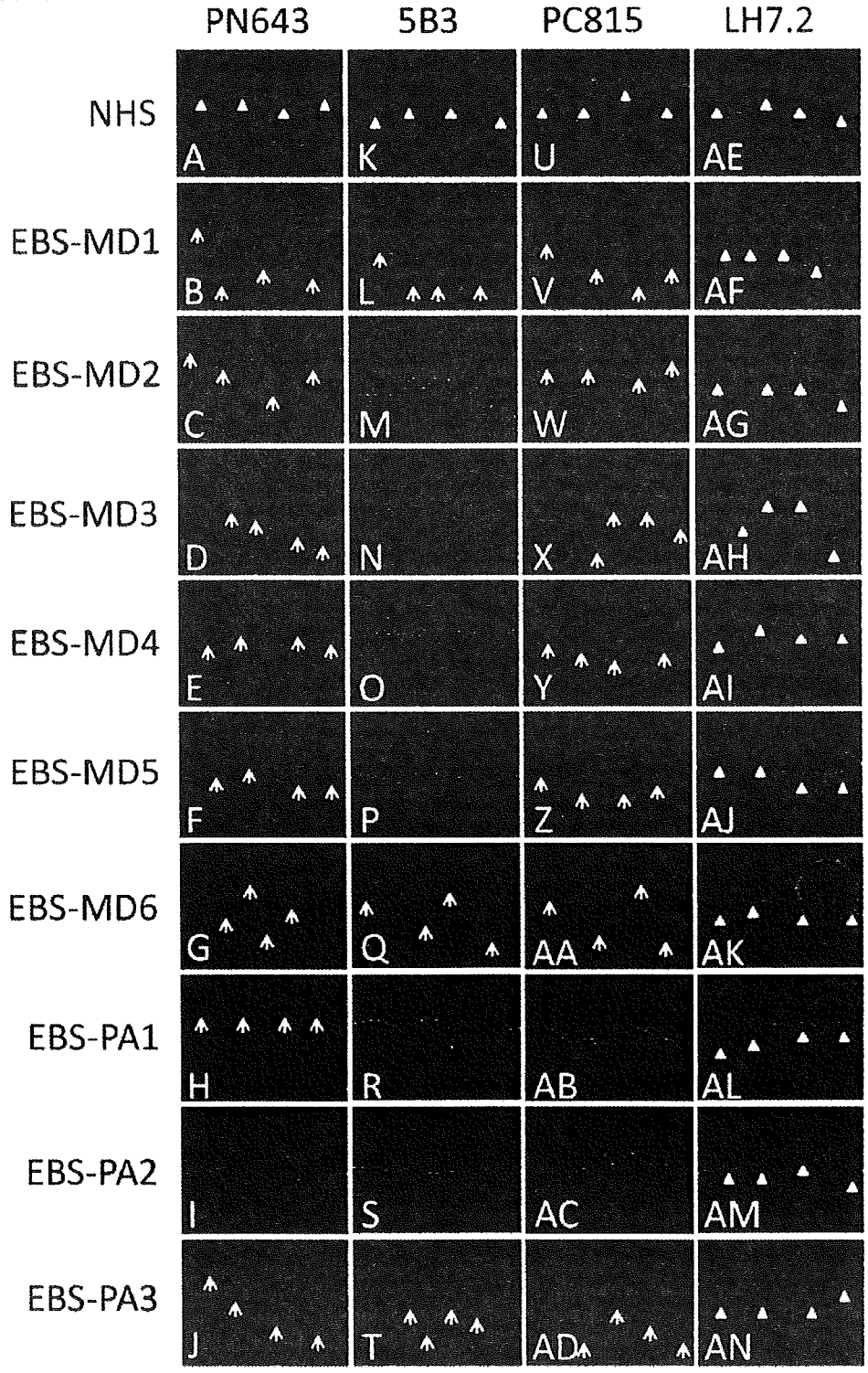

Figure 4. Immunofluorescence analysis of cutaneous plectin expression in EBS-MD and EBS-PA. In normal human skin (NHS), immunofluorescence shows that all mAbs against plectin (PN643, 5B3, and PC815) and type VII collagen (LH7.2) tested in the study bind to the dermal epidermal junction (DEJ) (**A, K, U, AE**). DEJ labeling of PN643 is weakly positive in all EBS-MD cases (**B–G**) and EBS-PA1, 3 (**H, J**), but negative in EBS-PA2 (**I**). DEJ labeling of 5B3 show faintly positive in EBS-MD1, 6 and EBS-PA3 (**L, Q, T**), and negative in EBS-MD2-5 and EBS1, 2 (**M–P, R, S**). DEJ labeling using PC815 is weakly positive in all EBS-MD cases and EBS-PA3 (**V-AA, AD**), but negative in EBS-PA1, 2 (**AB, AC**). Type VII collagen shows normal linear labeling in all EBS cases (**AF–AN**). Strong staining is indicated by arrowheads. Weak labeling is indicated by arrows. Negative labeling is indicated by dotted lines.

revealed loss of full-length plectin with the maintenance of a rodless plectin isoform in EBS-MD. EBS-PA skin specimens harbored greatly reduced amounts of both full-length and rodless plectin.

Protein and mRNA expression patterns of plectin in cultured cells from EBS-MD and EBS-PA patients

Plectin expression patterns of EBS-MD and EBS-PA cultured cells were assessed at both the protein and mRNA levels to confirm the comparative immunofluorescence analysis using skin biopsy specimens showing that the majority of EBS-MD patients expressed a rodless plectin variant, but not full-length plectin and that expression of both full-length and rodless-plectin variant peptides was remarkably reduced or completely abolished in EBS-PA patients. Immunoblot analysis of lysates from fibroblasts of patient EBS-MD5 failed to show any HD1-121 bands, although a band corresponding to rodless plectin was observed by using PN643 and C20 (Fig. 2). Lysates from cultured amniocytes from an aborted sibling of EBS-PA3 (EBS-PA3F) showed that a diminished amount of full-length plectin reacted with PN643, HD1-121, and C20 (Fig. 2).

