International Journal of
Dermatology

Medical genetics

# DNA-based prenatal diagnosis of plectin-deficient epidermolysis bullosa simplex associated with pyloric atresia

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Conflicts of interest: The authors declare no conflicts of interest.

## Abstract

**Background** Mutations in the plectin gene (*PLEC*) generally lead to epidermolysis bullosa simplex (EBS) associated with muscular dystrophy. It has been recently demonstrated that *PLEC* mutations can also cause a different clinical subtype, EBS associated with pyloric atresia (EBS-PA), which shows early lethality. Prenatal diagnosis (PND) of EBS-PA using mutation screening of *PLEC* has not been described.

**Objective** This study aimed to perform DNA-based PND for an EBS-PA family.

**Materials and methods** The EBS-PA proband was compound-heterozygous for a paternal c.1350G>A splice-site mutation and a maternal p.Q305X nonsense mutation. Genomic DNA was obtained from amniocytes taken from an at-risk fetus of the proband's family. Direct sequencing and restriction enzyme digestion of polymerase chain reaction products from the genomic DNA were performed.

**Results** Mutational analysis showed that the fetus harbored both pathogenic mutations, suggesting that the fetus was a compound-heterozygote and therefore affected with EBS-PA. The skin sample obtained by autopsy from the abortus confirmed the absence of plectin expression at the dermal–epidermal junction.

**Conclusions** This is the first successful DNA-based PND for an EBA-PA family.

## Introduction

Epidermolysis bullosa (EB) comprises a group of diseases that are classified into four categories – EB simplex (EBS), junctional EB (JEB), dystrophic EB, and Kindler syndrome – depending on the depth of the dermal-epidermal junction split.[1] The four categories are subcategorized into minor subtypes, some of which show severe prognosis and lead to early demise.

Prenatal diagnosis (PND) of lethal EB subtypes has been performed for more than two decades. Electron microscopy and immunofluorescence analysis of fetal skin samples were the mainstay for PND of EB fetuses.[2] However, morphologically based PND had technical difficulties and abortion risks from the fetal skin biopsies. As the genes responsible for EB have been indentified, DNA-based PND has been available for many lethal EB subtypes.[2,3] Recently, other techniques such as immunofluorescence analysis of villous trophoblasts,[4] preimplantation genetic analysis[5], and preimplantation genetic haplotyping[6] have been described as useful for PND of EB.

Among the lethal EB subtypes, EB associated with pyloric atresia (EB-PA) has been known to result from mutations in the genes encoding either plectin (*PLEC*), or α6 (*ITGA6*), or β4 integrin (*ITGB4*).[1] EB-PA can either manifest as JEB with PA (JEB-PA) or EBS with PA (EBS-PA) and is categorized as hemidesmosomal variant of EB. EB-PA due to *ITGA6* or *ITGB4* mutations is generally characterized by blister formation at the level of the lamina lucida as JEB-PA, although skin separation within basal keratinocytes has been described in a few cases.[1] In contrast, it has been recently reported that another subset of lethal EB-PA shows an intraepidermal level of cleavage consistent with EBS, caused by mutations in the gene encoding plectin (*PLEC*).[7–9] To date, PND of EBS-PA using mutation screening of *PLEC* has not been reported in the literature. This paper describes the first DNA-based PND for an EBS-PA family.

## Materials and Methods

### The EBS-PA family

We previously reported this family with EBS-PA, in which the first and second newborns exhibited the clinical features of blistering and PA and died shortly after birth.[7] We then identified the precise genetic abnormality in the family through immunohistochemical analysis and genetic screening using the candidate gene approach. *PLEC* mutation analysis of genomic DNA from the parents and the proband demonstrated a paternal c.1350G>A splice-site mutation and a maternal p.Q305X nonsense mutation.[7] c.1350G>A was originally described as c.1344G>A and corrected according to the latest sequence information (GeneBank Accession No. NM_000445), plectin isoform 1c.[10] The parents were found to be heterozygous carriers, and the proband was compound-heterozygous (Fig. 1). The parents sought PND for a subsequent pregnancy.

### PND

Amniocentesis was performed at 16 weeks gestation. Genomic DNA isolated from one-week-cultured amniocytes maintained in Amniomax medium (Invitrogen, Carlsbad, CA, USA) was subjected to polymerase chain reaction (PCR) amplification, followed by direct automated sequencing using an ABI Prism 3100 genetic analyser (Advanced Biotechnologies, Foster City, CA, USA). PCR amplification of the *PLEC* gene exons 9 and 12 was performed using the following primers. Primers 5′-GTCGCT GTATGACGCCATGC-3′ and 5′-TGGCTGGTAGCTCCATC TCC-3′ were used for amplification of exon 9, producing a 387-bp fragment. Primers 5′-CCCACTCGCCTTAGGACAGT-3′ and 5′-AAACCAACTCTGCCCAAAGC-3′ were used for amplification of exon 12, synthesizing a 428-bp fragment. PCR conditions were five minutes at 94 °C for one cycle, followed by 38 cycles of 45 seconds at 94 °C, 30 seconds at 57 °C or 60 °C, and one minute at 72 °C. The genomic DNA nucleotides, the cDNA nucleotides, and the amino acids of the protein were numbered based on the latest sequence information (GeneBank Accession No. NM_000445).

Written informed consent was obtained from the parents. PND was approved by the Institutional Ethical Committee of Hokkaido University Graduate School of Medicine. This study was conducted according to the Declaration of Helsinki Principles.

### Immunofluorescence analysis

Immunofluorescence analysis using a series of antibodies against basement-membrane-associated molecules on cryostat skin sections was performed as previously described.[11] Skin biopsy was performed for the aborted fetus and a healthy volunteer as the normal control. The following monoclonal antibodies (mAbs) were used: mAb HD1–121 (a gift from Dr K. Owaribe of Nagoya University) against plectin; mAb GoH3 (a gift from Dr A. Sonnenberg of the Netherlands Cancer Institute) against α6 integrin; and mAb 3E1 (Chemicon, CA, USA) against β4 integrin.

## Results

Mutation analysis of genomic DNA from amniocytes showed both paternal c.1350G>A splice-site mutation and maternal p.Q305X nonsense mutation (Fig. 2a). These mutation data were briefly mentioned in our recent paper on plectin expression patterns in patients with EBS.[12] Each mutation was confirmed by restriction enzyme digestion of PCR products. The c.1350G>A and p.Q305X mutations resulted in the loss of a restriction site for *Hph* I and *Pst* I, respectively (Fig. 2b). The prenatal molecular genetic diagnosis suggested that the fetus

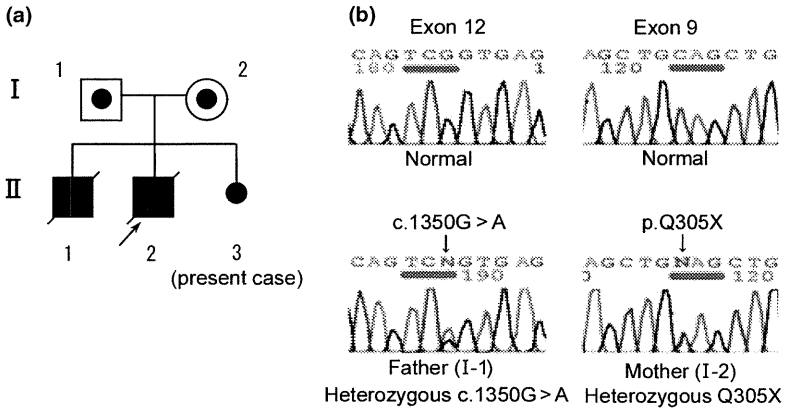


**Figure 1** Family tree of the present case and the causative *PLEC* mutations. (a) The first and second newborns exhibited clinical features typical of EBS-PA and died shortly after birth. The proband (the second newborn) is indicated by an arrow. (b) The paternal splice-site mutation was a c.1350G>A transition at the end of exon 12. The maternal nonsense mutation was a c.913C>T transition in exon 9, leading to the substitution of glutamine 305 with a nonsense codon (p.Q305X)

 

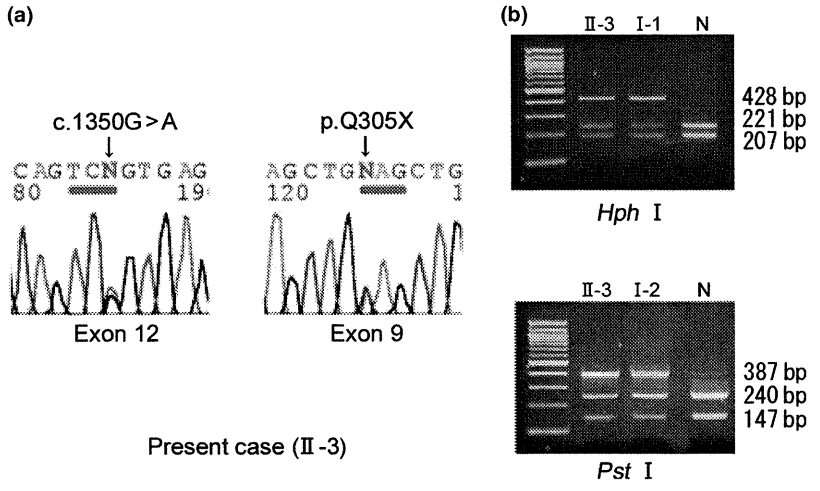


**Figure 2** Analysis of the plectin gene mutations in genomic DNA from amniocytes of a fetus at risk. (a) Mutation analysis of genomic DNA from amniocytes shows both the c.1350G>A mutation in exon 12 and p.Q305X mutations in exon 9. (b) The presence of the mutations was verified by restriction enzyme digestion. The paternal mutation abolished a recognition site for the *Hph*I restriction enzyme. In the case of the normal allele, the 428-bp fragment was digested to 221 bp and 207 bp (lane N), whereas in the case of the mutant allele, a 428-bp fragment resisted digestion in the PCR product (father: lane I-1; present fetus: lane II-3). The maternal mutation also abolished a recognition site for the *Pst*I restriction enzyme. In the case of the normal allele, the 387-bp fragment was digested to 240 bp and 147 bp (lane N), whereas in the case of the mutant allele, a 387-bp fragment resisted digestion in the PCR product (mother: lane I-2; present fetus: lane II-3)

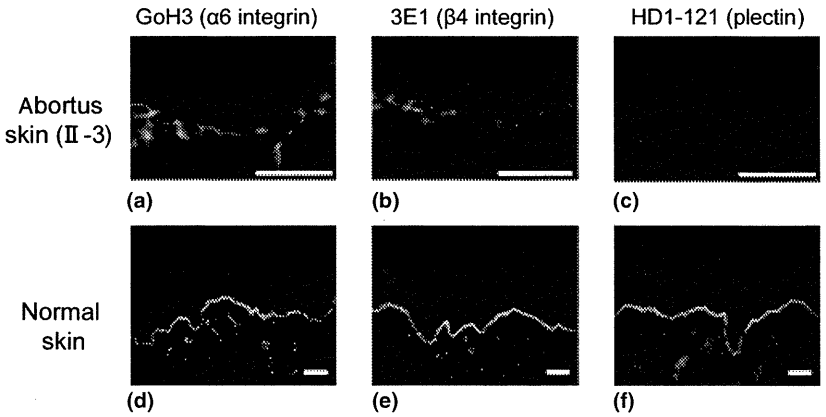


**Figure 3** Absence of plectin expression in the abortus. α6 integrin (mAb GoH3) and β4 integrin (mAb 3E1) are expressed in the abortus skin (a, b) and the control skin (d, e). Staining with monoclonal antibody for plectin (mAb HD1–121) shows positive in the control skin (f) but negative in the skin of the abortus (c: blue frame). Note that the skin tissue from the abortus was subject to degeneration before skin sampling. Thus, protein localization cannot be evaluated in the degenerated tissue. Scale bar: 50 μm

was a compound-heterozygote and affected by JEB-PA. The parents elected for the fetus to be terminated at 20 weeks gestation.

Immunofluorescence analysis showed that immunoreactivity using the mAbs HD1–121 (plectin), GoH3 (α6 integrin), and 3E1 (β4 integrin) was positive in the normal control skin (Fig. 3d–f). The skin sample obtained from the abortus tested positive for α6 integrin and β4 integrin (Fig. 3a,b) but negative for plectin (Fig. 3c).

## Discussion

This is the first successful PND of plectin-deficient EBS-PA, and the correct diagnosis was reconfirmed in the skin of the abortus. Given the universal mortality of EBS-PA due to *PLEC* mutations, there might be unreported PND cases for this form of EB. The prognosis of plectin-deficient EBS-PA is poor, and most patients commonly die within the first year of life,[13] as happened in the first- and

second-born progeny in the present family. Fetuses at risk of this condition are frequently terminated during pregnancy, and DNA-based PND plays an important role in prohibiting unnecessary termination of healthy fetuses at risk. Due to the recent elucidation of the causative genetic defects for genetic skin disorders, it has become possible to make DNA-based PND for severe genodermatoses by sampling of the chorionic villus or amniotic fluid in the earlier stages of pregnancy with a lower risk to fetal health and with a reduced burden on the mothers.

Plectin, a component of the hemidesmosome inner plaque, is involved in the attachment and crosslinking of the cytoskeleton and intermediate filaments to specific membrane complexes.[10] It has been described that EBS associated with muscular dystrophy (EBS-MD) results from *PLEC* mutations.[14,15] Mutations in the rod domain of *PLEC* are known to cause EBS-MD.[9,14,15] In addition, recent reports have confirmed that some *PLEC* mutations also lead to EBS-PA.[7–9,13] One alternative splice *PLEC* mRNA transcript that lacks exon 31 encoding the central core rod domain was identified in rat tissues.[16] By plectin-domain-specific reverse transcriptase-PCR, expression of this rodless alternative spliced form was confirmed in human keratinocytes.[17] Recently, our group demonstrated that loss of the full-length plectin with maintenance of the rodless plectin leads to EBS-MD, whereas complete loss or marked attenuation of full-length and rodless plectin expression underlies the EBS-PA phenotype.[12] The present family further supports the hypothesis that homozygotes or compound-heterozygotes for mutations that cause plectin truncation outside the rod domain show the EBS-PA phenotype.

In summary, this is the first report of DNA-based PND of EBS-PA. EBS-PA has now been added to the list of severe genodermatosis for which DNA-based PND is feasible.

## Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to H. Nakamura (Kiban C 1959129107) and H. Shimizu (Kiban A 21249063).

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30 June 2010
doi:10.1016/j.jdermsci.2010.11.014

## Letter to the Editor

### New insight into genotype/phenotype correlations in *ABCA12* mutations in harlequin ichthyosis

Harlequin ichthyosis (HI) is a severe and often fatal congenital ichthyosis with an autosomal recessive inheritance pattern [1]. The clinical features include thick, plate-like scales with ectropion, eclabium and flattened ears. *ABCA12* mutations underlie HI [2,3] and it was clarified that HI is caused by severe functional defects in the keratinocyte lipid transporter ABCA12 [2]. To date, various *ABCA12* mutations have been reported in HI patients [4]. However, genotype/phenotype correlations in *ABCA12* mutations have been poorly elucidated. In order to obtain clues to understand genotype/phenotype correlations in *ABCA12* mutations, we report two HI patients from two independent Japanese families, who were compound heterozygotes for *ABCA12* mutations.

Patient 1 is the second child of healthy, unrelated Japanese parents. The skin of the baby girl was covered with white, diamond shaped plaques at birth (Fig. 1a). After therapy with oral retinoids and local application of white petrolatum, in a humid incubator, the scales gradually detached and passive and spontaneous mobility of the joints increased. Now at the age of 1 year and 7 months, her general condition is good, although she still has white to grey scales on a background of erythematous skin over her entire body. Patient 2 is the fourth child of healthy, unrelated Japanese parents. Her older brother had a history of congenital ichthyosis and died in early infancy. The skin of the newborn showed serious symptoms with thick, white, diamond shaped plaques, partly bordered by bleeding fissures (Fig. 1c). Although she had therapy with oral retinoids and local application of white petrolatum, in a humid incubator, her clinical symptoms failed to show any apparent improvement and she died when she was 5 months old.

Skin biopsies showed thick stratum corneum in both patients (Fig. 1d–g). In Patient 2, parakeratosis was observed in the epidermis and a sparse inflammatory cell infiltration was seen in the superficial dermis (Fig. 1e inset). Electron microscopy (Hitachi, Tokyo, Japan) revealed a large number of abnormal, variously sized lipid droplets that accumulated in the cornified cells of both patients' epidermis.

Mutational analysis of *ABCA12* was performed in both patients and their families. Each genomic DNA sample was subjected to PCR amplification, followed by direct automated sequencing. Oligonucleotide primers and PCR conditions used for amplification of all exons 1–53 of *ABCA12* were originally derived from the report by Lefèvre et al. [5] and were partially modified for the present study. The entire coding region including the exon/intron boundaries for both forward and reverse strands from the patients, their parents and 50 healthy Japanese controls were also sequenced. Both patients had the same paternal novel nonsense mutation p.Arg1515X (Fig. 1h) which leads to truncation of the first ATP-binding cassette within ABCA12 likely resulting in ABCA12 loss of function (Fig. 2a). On the other allele, Patient 1 had a maternal recurrent splice acceptor site mutation c.3295-2A>G (Fig. 1h). This splice site mutation was reported in an unrelated Japanese family with HI and was shown to lead to comparable amounts of 2 splice pattern variants [2]. The first mutant transcript would result in a 3 amino acids deletion (1099_1101delYMK). These 3 amino acids are located in the first transmembrane domain and are highly conserved (Fig. 2b). The second mutant transcript lost a 170-bp sequence from exon 24, which led to a frameshift. Expression of a small amount of ABCA12 protein, although mutated, was detected in the granular layer keratinocytes of the patient's epidermis and cultured keratinocytes by immunofluorescent staining [2]. Thus, it is possible that Patient 1 expresses some mutated ABCA12 protein with a partial function. This might be the reason why Patient 1 survived beyond the perinatal and neonatal period and is still alive although this might also be in part due to the prompt oral retinoid treatment.