Using RT-PCR, the presence of an RNA message that does not encode the rod domain was demonstrated in the normal human control as well as the EBS-MD5 and EBS-PA3F cells (Figs. 1C and 5A) (30F/32R). Direct sequencing confirmed the skipping of exon31 in the PCR products (30F/32R) (Fig. 5B). mRNA encoding full-length plectin containing the rod domain was also detected in normal human control, EBS-MD5, and EBS-PA3F cells (Figs. 1C and 5A) (30F/31R and 31F/32R). Judging from the PCR analysis results, the quantity of full-length plectin transcript was greatly reduced in EBS-MD5 and EBS-PA3F compared with those in the normal human controls. In addition, the rodless plectin transcripts were markedly diminished in quantity in EBS-PA3F compared with those of the normal human controls, although expression of the rodless plectin transcripts was maintained in EBS-MD5.

These data suggest that EBS-MD5 fibroblasts express only rodless truncated forms of plectin without the full-length isoform, presumably because of nonsense-mediated mRNA decay (NMD) of the full-length plectin transcript induced by the mutations within *PLEC1* exon 31 (Table 1 and Fig. 1A). Conversely, EBS-PA3F amniocytes expressed a much lower level of plectin than normal human fibroblasts due to NMD of both full-length and rodless plectin transcripts induced by mutations within exons encoding the N-terminal globular domain.

The expression of a small amount of plectin in EBS-PA3 and EBS-PA3F is explained by the splice donor site mutation, c.1344G>A (Table 1 and Fig. 1A). The *PLEC1* cDNA corresponding to exons 9–14 was amplified by PCR using synthesized first-strand cDNA from EBS-PA3F and was cloned into a TA vector. Sequence analysis of the cloned PCR products revealed three different splicing patterns, one of which was a normal transcript from the wild-type allele without c.1344G>A (Fig. 6A and B). In addition to the normal transcript, most of the transcripts derived from the c.1344G>A mutant allele exhibited a 4-bp deletion at nucleotide position 1341–1344 in cDNA (Fig. 6C). This led to a frameshift followed by a PTC at amino acid position 475 (Fig. 6D), whereas small amounts of mRNA exhibiting a

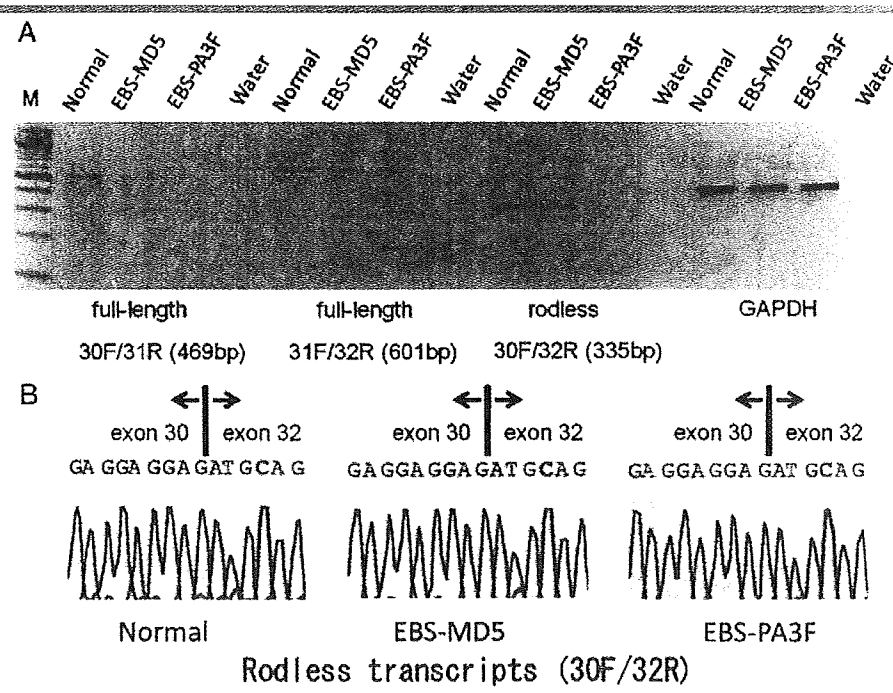

Figure 5. Semiquantitative RT-PCR on full-length and rodless plectin transcripts. **A:** Compared with the normal human control, the EBS-MD5 and EBS-PA3F cells revealed a reduced mRNA level of full-length plectin (30F/31R and 31F/32R). mRNA levels of rodless plectin in EBS-PA3F cells are reduced compared with EBS-MD5 and the normal human control (30F/32R). GAPDH mRNA expression was used as a loading control in these experiments. The negative control reaction (DNA-free water instead of cDNA) shows no PCR products. The molecular weight standard (lane M) is a 100-bp ladder. **B:** Direct sequencing demonstrates skipping of exon 31 in PCR products (30F/32R) from normal human, EBS-MD5, and EBS-PA3F.