Patient 2 carried a maternal missense mutation p.Gly1179Arg on the other locus (Fig. 1h). To confirm the presence of the mutation p.Gly1179Arg in Patient 2, we performed restriction enzyme digestion analysis using *Bcl*I (NEW ENGLAND BioLabs). Restriction enzyme digestion of PCR products was carried out according to the manufacture's protocols. The 255-bp PCR products from wild type alleles were not digested by *Bcl*I, although the PCR products from the allele with the mutation p.Gly1179Arg were digested into 173- and 82-bp fragments. The father's PCR product after *Bcl*I digestion showed a single 255-bp band, which indicated he had only normal alleles. In contrast, the PCR product after *Bcl*I digestion from the mother of Patient 2 showed 255-, 173- and 82-bp bands, which indicated that she was heterozygous for the p.Gly1179Arg missense mutation (supplementary Fig. S1). This mutation was reported in a

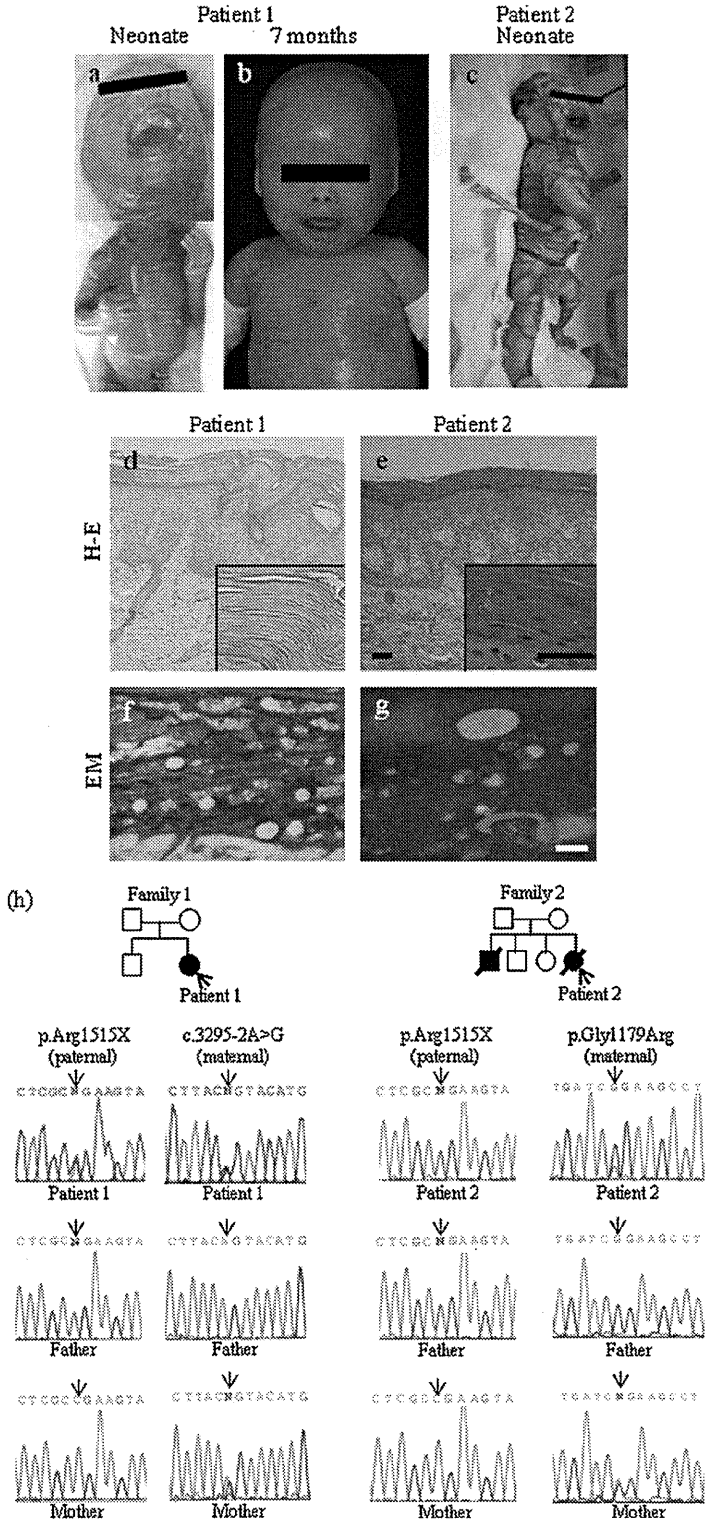


**Fig. 1.** (a–c) Clinical features of HI patients. Patient 1 showed the typical clinical phenotype of HI during the neonatal period, including the face and trunk (a). Her clinical symptoms remarkably improved at 7 months of age (b). Patient 2 showed more serious symptoms with thick plate-like scales and skin fissures in the neonatal period (c) and lived until the age of 5 months. (d–g) Histological features of the skin lesions of HI patients. Skin biopsies showed thick stratum corneum in both patients. Bars, 50 μm (d and e). In Patient 2, parakeratosis were observed (e, inset). By electron microscopy, abnormal variously sized lipid droplets had accumulated in the cornified cells of both patients' epidermis. Bars, 200 nm (f and g). (h) Families with HI and *ABCA12* mutations. Patient 1 was a compound heterozygote for two *ABCA12* mutations, a novel nonsense mutation p.Arg1515X and a recurrent splice site mutation c.3295-2A>G, and both her parents were heterozygous carriers. Patient 2 harboured two *ABCA12* mutations, p.Arg1515X and p.Gly1179Arg, and both her parents were heterozygous carriers of these defects.

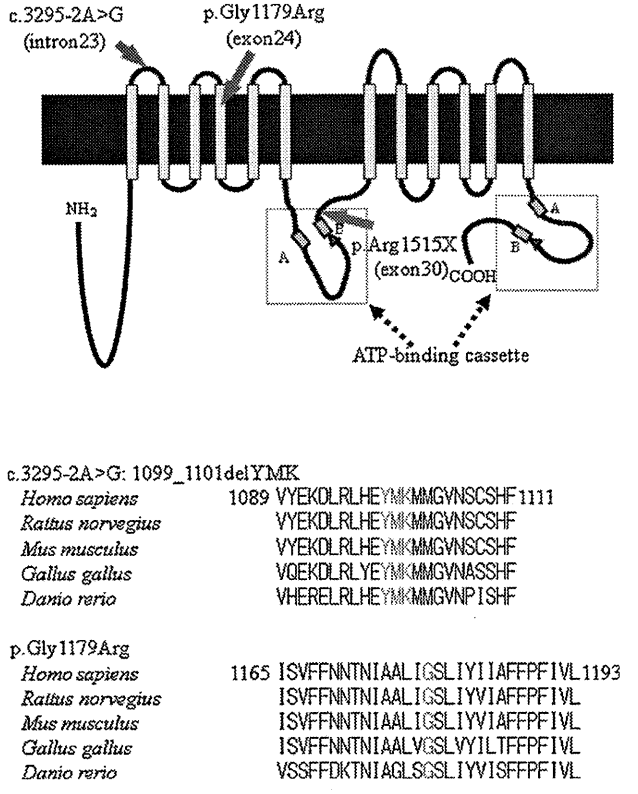


**Fig. 2.** (a) Structure of ABCA12 protein and the three mutations in present HI families. Dark blue area, cell membrane; bottom of dark-blue area, cytoplasmic surface. Note the mutation shared between the two patients is a truncation mutation in the first ATP-binding cassette (p.Arg1515X). The other mutation in Patient 2 is just a missense mutation in the first cluster of transmembrane domains (p.Gly1179Arg). (b) ABCA12 amino acid sequence alignment shows the level of conservation in diverse species of the amino acids, 1099_1101delYMK and p.Gly1179Arg (red characters).

Laotian family [6]. The glycine 1179 is a highly conserved amino acid residue (Fig. 2b) located in the first transmembrane ABCA12 domain (Fig. 2a), and this mutation substitutes an uncharged polar glycine residue for a positively charged arginine residue. The presence of these mutations was excluded in 100 alleles of 50 normal unrelated Japanese individuals.

Determinants of genotype/phenotype correlations resulting from *ABCA12* mutations, typically demonstrate that homozygotes or compound heterozygotes with truncation *ABCA12* mutations lead to an HI phenotype. Only a few exceptional cases have been reported such as the present case. The mutation p.Gly1179Arg might result in major loss of ABCA12 function and/or structure, leading to the severe phenotype in Patient 2.

Recently, long-term survival of patients with HI has been more frequently observed and documented [7,8]. The clinical symptoms of Patient 1 showed a remarkable improvement during infancy. In contrast, the symptoms of Patient 2 did not improve, and she died at the age of 5 months. The marked difference in the clinical severity of the two patients indicated that the p.Gly1179Arg has far bigger deleterious functional effects than c.3295-2A>G. The present study clearly demonstrates that some missense *ABCA12* mutations within highly conserved transmembrane regions are able to cause drastic changes in protein structure and function, leading to severe phenotypes, similar to truncation mutation patients. Further accumulation of similar cases is needed to confirm genotype/phenotype correlation in *ABCA12* mutations, especially in studies involving missense mutations underlying HI.

## Acknowledgments

We thank Dr. James R. McMillan for proofreading the manuscript. This work was supported in part by a grant-in-aid from the Ministry of Education, Science, Sports and Culture of Japan (Kiban B 20390304: to M.A.), a grant from Ministry of Health, Labour and Welfare of Japan (Health and Labour Sciences Research Grants; Research on Intractable Disease: H22-177: to M.A.).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2010.11.010.

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DOI:10.1111/j.1600-0625.2010.01177.x
www.blackwellpublishing.com/EXD

Letter to the Editor

# A founder effect of c.1938delC in *ITGB4* underlies junctional epidermolysis bullosa and its application for prenatal testing

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**Abstract:** Junctional epidermolysis bullosa associated with pyloric atresia (JEB-PA) is one of the most severe inherited skin diseases, characterized by generalized blister formation and occlusion of the pylorus at birth. Most JEB-PA patients have mutations in the gene encoding β4 integrin (*ITGB4*). No recurrent mutations in *ITGB4* have been described as having founder effects. We collected three JEB-PA families with c.1938delC in *ITGB4*. Haplotype analysis using single nucleotide polymorphism markers throughout *ITGB4* suggested one rare haplotype (2.8% of the Han Chinese and ethnic Japanese populations) in all alleles with c.1938delC. The parents of one of the three families sought prenatal diagnosis for a subsequent pregnancy. We succeeded in performing prenatal exclusion of JEB-PA using the foetal genomic DNA. Our study clearly demonstrated that recurrent c.1938delC in *ITGB4* is a founder mutation in JEB-PA patients, and that genotyping of the mutation can be utilized for prenatal diagnosis of JEB-PA.

**Key words:** basement membrane zone – haplotype analysis – single nucleotide polymorphism

*Accepted for publication 20 July 2010*

## Background

Recurrent mutations in a population might be explained by founder effects, in which the mutant alleles of a few ancestors spread in the population because of limited gene pool, genetic drift and healthy carrier migration (1).

Epidermolysis bullosa (EB) comprises a group of disorders characterized by congenital skin fragility. EB has been classified into EB simplex, junctional epidermolysis bullosa (JEB), dystrophic EB and Kindler syndrome (2–4). JEB is subclassified into three clinical subtypes: Herlitz JEB, non-Herlitz JEB and JEB with pyloric atresia (JEB-PA). JEB-PA is characterized by generalized blistering and occlusion of the pylorus at birth, which usually leads to early demise (5). Mutations in the gene encoding α6 (*ITGA6*) or the β4 integrin subunit (*ITGB4*) are responsible for JEB-PA (6,7). Most patients with JEB-PA have mutations in *ITGB4* (8). No frequent prevalent mutations have been noted, except in the Hispanic population, where c.1802G>A (p.Cys601Tyr) is present on five of 10 alleles of JEB-PA patients (9).

Here, we have collected three JEB-PA families, in which c.1938delC in *ITGB4* is present. Haplotype analysis revealed c.1938delC as a founder mutation in JEB patients. Based on these data, we successfully performed prenatal exclusion of JEB-PA with this mutation.

## Experimental design

### Patients

Three unrelated non-consanguineous Japanese families (A, B and C) with JEB-PA in this study are summarized in Fig. S1a. Family A and B originate from Shikoku Island in Japan and family C is from other part of the country. A-1 and B-1 are newly identified JEB-PA patients. They died of disseminated intravascular coagulation 1 and 2 months after birth, respectively. Immunofluorescence study of skin specimens from both of the patients showed the absence of β4 integrin and weak expression of α6 integrin subunits (data not shown). Immunostaining for laminin 332, type IV collagen, type VII collagen, type XVII collagen, plectin and BP230 revealed normal linear labelling patterns (data not shown). C-2 is a patient with non-lethal variant of JEB-PA. The case description and mutational data of C-2 have been reported previously (10).

### Mutation detection

Genomic DNA (gDNA) was extracted from blood cells of the probands and their parents. Mutation detection was performed after polymerase chain reaction (PCR) amplification of all exons and intron–exon borders of *ITGB4*, followed by direct sequencing using an ABI Prism 3100 genetic analyzer (Advanced Biotechnologies Inc., Columbia, MD, USA) (11–13). The genomic DNA nucleotides, the complementary DNA nucleotides and the amino


 

acids of the protein were numbered based on the following sequence information (GenBank accession No. NM_000213).

### Haplotype analysis

To determine whether c.1938delC is a founder mutation, we performed haplotype analysis of three JEB-PA families. We constructed linkage disequilibrium (LD) blocks containing *ITGB4* using genotype data from the HapMap database (International HapMap Consortium, 2005). The haplotype structure with its tag-single nucleotide polymorphisms (SNPs) was determined using Haploview (14). We genotyped 15 tag-SNPs (Fig. S1b) using the ABI Prism 3100 genetic analyzer (Advanced Biotechnologies Inc.).

### Prenatal diagnosis

We performed prenatal diagnosis of a foetus (A-2) at risk for JEB-PA from family A. A total of 30 ml of amniotic fluid was obtained under ultrasound guidance at 16 weeks' gestation. Foetal DNA was extracted from fresh cells from 10 ml of amniotic fluid. Genomic DNA isolated from amniotic fluid cells was subjected to polymerase chain reaction (PCR) amplification, followed by direct automated sequencing as described. The mutation site was sequenced using both forward and reverse strands and verified by *PmlI* (New England Biolabs Inc., Beverly, MA, USA) enzyme digestion of the PCR products.

The medical ethical committee of Hokkaido University and National Center for Child Health and Development approved all described studies. The study was conducted according to Declaration of Helsinki Principles. Participants gave their written informed consent.

## Results

### Recurrent c.1938delC in *ITGB4*

*ITGB4* mutation analysis revealed that A-1 was homozygous for c.1938delC (Fig. 1c). The father and mother of A-1 were heterozygous for c.1938delC (Fig. 1a, b). B-1 was heterozygous for paternal c.1938delC and maternal c.4050_4057del (data not shown). c.1938delC was previously described in a patient with non-lethal variant of JEB-PA who is compound heterozygous for c.1938delC and c.2168C>G (p.Pro723Arg) (C-2) (10). c.4050_4057del was also reported in a JEB-PA patient who is compound heterozygous for c.4050_4057del and c.3434delT (12).

### Founder effects of c.1938delC

The haplotype structure containing *ITGB4* was constructed using genotype data from the HapMap database (Fig. S1b, c). The haplotype block was represented by 16 haplotypes with >2% frequency (Fig. S1b, c). The chromosome containing c.1938delC in A-1 and B-1 had haplotype XI (GGGACGGGCGTCACC), which is seen in 2.8% of the Han Chinese and ethnic Japanese populations. The chromosome containing c.1938delC in C-2 might have had this haplotype although the phase was not determined.

### Prenatal exclusion of JEB-PA

Direct sequencing of PCR products from the foetal gDNA (A-2) revealed the presence of c.1938delC in one allele and wild-type sequence in another allele (Fig. 1d). To confirm the results of direct sequencing, we performed restriction enzyme analysis. c.1938delC was found to result in the loss of a restriction enzyme for *PmlI*. The PCR product from the proband (A-1) after *PmlI* digestion revealed a 285-bp band, which indicated that she was homozygous for c.1938delC (Fig. 1f). In contrast, the PCR product from the parents and the foetus (A-2) after *PmlI* digestion showed 285-, 195- and 90-bp bands, which indicated that they were heterozygous for c.1938delC (Fig. 1f). Haplotype analysis of this family using microsatellite markers excluded maternal contamination of foetal cells (data not shown). These results predicted that the foetus would not be affected, and the pregnancy was continued. A neonate was born at full term in good health with completely normal skin.

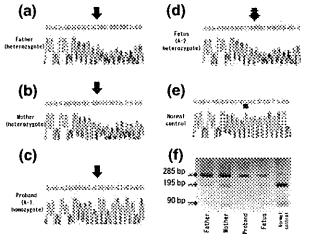


**Figure 1.** Prenatal diagnosis of junctional epidermolysis bullosa with pyloric atresia (family A). (a–e) Direct sequencing of *ITGB4*. The parents were heterozygous for c.1938delC in *ITGB4* (a, b). A-1, the proband, was homozygous for that mutation (c). A-2, the foetus, was found to be a heterozygous carrier (d). A cytosine at cDNA position 1938 in normal control is underlined (e). Arrows indicate a deleted cytosine in *ITGB4* sequence. (f) *PmlI* restriction enzyme digestion of the PCR products from the family members' genomic DNA. c.1938delC results in the loss of a site for *PmlI*. *PmlI* restriction enzyme digestion of the PCR products from normal control reveals 195- and 90-bp bands. Only a 285-bp band is observed in A-1 (the proband), who is homozygous for c.1938delC. In contrast, 285-, 195- and 90-bp bands are detected in the father, mother and A-2, suggesting that they are heterozygous for c.1938delC.

## Conclusions

There are no recurrent *ITGB4* mutations that have been demonstrated to have founder effects in JEB-PA patients. Our study detected recurrent c.1938delC in *ITGB4* and revealed this to be a founder mutation in JEB-PA patients.

DNA-based prenatal testing of JEB-PA has been described (15–18). Our study has demonstrated the successful prenatal exclusion of JEB-PA with c.1938delC through mutation analysis of the foetal genomic DNA.

In summary, our study identified a founder c.1938delC in *ITGB4* and showed that this mutation can be applied for prenatal diagnosis of JEB-PA.

## Acknowledgements

We thank Ms Yuko Hayakawa and Ms Yuki Miyamura for their technical assistance. This work was supported by Health and Labor Sciences Research grants for Research on Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan (to H.S.).

## Conflicts of interest

The authors declare no conflicts of interest.

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## Supporting Information

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

**Figure S1.** Haplotype analysis of the junctional epidermolysis bullosa families.

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DOI:10.1111/j.1600-0625.2010.01172.x
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Letter to the Editor

# IL-1 signalling is dispensable for protective immunity in *Leishmania*-resistant mice

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**Abstract:** Leishmaniasis is a parasitic disease affecting ~12 million people. Control of infection (e.g. in C57BL/6 mice) results from IL-12-dependent production of IFNγ by Th1/Tc1 cells. In contrast, BALB/c mice succumb to infection because of preferential Th2-type cytokine induction. Infected dendritic cells (DC) represent important sources of IL-12. Genetically determined differences in DC IL-1α/β production contribute to disease outcome. Whereas the course of disease was not dramatically altered in IL-1RI$^{-/-}$ mice, local administration of IL-1α to infected C57BL/6 mice improved disease outcome. To definitively elucidate the involvement of IL-1 in immunity against leishmaniasis, we now utilized IL-1α/β-double-deficient C57BL/6 mice. C57BL/6 mice are believed to be a good surrogate model for human, self limited cutaneous leishmaniasis (CL). *Leishmania major*-infected IL-1α/β$^{-/-}$ mice were resistant to experimental CL comparable to controls. In addition, DC-based vaccination against leishmaniasis in C57BL/6 mice was independent of IL-1. Thus, in *Leishmania*-resistant C57BL/6 mice, IL-1 signalling is dispensable for protection.