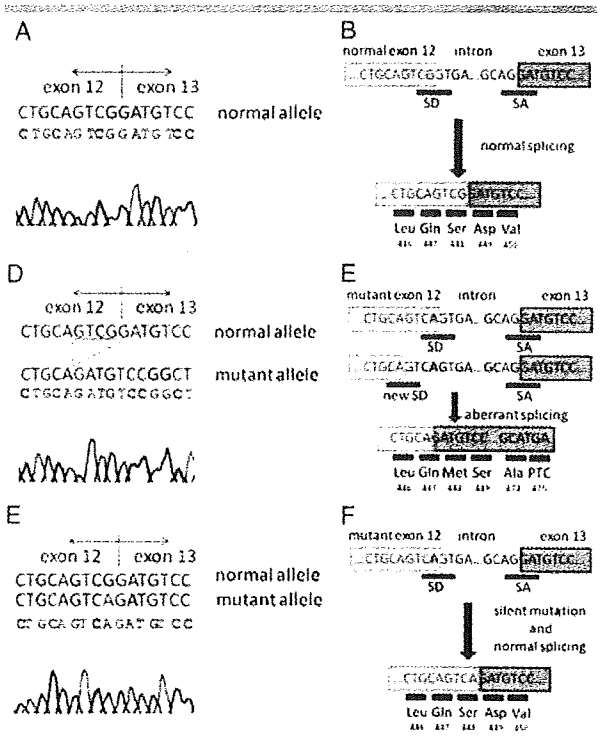

Figure 6. Abnormal splicing due to c.1344G>A mutation in EBS-PA3F, and its consequences **A:** Normal transcripts of the exon 12–exon 13 junction derived from EBS-PA3F cells. **B:** Normal splicing at the exon 12–exon 13 junction. Boxes represent exons, blue underlines are splice sites (SD: splice donor site; SA: splice acceptor site) and black underlined regions are amino acids. **C:** Mutant transcripts with deletion of four nucleotides from exon 12. Deleted nucleotides are underlined. **D:** c.1344G>A mutation altered the G nucleotide of the original splice donor site at the end of exon 12 and activated a cryptic splice donor site (red underline) four nucleotides upstream, leading to aberrant splicing with 4-bp deletion and subsequent frameshift, resulting in a premature termination codon at the amino acid position 475 in the N-terminal globular domain. **E:** Mutant transcripts with c.1344G>A. **F:** A small amount of mRNA carrying a silent nucleotide alteration c.1344G>A at amino acid position 448 Ser was also expressed by the original wild-type splicing.

normal splicing pattern with a silent mutation c.1344G>A at amino acid position 448 Ser were expressed (Fig. 6E and F).

Discussion

This study has demonstrated that two distinct plectin isoforms function in the skin, and that their truncation by *PLEC1* mutations causes the distinct EBS subtypes of EBS-MD and EBS-PA, depending on the pattern of remaining plectin peptide expression.

Plectin has a large rod domain encoded by *PLEC1*exon 31. Alternative splicing of transcripts lacking exon 31 results in a rodless plectin isoform, and it has been demonstrated that the rodless variant is expressed in various rat tissues, including skin, heart, brain, muscle, testis, and liver [Elliott et al., 1997; Fuchs et al., 2005; Steinboeck and Kristufek, 2005]. In addition, the rodless plectin isoform has been found in human muscle cells and keratinocytes [Koster et al., 2004; Schroder et al., 2000]. The significance of this rodless plectin splice variant in the skin remains unclear, but accumulation of *PLEC1* mutational data has revealed that most EBS-MD patients have mutations in exon 31 encoding the large rod domain of plectin, suggesting that conserved expression of the rodless variant plectin could be related to the pathogenesis of EBS-MD in patients with mutations in exon 31 [Pfendner et al., 2005; Sawamura et al., 2007]. However, little data that clarify this hypothesis has been reported, and only one report noted that cultured keratinocytes from one EBS-MD patient were able to express both N- and C-termini plectin epitopes without the expression of rod domain [Koster et al., 2004]. Our data including plectin isoform expression patterns in six EBS-MD patients clearly demonstrate that loss of full-length plectin with conserved rodless plectin isoform expression leads to an EBS-MD phenotype, which is consistent with accumulated clinical and genetic data. We also analyzed the relative amounts of two isoforms of plectin in normal human fibroblasts, keratinocytes, and skeletal muscle (Fig. 3). Our data revealed that the amount of full-length plectin is much greater than that of rodless plectin in fibroblasts and keratinocytes. In contrast, the full-length/rodless ratio in skeletal muscle is a little more than 1. These data are compatible with the fact that EBS-MD patients have skin fragility at birth and develop muscular dystrophy later in life. These data suggests that substantial amounts of rodless plectin in skeletal muscle might delay muscular symptoms while EBS-MD patients are in infancy.

In contrast to the EBS-MD patients, EBS-PA patients are significantly more likely to have mutations in domains outside exon 31 [Pfendner et al., 2005; Sawamura et al., 2007]. The majority of EBS-PA patients included in this study also exhibited *PLEC1* mutations in the gene outside exon 31(Table 1 and Fig. 1A). In the EBS-PA patients in this study, at least one allele is expected to have a stable product (the normal splicing variant from c.1344G>A in EBS-PA1; p.Gln2538X in EBS-PA2, and p.Gln2545X in EBS-PA3). There are three examples in which there are nulls in both alleles that have the PTC outside exon 31 but not in the terminal exon: (1) c.[2727_2740del]+c.[2727_2740del] (exon 22) [Charlesworth et al., 2003], (2) c.[1567_1570del]+c.[1567_1570del] (exon 14) [Pfendner and Uitto, 2005], and (3) p.[Gln305Term]+p.[Gln305Term] (exon 9) [Pfendner and Uitto, 2005]. All three patients had early deaths. Patients (2) and (3) had the EBS-PA phenotype [Pfendner and Uitto, 2005]. Patient (1) had the EBS phenotype, but the occurrence of PA was not substantiated [Charlesworth et al., 2003]. Due to the limited number of EBS-PA patients available, detailed expression patterns of plectin in the skin of EBS-PA patients has not been performed. In addition, comparative analysis of EBS-MD and EBS-PA skin specimens has not been performed. To our knowledge, the present report is the first to compare cutaneous plectin expression in EBS-MD and EBS-PA subtypes using multiple tissues and cells with antibodies that span a range of plectin domains including the N-terminus, rod domain, and C-terminus of plectin. This comparison between EBS-MD and EBS-PA enabled us to identify the differences in these EBS subtypes and to gain a better understanding of the consequences that complete loss or markedly attenuated expression of plectin has. These data are also consistent with the fact that EBS-PA patients generally show more severe skin symptoms than EBS-MD cases, in which expression of a rodless plectin isoform is maintained at least in the skin, although one EBS-PA patient (EBS-PA1) showed a relatively mild skin phenotype [Sawamura et al., 2007]. Also, in some cases of JEB-PA, another subtype of EB with pyloric atresia, the skin manifestations have been reported to be relatively mild and to improve with age, and surgical correction of the PA allowed growth of the patients [Pulkkinen et al., 1998]. It is possible that EBS-PA patients could develop muscular dystrophy if they survived longer. However, to our knowledge, such EBS-PA patients have not been reported in the literature. Figure 7A–C