**Key words:** IL-1 – dendritic cells – *L. major*

*Accepted for publication 26 July 2010*

## Background

Leishmaniasis is a parasitic disease transmitted by the bite of a sand fly. The disease ranges from cutaneous leishmaniasis (CL) to visceral leishmaniasis and ~12 million people are affected worldwide (1). In murine experimental leishmaniasis, control of infection results from IL-12-dependent production of Th1/Tc1-derived IFNγ that activates infected macrophages (MΦ) to eliminate parasites (2–5). In disease-resistant C57BL/6 mice, skin DC infected with *Leishmania major* represent important sources of IL-12 (6). In contrast, BALB/c mice respond to infection with preferential Th2-type cytokine production, which is associated with disease progression. Genetically determined DC-derived factors that influence disease susceptibility of BALB/c mice include elevated levels of inhibitory IL-12p80 (7) and decreased release of IL-1α/β (8,9). Previously, we demonstrated that IL-1α/β facilitates Th1 induction in several inflammatory disease models (9–11). Treatment of BALB/c mice with IL-1 during T cell priming inhibited progressive disease by shifting the immune response towards Th1 (9). However, prolonged administration of IL-1α promoted Th2 expansion in already established infections and worsened disease outcome (11).

**Abbreviations:** CL, cutaneous leishmaniasis; DC, dendritic cells; MΦ, macrophages.

## Question addressed

IL-1 is a key mediator of inflammation (12,13). IL-1α and IL-1β exert similar biological functions by binding to the IL-1 type I receptor (IL-1RI) (14). To definitively elucidate the involvement of IL-1 in immune responses in CL, we utilized IL-1α/β-double


 

thickness of the mucous layer of small intestines, resulting in the inhibition of small intestinal absorption.[4] In addition, PGE1 increases blood flow in the stomach and upregulates the digestion in the stomach. During the provocation test in our case, serum gliadin levels were not increased by administering misoprostol. However, sodium cromoglicate, a mast cell stabilizer commonly used to treat allergic rhinitis, allergic conjunctivitis, and asthma, could not affect serum gliadin levels in the provocation test, and therefore allowed the symptoms to occur. We consider that the effects of misoprostol on the alimentary tract are crucial for the prevention of FDEIA. Our observation indicates that the exacerbating effect of aspirin in FDEIA comes from the inhibitory effects of aspirin on PGE1 in the gastrointestinal milieu. Thus, misoprostol would be a promising prophylactic drug for FDEIA.

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**Type XVII collagen ELISA indices significantly decreased after bullous pemphigoid remission**

The major pathogenic epitope of bullous pemphigoid (BP) is known to be the noncollagenous extracellular domain (NC16A) of type XVII collagen (COL17).[1] Here we investigated indirect immunofluorescence (IIF) and COL17 NC16A domain enzyme-linked immunosorbent assay (ELISA)[2–5] data before treatment and after remission to evaluate the usefulness of ELISA analyses as indicators for BP disease activity.

We included ten consecutive BP patients [eight women and two men: between 33 and 80 years old (mean; 59 years old)] who showed typical clinical features before treatment and were successfully treated, resulting in complete or partial remission at our institute. The first day of each patient visit was within the last three years. In all patients, the diagnosis was confirmed by histopathological observation and immunofluorescence study, i.e. histopathological subepidermal blister formation was observed and direct and IIF studies revealed the presence of autoantibodies along the dermal–epidermal junction. All patients were successfully treated with oral prednisolone therapy of 30–50 mg/d with or without azathioprine or a combination therapy using tetracycline and nicotinamide. Treatment periods from initial diagnosis to remission ranged from four months to 35 months (mean; 14.6 ± 10.8 months). Serum samples were obtained for ELISA and IIF at least twice during the disease course for each patient.

Concentration of autoantibodies in the patients' sera directed against the NC16A domain of COL17 was measured using the COL17 NC16A ELISA kit following the kit's instructions.[6] IIF staining and evaluation were performed as previously described using normal human skin as a substrate.[7]

In all the cases, the ELISA indices showed a decrease during the successful treatment course. ELISA indices after remission were significantly reduced compared with those before treatment ($P < 0.0001$) (Fig. 1a). IIF titers also decreased after remission in six cases, but the titers were not apparently reduced in the other four cases, although a statistically significant reduction in combined IIF titer was observed after remission compared with those before treatment ($P < 0.05$) (Fig. 1b).

Positive correlation between ELISA indices and BP disease activity has been reported previously in the literature. Di Zenzo *et al.*[8] demonstrated that disease severity before treatment was well correlated with ELISA indices in BP patients. Izumi *et al.*[9] described ELISA indices and alteration of disease activity of five BP patients during various treatments. In this study, we compared the ELISA

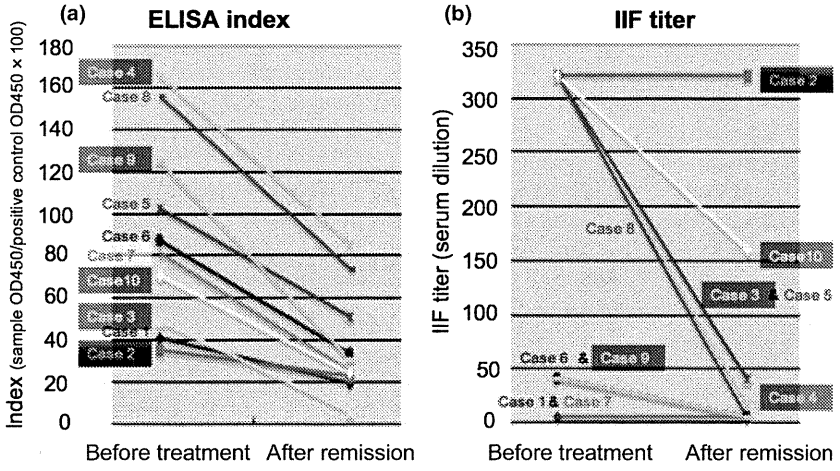


**Figure 1** ELISA indices and indirect immunofluorescence (IIF) titers before treatment and after remission. (a) ELISA indices of successfully treated BP patients. Disease remission was defined as when erythema, bullae and erosions had completely healed (complete remission) or no more than three bullae or erythema were seen in a week (partial remission) and only a low dose of oral prednisolone (<5 mg/d) or no treatment was needed to maintain this condition. As ELISA indices after remission, we adopted ELISA indices at the time when each patient's disease activity was evaluated as being in "complete remission" or "partial remission" (as defined above) for the first time after treatment. Mean ELISA index of the 10 patients before treatment was 91.3 ± 45.7 (range: 35.6–165.6) and the mean index after remission was 37.4 ± 25.3 (range: 6.0–86.4). After complete or partial remission, the ELISA indices were significantly reduced ($P < 0.0001$). (b) IIF titers of the same patients. Apparent decreases in IIF titers after remission were seen only in six patients. Mean IIF titer of the 10 patients before treatment was 201 ± 154 (range: 5–320) and the mean titer after remission was 60.5 ± 102.8 (range: 5–320). A statistically, significant reduction was observed in combined IIF titers after remission compared with those before treatment ($P < 0.05$). Colors of the lines are specific for each patient in both figures (a) and (b)

indices before treatment and after remission in our BP patient cohort and clearly demonstrated that ELISA indices significantly decreased after remission. Feng *et al.*[10] reported similar results on correlation of ELISA indices with disease course in BP patients, although the time points for ELISA after treatment were just before the decrease in corticosteroid and when the dosage of corticosteroid was successfully decreased to half the initial dose in the report. In this study, we employed ELISA indices at the time when each patient's disease activity was evaluated as "complete remission" or "partial remission" for the first time after treatment. Thus, this study is unique in the point that we evaluated exact correlation between ELISA indices and disease remission.

In conclusion, the present results further support the idea that the COL17 NC16A ELISA indices demonstrate a correlation with the BP disease remission more accurately than IIF titers and are a useful tool to detect BP disease remission and to assess the efficacy of BP treatment.

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Conflict of interest: the authors state no conflict of interest.

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# Case letters

## Extremely severe palmoplantar hyperkeratosis in a generalized epidermolytic hyperkeratosis patient with a keratin 1 gene mutation

*To the Editor:* Epidermolytic hyperkeratosis (EHK; OMIM#113800), also called bullous congenital ichthyosiform erythroderma, is a rare genetic disorder of keratinization. We report a patient with generalized EHK showing extremely severe palmoplantar hyperkeratosis with digital contractures.