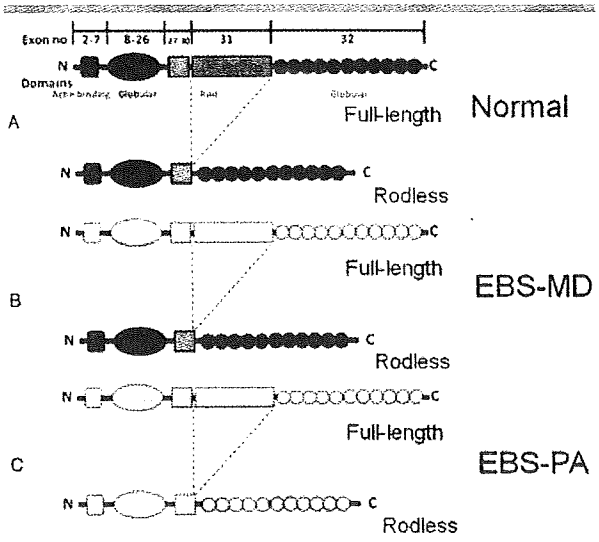

Figure 7. Schematic diagram of cutaneous plectin expression patterns in normal human skin and in skin from EBS-MD and EBS-PA patients. **A:** Two distinct isoforms of plectin—full-length and rodless—are expressed in the normal human control. **B:** Only rodless plectin is expressed in EBS-MD. **C:** Both the full-length and rodless plectin isoforms are greatly diminished or completely lost in EBS-PA. The peptides in light gray are not expressed or are markedly diminished in the patients.

depicts a schematic diagram of the predicted plectin expression pattern among the normal human control, EBS-MD, and EBS-PA.

As described above, almost all EBS-MD patients have one or two truncated mutations in exon 31 encoding the large rod domain of plectin, whereas most *PLEC1* mutations detected in EBS-PA are outside exon 31. To our knowledge, we have three cases of EBS-MD and one case of EBS-PA in the literature whose mutations are not explained by our data: (1) c.[2719_2727del] (exon 21)+c.[2719_2727del] (exon 21) (EBS-MD) [Pulkkinen et al., 1996], (2) c.[1541_1576del] (exon 14)+c.[2677_2685del] (exon 21) (EBS-MD) [Uitto and Pfendner, 2004], (3) c.[2769_2789del] (exon 21)+c.[2769_2789del] (exon 21) (EBS-PA) [Uitto and Pfender, 2005], and (4) c.[13803_13804ins16] (exon 32)+c.[13803_13804ins16] (exon 32) (EBS-MD) [Schroder et al., 2002]. The former three EBS patients had in-frame *PLEC1* deletion mutations outside exon 31 but not in the terminal exon. The last EBS-MD patient was homozygous for out-of-frame mutation in the terminal exon predicting a premature stop-codon within the exon. c.[2719_2727del] was in the nucleotide sequence where CAGGAGGCC was tandemly repeated. Therefore, this in-frame deletion was predicted to result in slipped misparing of DNA [Krawczack and Cooper, 1991; Pfendner and Uitto, 2005]. It is hard to figure out how altered plectin is synthesized from c.[1541_1576del]+c.[2677_2685del] and c.[2769_2789del]+c.[2769_2789del]. It is noteworthy that the phenotype of the EBS-MD patient with c.[1541_1576del]+c.[2677_2685del] was relatively mild, and that muscular dystrophy did not develop until the age of 42 [Uitto and Pfendner, 2004].

In previous studies, the expression of plectin was mainly evaluated by monoclonal antibodies raised against the rod domain. However, several splicing variants had previously prevented us from identifying whether plectin is completely lost or expressed in a truncated protein form in EBS patients with *PLEC1* mutations. Antibodies including those raised against both the plectin N- and C-termini are required to distinguish the expression of rodless splicing variants from a complete protein loss. Nevertheless, we have now elucidated how differences in plectin expression can lead to the two distinct skin blistering-associated phenotypes of muscular dystrophy and pyloric atresia.

Our former study on an EBS-PA3 patient [Nakamura et al., 2005] described different predicted transcripts of the c.1344G>A splice-site mutation from those of the present study. Our previous report employed an exon-trapping system, which is a tool to predict the transcripts that arise from a splice-site mutation when mRNA samples from patient tissues or cells are not available [Buckler et al., 1991]. In that system, the gDNA that is to be screened is subcloned into the exon trapping vector. The subcloned vector is transfected into cells, and mRNA is extracted from the cells to elucidate the splicing consequences. The system is useful, but it is such an artificial way of predicting the splicing products that the induced splicing patterns in the cell culture system are not necessarily correct nor are they the same as those in patient tissues or cultured cells [Schneider et al., 2007]. Because we used cultured amniocytes from EBS-PA3F in the present study, the results shown in Figure 6 supersede the results that were obtained by using an exon-trapping system in the previous report.

To summarize, EBS-MD patients typically express a rodless plectin isoform, although the full-length plectin is lost. In contrast, both full-length and rodless plectin isoforms are deficient in the EBS-PA patients, leading to a more severe disease phenotype. These findings demonstrate that deficiency of both plectin isoforms—full-length and rodless—leads to the severe phenotype of EBS-PA, and in contrast, conserved expression of the rodless isoform results in muscular dystrophy without pyloric atresia. The present results provide important insights toward further understanding the pathomechanisms of muscular dystrophy and pyloric atresia in plectin-deficient patients.