A 45-year-old Japanese man had erythroderma at birth. He exhibited skin blistering, erosions, and hyperkeratosis on the erythrodermic skin since infancy. The blistering and erosions gradually diminished with age. He developed severe palmoplantar hyperkeratosis and digital contractures at 7 years of age. At 24 years of age, surgery was performed to improve the contraction of his toes. A physical examination revealed hyperkeratosis of the entire body, especially at the ankles, elbows, and knees, and erosions were observed on the inner side of the elbows and knees (Fig 1, *A-D*). Palmoplantar hyperkeratosis was severe with digital contractures. The morphology of his hair, nails, and teeth was normal. There was no known family history of skin disease. Skin biopsy from the upper portion of the left arm showed severe granular degeneration in all the suprabasal layers (Fig 1, *E*). Ultrastructural analysis revealed clumping of the intermediate filaments within keratinocytes of the suprabasal layers (Fig 1, *F*).

Direct sequencing of the whole coding regions of *KRT1* and *KRT10* (GenBank accession numbers NT029419.11 and NT010755.15) was performed as previously described,[1] and a novel heterozygous *KRT1* missense mutation c.1457T>G (p.Leu486Arg) was identified in exon 7. This mutation was verified by restriction enzyme *Msp*I digestion. The mutation p.Leu486Arg was not found in 100 normal, unrelated Japanese alleles (50 healthy unrelated individuals) using sequence analysis (data not shown).

The present novel *KRT1* mutation p.Leu486Arg is in the 2B segment of keratin 1 (Fig 2, *A* and *B*). This mutation occurred within the highly conserved helix termination motif (HTM) of the K1 protein. The palmoplantar hyperkeratosis was extremely severe. It is noteworthy that another mutation at the identical

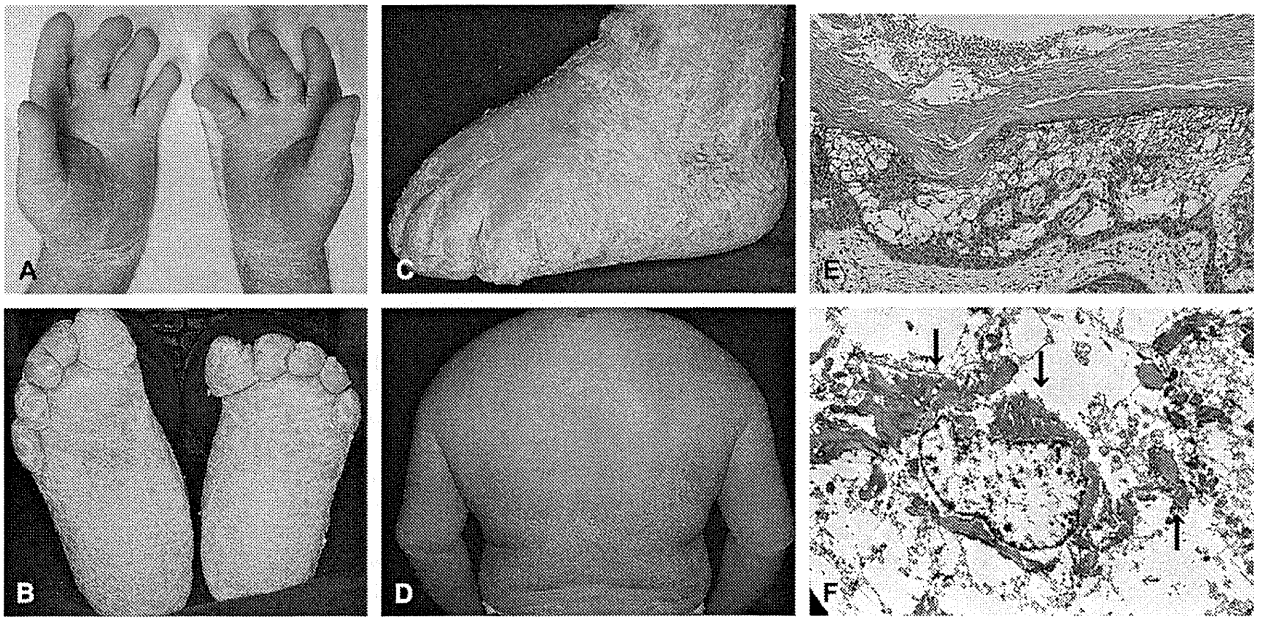
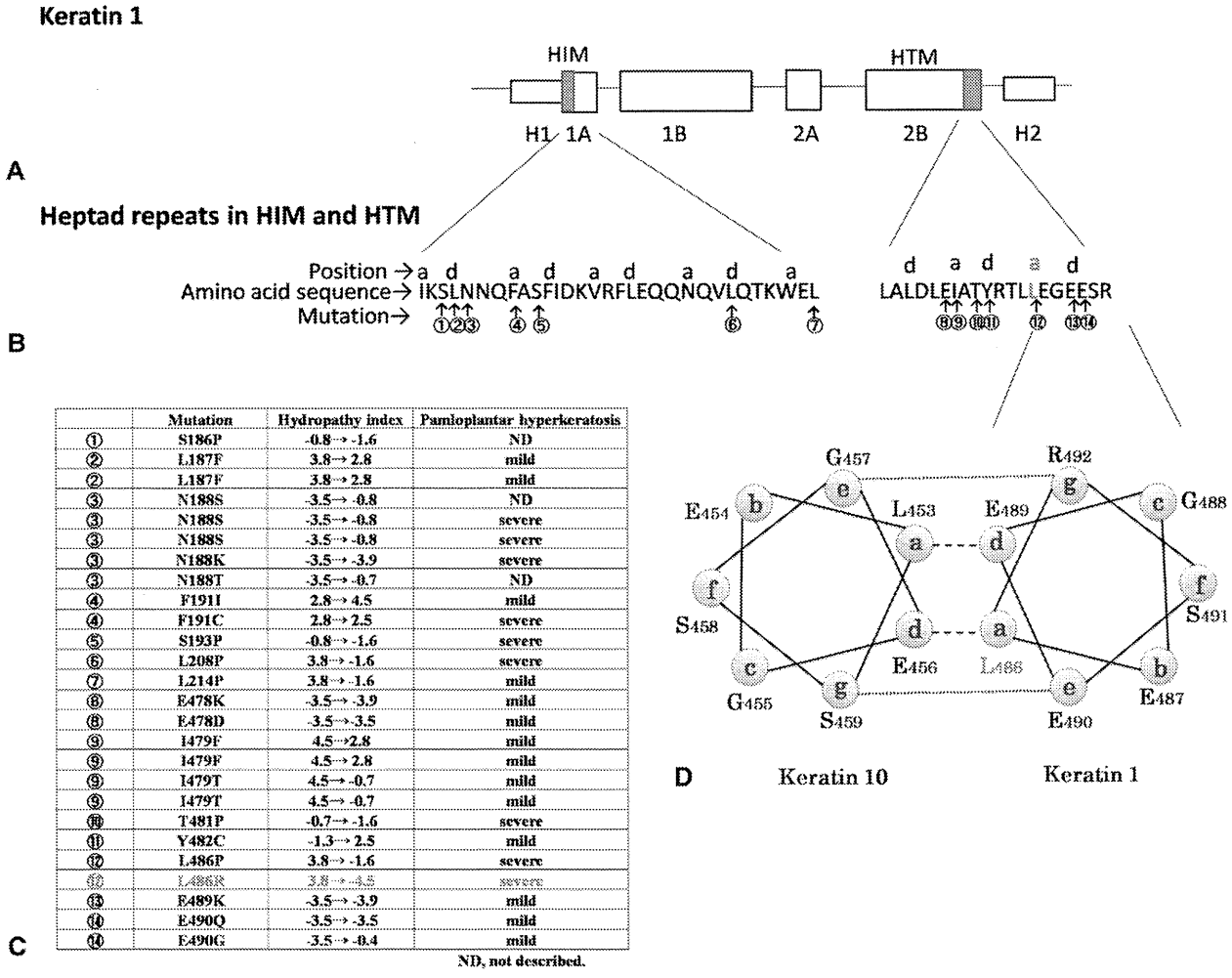



**Fig 1.** Clinical, histopathologic, and ultrastructural features of the patient. Severe diffuse hyperkeratosis and scale are seen on the palms (**A**) and soles (**B**). Warty brown hyperkeratosis and scale are present on the margins and the dorsal surface of the foot (**C**). Generalized erythroderma and scaling is seen on the trunk (**D**). The histopathologic examination revealed acanthosis and hyperkeratosis, coarse keratohyaline granules, and severe granular degeneration in the entire spinous and granular layers of the epidermis (**E**). Ultrastructurally, clumping of the keratin filaments *(arrows)* is seen within an upper epidermal keratinocyte (**F**).




C

| | Mutation | Hydropathy index | Palmoplantar hyperkeratosis |
|---|---|---|---|
| ① | S186P | -0.8→ -1.6 | ND |
| ② | L187F | 3.8→ 2.8 | mild |
| ② | L187F | 3.8→ 2.8 | mild |
| ③ | N188S | -3.5→ -0.8 | ND |
| ③ | N188S | -3.5→ -0.8 | severe |
| ③ | N188S | -3.5→ -0.8 | severe |
| ③ | N188K | -3.5→ -3.9 | severe |
| ③ | N188T | -3.5→ -0.7 | ND |
| ④ | F191I | 2.8→ 4.5 | mild |
| ④ | F191C | 2.8→ 2.5 | severe |
| ⑤ | S193P | -0.8→ -1.6 | severe |
| ⑥ | L208P | 3.8→ -1.6 | severe |
| ⑦ | L214P | 3.8→ -1.6 | mild |
| ⑧ | E478K | -3.5→ -3.9 | mild |
| ⑧ | E478D | -3.5→ -3.5 | mild |
| ⑨ | I479F | 4.5→ 2.8 | mild |
| ⑨ | I479F | 4.5→ 2.8 | mild |
| ⑨ | I479T | 4.5→ -0.7 | mild |
| ⑨ | I479T | 4.5→ -0.7 | mild |
| ⑩ | T481P | -0.7→ -1.6 | severe |
| ⑪ | Y482C | -1.3→ 2.5 | mild |
| ⑫ | L486P | 3.8→ -1.6 | severe |
| ⑬ | L486R | 3.8→ -4.5 | severe |
| ⑬ | E489K | -3.5→ -3.9 | mild |
| ⑭ | E490Q | -3.5→ -3.5 | mild |
| ⑭ | E490G | -3.5→ -0.4 | mild |

ND, not described.

**Fig 2.** Summary of mutations in the helix initiation motif (HIM) and helix termination motif (HTM) of K1 from the Human Intermediate Filament Database (www.interfil.org/). **A**, Molecular structure of K1. **B**, Heptad repeats in HIM and HTM of K1 and mutation sites. The majority of cases (22 of 26) had mutations in the heptad repeat position *a, d, e,* and *g*. The present mutation is located at the *a* position leucine residue at codon no.486 *(red characters)* in the C-terminal—most heptad repeat. **C**, Summary of the *KRT1* mutations in HIM and HTM, alterations of hydropathy index, and levels of palmoplantar hyperkeratosis. Eight cases, including the present one, were reported as showing severe palmoplantar hyperkeratosis, and seven of those nine patients harbored mutations in the important *a, d, e,* and *g* position of heptad repeats. Mutations in this 486-leucine residue may seriously perturb the stability of keratin intermediate filaments. The substitution of arginine for leucine alters the character of the amino acid from that of a hydrophobic, apolar amino acid (hydropathy index of leucine, +3.8) to that of the most hydrophilic, basic amino acid (hydropathy index of arginine, −4.5). **D**, Heptad structure of the rod domain: schematic of a transverse cut through the last heptad (*abcdefg*) of the HTM of K1 and K10, showing hydrophobic interactions between positions *a* and *d (dashed lines)* and ionic hydrogen interactions between positions *e* and *g (dotted lines)*. Position *a* is occupied by apolar, hydrophobic amino acids. The *a* residues are thought to interact with amino acids located in the *d* position of the partner molecule of the heterodimer through hydrophobic interactions which stabilize the two-chain coiled-coil molecules. When the two strands coil around each other, positions *a* and *d* are internalized, stabilizing the structure, while positions *b, c, e, f,* and *g* are exposed on the surface of the protein. Residues at positions *e* and *g* stabilize dimer formation through ionic and hydrogen bonds.

position of K1, p.Leu486Pro, was reported in patients with EHK and severe palmoplantar hyperkeratosis (Fig 2, *C*) and digital contractures, and the affected individuals exhibited clinical features similar to our patient.[2] Therefore, our data suggest that a nonconservative amino acid change at codon 486 of K1 results in a severe form of generalized EHK.

The rod domains consist of four alfa-helical segments that possess a repeating heptad amino acid residue peptide motif *(a-b-c-d-e-f-g)n* that has the potential to form a two-chain coiled coil with a corresponding sequence (Fig 2, *D*).[3-5] The residues at position *a, d, e,* and *g* are considered to be highly sensitive to mutations.[6]

Our patient with generalized EHK had most severe palmoplantar hyperkeratosis compared to previously reported cases with mutations in *KRT1*. The leucine residue at codon 486 is located in the *a* position of the heptad repeat at the C-terminal end of the 2B helix, and the substitution of arginine for leucine seriously alters the character of amino acid. It is therefore reasonable to say that this mutation caused generalized EHK with severe palmoplantar hyperkeratosis, compared with that seen in patients harbouring mutations in the other residues.

Twenty-six EHK cases, including the present case with point mutations at the helix initiation motif (HIM) and HTM of *KRT1*, have been reported to date (Fig 2, *C*); Human Intermediate Filament Database [www.interfil.org/]). Only nine cases, including the present case, were diagnosed as generalized EHK with severe palmoplantar hyperkeratosis, and seven cases out of nine harbored missense mutations in the heptad repeat position *a, d, e,* and *g*. These facts indicate that the mutation site and the nature of amino acid alterations in K1 may determine the level of severity of palmoplantar hyperkeratosis.

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*Supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan to Dr Akiyama (Kiban 20390304).*

*Conflicts of interest: None declared.*

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doi:10.1016/j.jaad.2009.04.019

## Scrotal elephantiasis secondary to hidradenitis suppurativa

*To the Editor:* We read with great interest the continuing medical education article by Alikhan et al[1] in the April 2009 issue of the *Journal* providing a comprehensive review of hidradenitis suppurativa (HS). We found the mention of scrotal lymphedema caused by HS to be of particular interest and would like to share our experience with a patient who developed the unusual complication of scrotal elephantiasis caused by longstanding HS.

A 58-year-old white man with no recent travel history who lived in social isolation presented with scrotal enlargement that began about a decade ago but had become notably worse over the past 3 years. He denied any history of sexually transmitted infections and recalled having had HS his entire adult life. The patient underwent several incision and drainage procedures in the emergency department over the years, had recently been treated briefly with oral antibiotics without improvement, and was finally referred for dermatologic evaluation. The physical examination revealed a massively enlarged, indurated scrotum that obscured his penis and had multiple sinus tracts draining clear, foul-smelling fluid (Fig 1). He had many violaceous nodules with open comedones in the intertriginous regions, including the left and right axillae, bilateral groins, bilateral inner thighs, and beneath the skin folds on his lower abdomen.

Punch biopsy specimens from the patient's left inner thigh and left lower abdomen revealed findings

BJD
British Journal of Dermatology

## Childhood subepidermal blistering disease with autoantibodies to type VII collagen and laminin-332

DOI: 10.1111/j.1365-2133.2010.10065.x

MADAM, Autoimmune subepidermal blistering diseases include bullous pemphigoid, pemphigoid gestationis, linear IgA bullous dermatosis, mucous membrane pemphigoid (MMP), anti-p200 pemphigoid, epidermolysis bullosa acquisita (EBA) and bullous systemic lupus erythematosus.[1] Patients with EBA have IgG autoantibodies to type VII collagen while some patients with MMP have autoantibodies to laminin-332.[2,3] We describe a juvenile case of subepidermal blistering disease with autoantibodies to both type VII collagen and laminin-332. The present case is unique because of its childhood onset and successful remission following only topical steroid therapy.

A 12-year-old Japanese girl presented with pruritic eruptions on her scalp. A few weeks later, widespread pruritic vesicles gradually developed over her whole body. The vesicles were seen both on erythematous and normal skin (Fig. 1a, b). Blisters and erosions also appeared in her oral mucosa, but there was no involvement of genital or ocular mucous membranes (Fig. 1c). Neither nail changes nor alopecia were observed. She had no family history of any blistering disorders or autoimmune disease. There was no preceding illness or history of medication/vaccination that might have triggered her disease.

General laboratory examinations revealed no apparent abnormalities except for an increased serum IgE level (668·8 IU $mL^{-1}$; normal < 100 for age 7–14 years). A skin biopsy was taken from the edge of one blister on her right forearm. Light microscopy showed a subepidermal blister with an inflammatory cell infiltrate consisting of mainly neutrophils in the upper dermis (Fig. 2a). Direct immunofluorescence of the patient's lesional skin showed *in vivo* linear deposits of IgG and C3 at the epidermal basement membrane zone (Fig. 2b). On the blistered area, deposition of IgG and C3 was demonstrated on the dermal side of the separated skin (arrows, Fig. 2b). Indirect immunofluorescence with the patient's serum on 1 mol $L^{-1}$ NaCl-split normal human skin showed IgG antibodies bound to the dermal side of the blister (Fig. 2c). Immunoblot analysis revealed that the patient's serum reacted with a 290-kDa protein in dermal extracts, and further with purified laminin-332 α3 protein (145, 165 kDa) (Fig. 2d, e). Laminin-332 was obtained from human keratinocytes and was purified using an antilaminin-332 affinity column as

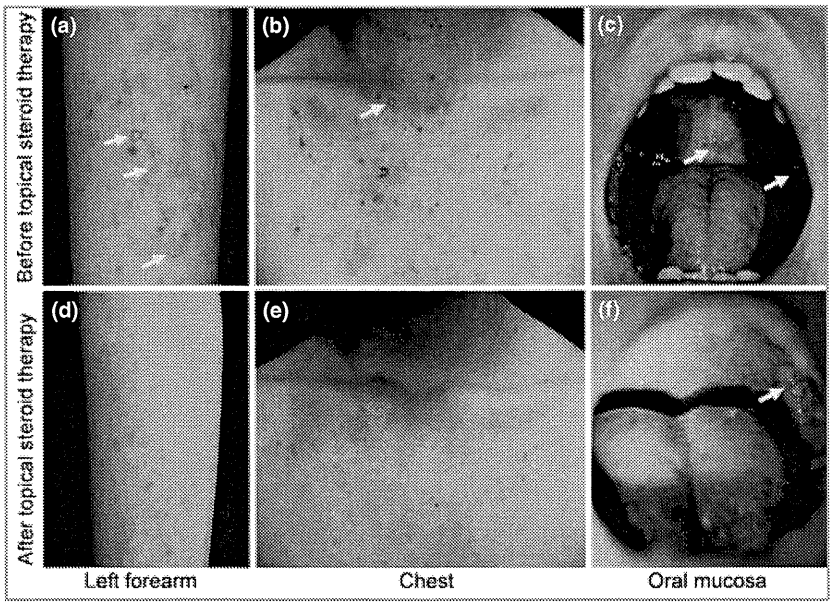


**Fig 1.** Clinical manifestations of the skin and oral mucosa. (a–c) Before topical steroid therapy. Erythema and tense vesicles on the left forearm and chest (a and b, arrows). Blisters and erosions over the oral mucosa (c, arrows). (d–f) After topical steroid therapy. Skin lesions healed within 9 days of the beginning of treatment, leaving residual pigmentation, scars and milia (d and e). Blisters and erosions on the oral mucosa subsided (f, arrow).