Acknowledgments

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特集 専門医にきく子どもの皮膚疾患

Ⅳ．角化症・遺伝性皮膚疾患

先天性表皮水疱症

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Key Words
表皮水疱症
先天性皮膚疾患
ケラチン5/14
ラミニン332
Ⅶ型コラーゲン

要旨

表皮水疱症は，軽微な外力で容易に皮膚に水疱を形成する疾患である．水疱の深さにより，単純型，接合部型，栄養障害型の3型に大別され，さらに責任遺伝子と臨床症状により10型の主要病型に分類されている．生命にかかわる重篤なものから非常に軽微なものまであるため，電子顕微鏡的検索，各種基底膜部抗体による水疱形成位置のマッピング，そして病型によっては遺伝子診断が必要となる．臨床的な診断のポイントを概説した．

先天性表皮水疱症の臨床症状

先天性表皮水疱症は軽微な外力で容易に皮膚に水疱を形成する先天性皮膚疾患であり，表皮と真皮の結合に関与する蛋白分子の遺伝的な異常により発症する．表皮真皮結合部（図1）は，基底細胞膜に存在するヘミデスモソームと真皮側に存在する基底板を中心とした構造により維持され，図1-Aに示すような分子構築をしている．その構成分子の異常により表皮水疱症を発症するが，責任蛋白分子の種類やその遺伝子変異の種類の違いによりさまざまな病型が存在し，それぞれに水疱の程度や合併症，生命予後が異なる．水疱のできる深さにより，表皮内（図1-B①）に水疱ができる単純型，表皮真皮結合部の透明層（図1-B②）に水疱ができる接合部型，真皮内（図1-B③）に水疱ができる栄養障害型の3型に大別され，さらに臨床症状により10型の主要病型に分類されている（表）．

生命にかかわる重篤なものから非常に軽微なものまであるため，正確な病型診断が要求されるが，それには電子顕微鏡的検索，各種基底膜部抗体による水疱形成位置のマッピング，そして病型によっては遺伝子診断が必要となる．病型分類に関しては，専門としている施設にコンサルテーションすることが望ましい．

1．単純型表皮水疱症

表皮内に水疱を形成する病型で，長期歩行や摩擦，打撲などにより水疱が生じるが，短期間に治癒し，瘢痕や稗粒腫を残さない．水疱が手掌足底に限局するWeber-Cockayne型，体幹にも水疱を生じるKöbner型，比較的広範囲に小水疱が集簇性に生じるDowling-Meara型はいずれも，表皮基底細胞の細胞骨格を担うケラチン5またはケラチン14の遺伝子変異によって生じ，常染色体優性遺伝である．変異の部位の違いにより

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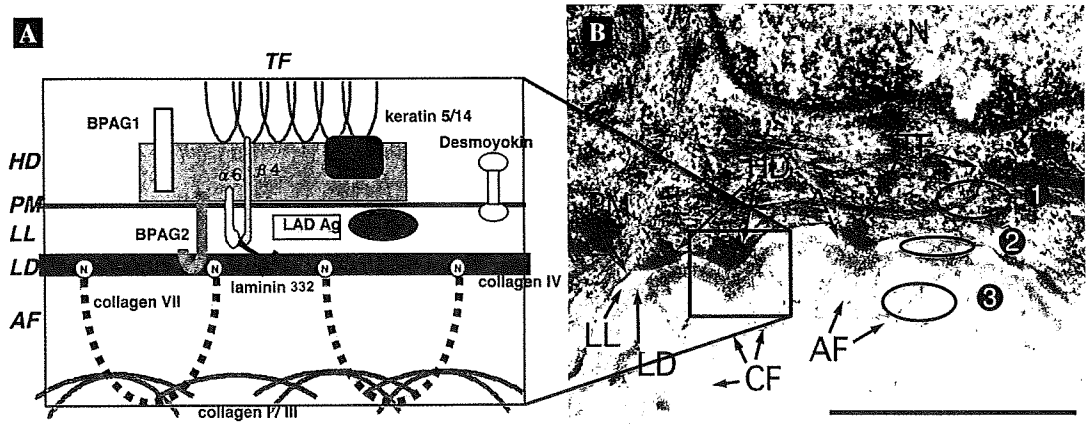

図1 表皮基底膜部の電顕像と分子構造

A：基底膜部の分子構造，B：基底膜部電顕像．水疱ができる位置により，表皮内（❶），接合部（❷），真皮内（❸）に分類される．
N：基底細胞核，TF：トノフィラメント，HD：ヘミデスモソーム，PM：基底細胞膜，LL：透明層，LD：基底板，AF：係留線維，
CF：膠原線維，Af：係留細線維

表 表皮水疱症の分類

大病型	主要病型	責任蛋白・遺伝子
単純型	Weber-Cockayne型	ケラチン5または14
	Köbner型	ケラチン5または14
	Dowling-Meara型	ケラチン5または14
	筋ジストロフィー合併型	プレクチン
接合部型	Herlitz型	ラミニン332
	非Herlitz型	ラミニン332, BP180
	幽門閉鎖症合併型	α6β4インテグリン
栄養障害型	優性型	Ⅶ型コラーゲン
	Hallopeau-Siemens劣性型	Ⅶ型コラーゲン
	非Hallopeau-Siemens劣性型	Ⅶ型コラーゲン

症状の程度に差ができると考えられている．特殊な病型として遅発性に筋ジストロフィーを発症するものがあるが，これはヘミデスモソームとケラチンをつなぐ分子であるプレクチンの遺伝子変異による．

Weber-Cockayne型（図2）は「限局型」ともいわれ，もっとも頻度が高く，おもに夏期に足底に水疱，びらんを認め，水疱形成時には軽度の疼痛を伴う．爪甲，頭髪，歯牙，口腔粘膜には異常を認めない．出生時には症状はないことが多く，乳児期も時に足趾背に微小な水疱を認める程度（図2-A）であるが，幼児期になって活発に歩行するようになると顕症化する（図2-B）．ブドウ球菌など表在性細菌の二次感染によりリンパ管炎をきたすことがあるので注意を要するが，成人期以降は症状が軽くなることが多い．夏季に増悪し，冬期には治療を必要としないことが多い．

Köbner型は「その他汎発型」ともいわれ，手掌，足底以外，体幹部や四肢にも水疱を生じる．出生時より症状があることが多く，接合部型や栄養障害型との鑑別が必要となってくる．爪甲の異常を伴うことはあまりなく，臨床的に参考になるが，皮膚生検のうえ，水疱形成部位を電顕または蛍光抗体法にて確認することが重要である．小児期においては，夏期には，本症の増

悪か伝染性膿痂疹の合併か判断がむずかしいことがあるが，顔面などの非間擦部位にびらんが生じたときには膿痂疹を強く疑う．

Dowling-Meara型は単純型の中でもっとも重症な病型であり，出生時より体幹部に水疱を生じる．爪甲の脱落や萎縮をみることもあり，他病型との鑑別が重要である．紅斑局面の辺縁部に小水疱が集簇性に配列する所見は，本病型に特徴的な皮疹である．

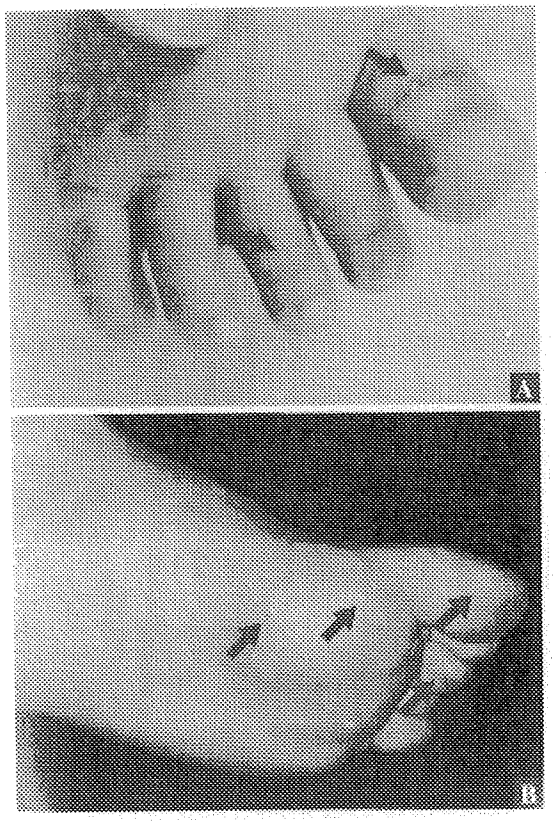

図2 単純型表皮水疱症（Weber-Cockayne型）
A：乳児期に趾背にみられた微小な水疱
B：成人の足底にみられた水疱

2. 接合部型表皮水疱症

表皮真皮境界部が離開する接合部型は重篤なものが多い．Herlitz型は致死型で，ほとんどの症例は1年以内，多くは半年以内に敗血症をきたし死亡する．ラミニン332の欠損により発症し，α，β，γ鎖をコードする遺伝子*LAMA3*, *LAMB3*, *LAMC2*のいずれかの機能喪失変異によって生じる常染色体劣性遺伝性疾患である．出生時は体幹部に少数の水疱を生じるが（**図3-A**），個々の水疱は比較的速やかに軽快する．しかし，水疱の新生は続き，徐々にその範囲を拡大して大きなびらん面を形成する（**図3-B**）．爪甲の脱落，萎縮を伴うことが多い．下痢，下血，嗄声，眼充血などを伴い，体重増加不良となる．

非Herlitz汎発型はかつては良性汎発萎縮型とよばれ，生命予後は良好な病型である．ラミニン332またはBP180の遺伝子異常により発症し，頭髪異常，歯牙異常，瘢痕形成，爪甲変形をきたす．

特殊型の幽門閉鎖症合併型はα6インテグリ

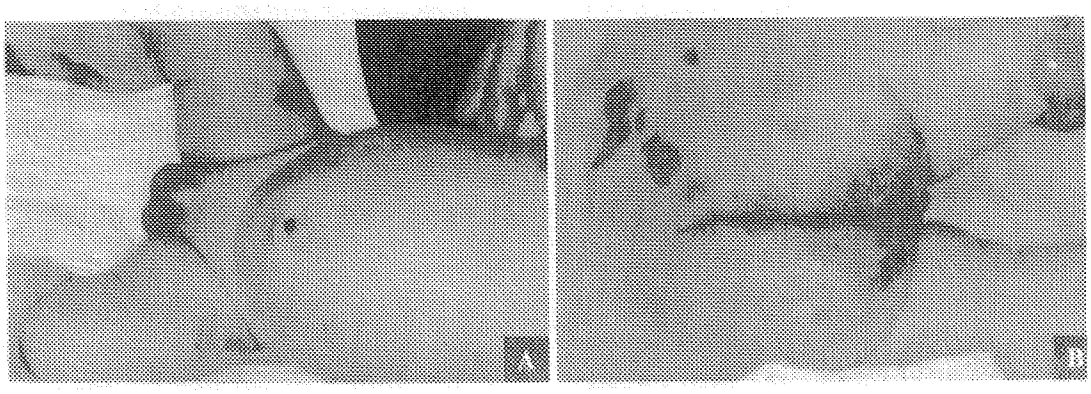

図3 接合部型表皮水疱症（Herlitz型）
A：病初期には体幹にびらんが認められるが治癒傾向もみられる
B：生後3カ月時，びらん，水疱は拡大傾向にある

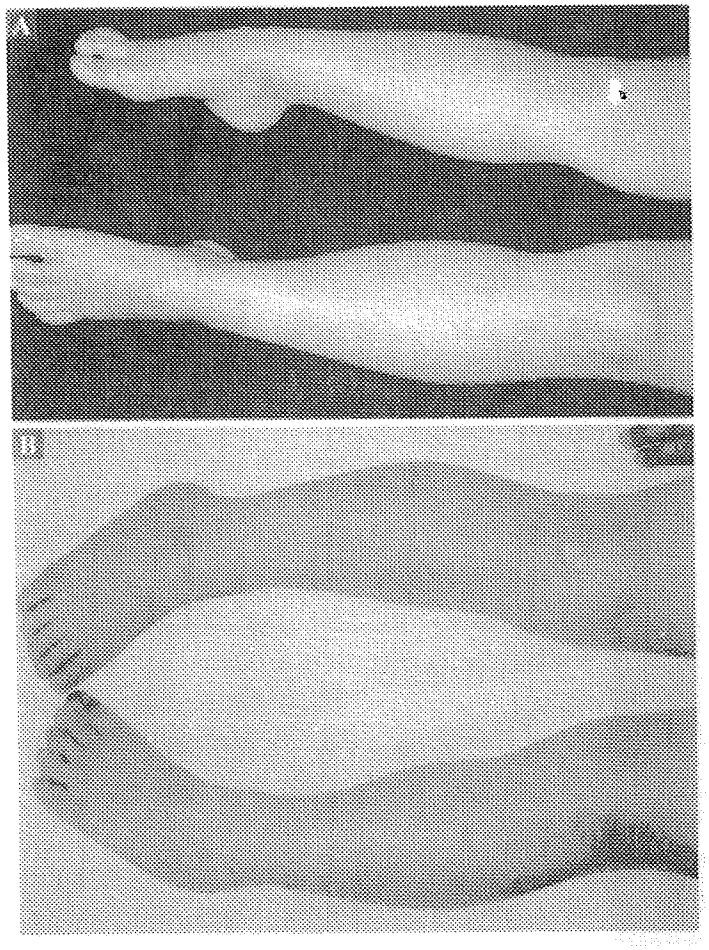

図4 栄養障害型表皮水疱症（軽症型）
A：3歳女児．優性栄養障害型表皮水疱症，B：3歳男児．非Hallopeau-Siemens劣性栄養障害型表皮水疱症．膝蓋，足背にびらん，稗粒腫を伴う萎縮性瘢痕あり，足趾の爪は萎縮している．臨床的に両者を区別することは困難である

ン，またはβ4インテグリンの遺伝子変異によって生じ，出生後，死に至ることが多い．

3．栄養障害型表皮水疱症

真皮内に水疱を形成する栄養障害型表皮水疱症は，いずれもⅦ型コラーゲンの遺伝子変異による．優性遺伝型は一般に軽症で，劣性遺伝型は重症のHallopeau-Siemens型と比較的軽症な非Hallopeau-Siemens型とに分類される．

優性型（**図4-A**）と非Hallopeau-Siemens劣性型（**図4-B**）の皮膚症状は類似していることに加え，優性型に突然変異による孤発例があるため，家族内に同症がない場合は鑑別に遺伝子検査が必要である．四肢伸側の瘢痕形成，稗粒腫の形成，爪甲の萎縮～欠損，口腔粘膜のびらんを伴う．食道狭窄を併発することもある．

Hallopeau-Siemens劣性型は最重症型で，出生直後より体幹，四肢に水疱，びらんを形成し（**図5-A**），治癒後に瘢痕，稗粒種を残す．びらんの形成と治癒を繰り返すことにより手指・足趾は次第に癒合し，棍棒状となる（**図5-B**）．そのほか，口腔粘膜の広範なびらん，脱毛，食道狭窄，貧血，低体重をきたし，感染症，腎障害，扁平上皮がんの続発（**図5-C**），重度の貧血に伴う心不全などにより青年期に死に至ることもある．

診断手順と鑑別診断

生下時もしくは幼児期に，外力の加わる部位に繰り返し水疱，びらんが生じる際には本症を

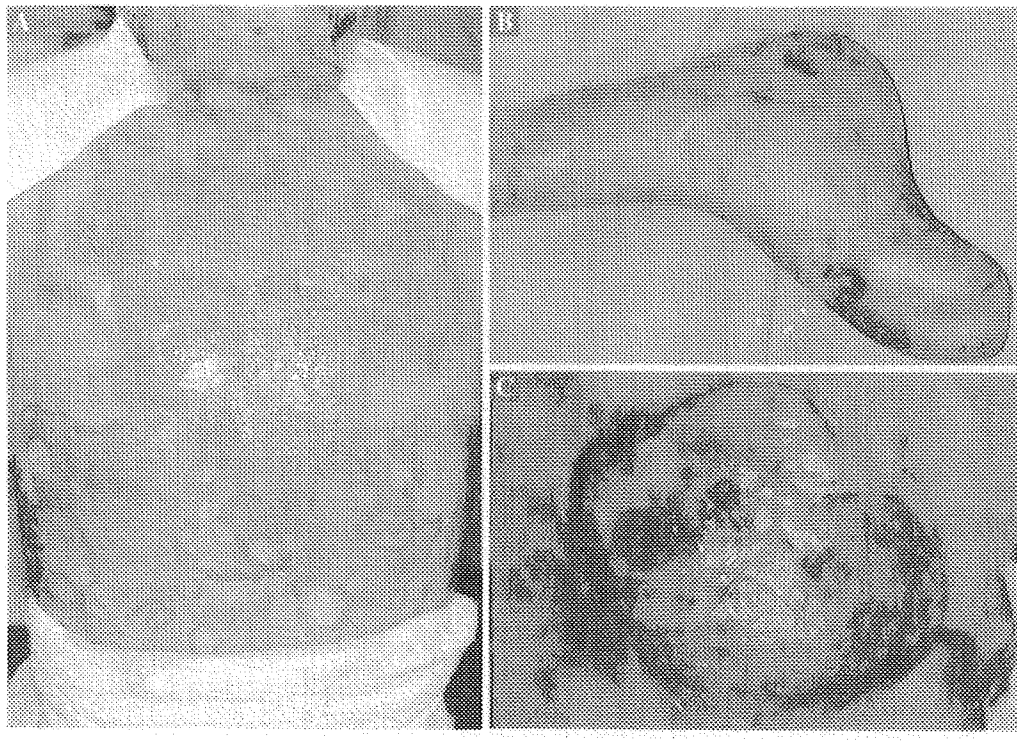

図5 栄養障害型表皮水疱症（重症型，Hallopeau-Siemens劣性栄養障害型表皮水疱症）
A：体幹四肢に難治性のびらん，潰瘍が癒合性に多発している
B：足趾は癒合し棍棒状となっている
C：20歳時に背部に生じた有棘細胞がん

疑い，皮膚生検を施行すべきである．通常のHE染色所見のみでは病型分類ができず，凍結切片による各種の抗基底膜蛋白抗体を用いた蛍光抗体法と，電顕による基底膜部の観察が必要である．

蛍光抗体法は各種基底膜構成分子に対するモノクローナル抗体を用いて，基底膜構成蛋白が正常に発現しているかどうか，また，水疱と基底膜蛋白の位置関係をみる．電顕では水疱形成の位置を確認するとともに，ケラチン線維の凝集，ヘミデスモソーム・係留線維の低形成の有無を観察する．

鑑別すべき疾患と鑑別点は以下のとおりである．

1．水疱型先天性魚鱗癬様紅皮症（図6-A）

ケラチン1または10の遺伝子変異により生じる常染色体優性遺伝性疾患で，生下時は水疱が主症状となることがある．皮膚生検のHE染色所見で特徴的な顆粒変性の像がみられるため，表皮水疱症と鑑別可能である．全身の潮紅と落屑が次第に増強することが臨床的な鑑別点であり，成長するに従い水疱形成は少なくなり，魚鱗癬症状が前面に出てくる．

2．色素失調症（図6-B）

*Nemo*遺伝子の変異により生じるX染色体優性遺伝性疾患で，罹患児のほとんどが女児である．生下時もしくはその直後より，線状，渦巻き状の配列をとる小水疱が多発する．皮膚生検のHE染色所見では，好酸球の表皮内浸潤と液状変性が特徴的である．経過を観察すると，次第に水疱新生はおさまり，褐色の色素斑が特有の縞模様を形成する．

3．伝染性膿痂疹（図6-C）

*Staphylococcus aureus*などの感染により，水

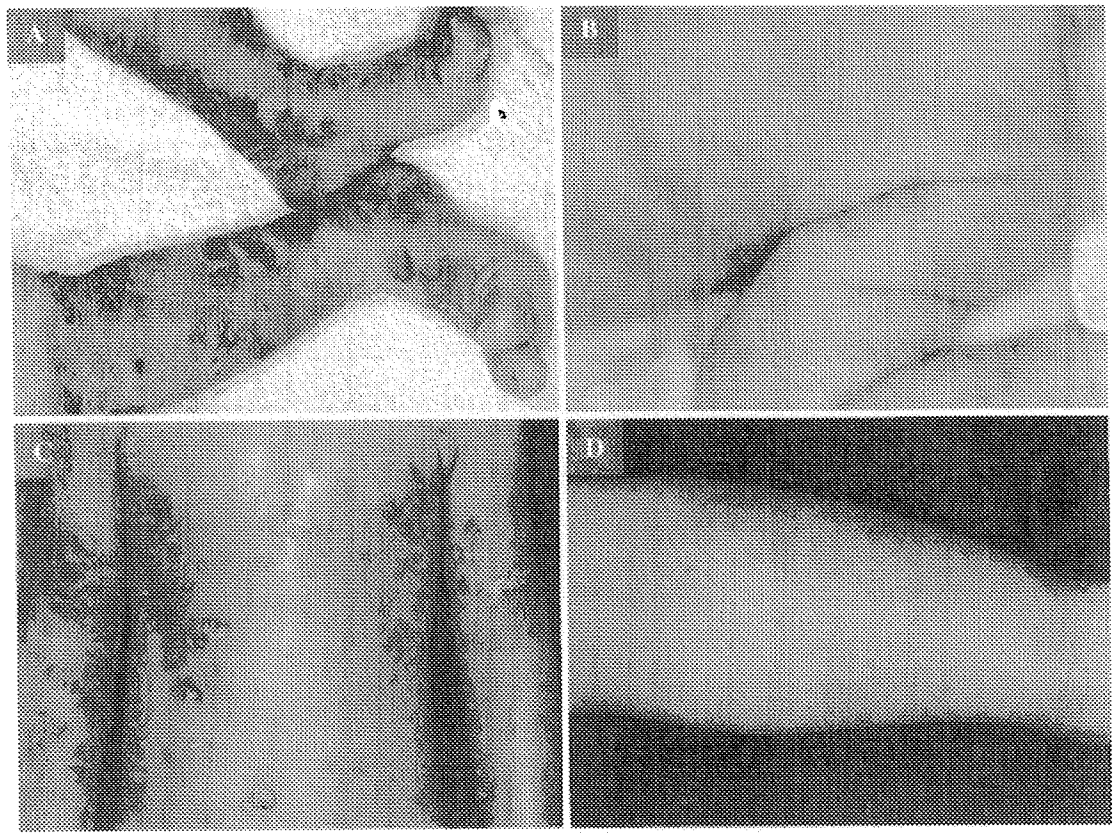

図6　表皮水疱症と鑑別を要する疾患
A：水疱型先天性魚鱗癬様紅皮症．水疱，びらんに加え，周囲皮膚に落屑を伴う紅皮症がある
B：色素失調症．線状に小水疱が配列している
C：伝染性膿痂疹．水疱，びらんに加え，紅斑，痂皮，膿疱をみる
D：Kindler症候群．水疱形成に加え，四肢末梢優位に多形皮膚萎縮を伴う

疱，痂皮，びらんを形成する．水疱形成は著明ではなく，びらんと痂皮がおもな症状である．培養により菌が証明され，抗生物質治療に反応する．

4. Kindler症候群（図6-D）

*Kind1*遺伝子の変異によって生じる疾患で，四肢末梢優位の皮膚萎縮と水疱形成を主症状とする．生検組織像ではⅣ型コラーゲンの免疫染色などにより基底板の重層化がみられる．成長とともに次第に水疱形成は少なくなり，皮膚萎縮は末梢から徐々に中枢に拡大してくる．

治療

1. 単純型表皮水疱症

新生した水疱は針などで内容液を抜き，水疱蓋は残すようにする．水疱，びらんが大きいときはアズノール®軟膏，バラマイシン®軟膏などを塗布したガーゼを貼布する．びらんは浅く，容易に治癒へと向かう．リンパ管炎や蜂窩織炎，伝染性膿痂疹などの二次感染が生じたときには，抗生物質の全身投与により加療する．

2. 接合部型表皮水疱症

Herlitz型は出生時より水疱を形成する．水疱内容を穿刺し，アズノール®軟膏やバラマイシン®軟膏を非固着性ガーゼ（メロリンガーゼな