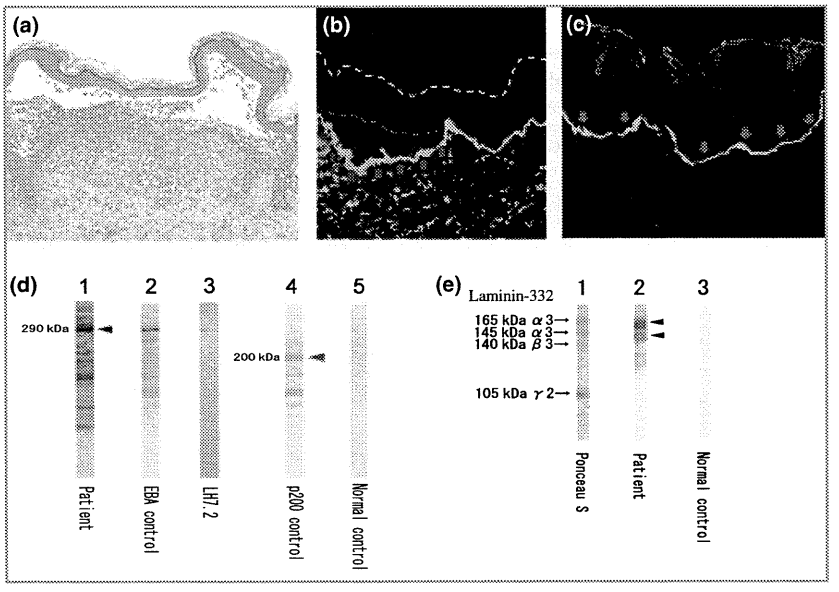


**Fig 2.** Histopathological findings, immunofluorescence staining and immunoblot analyses. (a) A subepidermal blister with an inflammatory cell infiltrate composed of mainly neutrophils in the upper dermis (haematoxylin and eosin; original magnification × 40). (b) Direct immunofluorescence showed *in vivo* linear deposits of IgG along the basement membrane zone. On the blister area, deposition of IgG was shown to be towards the dermal side of separated skin (arrows) (original magnification × 40; white dotted line is the skin surface and red dotted line is the roof side of separated skin). (c) Indirect immunofluorescence with the patient's serum on 1 mol $L^{-1}$ NaCl-split normal human skin showed IgG antibodies bound to the dermal side (arrows) (original magnification × 40). (d) Immunoblot analysis revealed that the patient's serum (lane 1), like both serum from a reference patient with epidermolysis bullosa acquisita (EBA, lane 2) and monoclonal antibody LH7.2 to type VII collagen (lane 3), reacted with a 290-kDa protein in dermal extracts (arrowhead). Control anti-p200 serum did not react with the 290-kDa but with a 200-kDa protein (red arrowhead) (lane 4). Normal control serum (lane 5) showed reactivity with neither. (e) In immunoblotting of purified laminin-332, lane 1 shows Ponceau S stain (protein staining using amido black). Reactivity with 145-kDa and 165-kDa purified laminin-332 α3 protein (arrowheads) was indicated in the patient's serum (lane 2), but not in the normal control serum (lane 3).

previously described.[4,5] Purified laminin-332 was a generous gift from Dr S. Amano, Shiseido Life Science Research Centre, Yokohama, Japan. The patient was diagnosed as having an autoimmune subepidermal blistering disease with circulating autoantibodies to type VII collagen and laminin-332.

Treatment was initiated with 0·05% clobetasol propionate ointment 20 g daily to skin lesions, which healed within 9 days after the beginning of treatment, leaving residual pigmentation, scars and milia (Fig. 1d, e). Blisters and erosions on the oral mucosa subsided without any topical therapy (Fig. 1f). The dose of topical corticosteroids was progressively decreased, and no recurrence of skin lesions was observed. The titre of antibasement membrane zone antibodies in indirect immunofluorescence studies decreased from 1 : 320 to 1 : 40 over 2 months. We performed further immunoblot analyses on five serial serum samples obtained from the patient after her antibasement membrane zone antibodies decreased. All five samples showed similar reaction bands to both 290-kDa protein in dermal extracts and purified laminin-332 α3 protein (145, 165 kDa) (data not shown). Hence it is difficult to speculate the major target antigen in this patient from these results. No local or systemic side-effects of topical corticosteroids were noticed during the entire treatment duration.

EBA and MMP are distinct autoimmune bullous diseases that are both characterized by autoantibodies to dermoepidermal junction components.[1] Detection of autoantibodies to either type VII collagen or laminin-332 differentiates these two diseases.[1] Interestingly, besides antitype VII collagen antibodies, circulating antilaminin-332 α3 antibodies were also found in our patient's serum. According to our survey of the literature, three other previous cases of subepidermal blistering disease with circulating antibodies to both type VII collagen and antilaminin-332 have been reported (Table 1).[6–8] All of the reported cases are of adult onset, thus our report is the first juvenile case. Similar to our patient, these reported patients all presented with mucosal involvement.

Our case is unique in its course and prognosis as well as age at onset. All of the previously reported patients needed systemic corticosteroids or immunosuppressant agents for proper disease control. In the studies by Jonkman *et al.*[6] and Umemoto *et al.*,[7] the bullous lesions of the patients relapsed after systemic prednisolone was tapered. The skin lesions of the patient reported by Baican *et al.*[8] were refractory to systemic prednisolone, azathioprine and dapsone. However, our juvenile case was successfully treated with only topical steroids, and no recurrence was observed in the following 6 months. Our case suggests that the treatment outcome and prognosis of juvenile cases are better than those of adult-onset cases. Further accumulation of similar juvenile cases is needed to confirm this hypothesis. The differ-

Table 1 Comparison of four reported patients with circulating antitype VII collagen and antilaminin-332 antibodies

| Patient | Age (years)/sex | Skin lesion | Mucosal involvement | Treatment | Outcome | Immunoblot analysis | Reference |
|---|---|---|---|---|---|---|---|
| 1 | 64/F | Blisters and erythema on the hands and feet | Oral/genital | Oral prednisolone 80 mg daily | Lesions resolved without scars/milia. Mild relapse occurred when tapering to oral prednisolone 5 mg daily | Type VII collagen, laminin-332 α3 | Jonkman *et al.*[6] |
| 2 | 46/M | Erythematous plaque, blisters, erosions and crusts on the trunk and extensor aspects of extremities | Oral | Oral colchicine 1·5 mg daily (refractory to prednisolone, azathioprine and dapsone) | Previous lesions healed with milia and scars. Free of new blisters but erythematous plaque persisted with erosions and crusts | Type VII collagen, laminin-332 α3, γ2 | Baican *et al.*[8] |
| 3 | 35/F | Vesicular lesions on the face, neck and upper back | Oral/genital | Oral prednisolone 40 mg daily | Lesions resolved without scars/milia. Mild relapse occurred when tapering to oral prednisolone 25 mg daily | Type VII collagen, laminin-332 α3, β3 | Umemoto *et al.*[7] |
| 4 | 12/F | Blisters, erosions and erythema on the face, trunk, hands and feet | Oral | Topical clobetasol propionate ointment 20 g daily | Lesions resolved with scars and milia. No recurrence was found | Type VII collagen, laminin-332 α3 | Our patient |

ences between childhood-onset and adult-onset cases seem to mirror those of EBA at different ages. Compared with adult cases, childhood EBA cases respond relatively better to treatment, and usually low-dose oral prednisolone and dapsone are effective and sufficient.[1]

In conclusion, we report the first juvenile case with autoantibodies to both type VII collagen and laminin-332, successfully treated with only topical steroid therapy. Our case suggests that juvenile cases have different characteristics from those of adult-onset cases in their course, including treatment outcome and prognosis. As topical steroid therapy has several advantages over systemic corticosteroids due to less severe complications, we consider topical steroids as preferable to systemic steroids for childhood-onset autoimmune subepidermal bullous disease.

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Funding sources: none.

Conflicts of interest: none declared.

STUDY

# Clinical Correlations With Dermatomyositis-Specific Autoantibodies in Adult Japanese Patients With Dermatomyositis

## *A Multicenter Cross-sectional Study*

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**Objective:** To clarify the association of clinical and prognostic features with dermatomyositis (DM)-specific autoantibodies (Abs) in adult Japanese patients with DM.

**Design:** Retrospective study.

**Setting:** Kanazawa University Graduate School of Medical Science Department of Dermatology and collaborating medical centers.

**Patients:** A total of 376 consecutive adult Japanese patients with DM who visited our hospital or collaborating medical centers between 2003 and 2008.

**Main Outcome Measures:** Clinical and laboratory characteristics of adult Japanese patients with DM and DM-specific Abs that include Abs against Mi-2, 155/140, and CADM-140.

**Results:** In patients with DM, anti–Mi-2, anti–155/140, and anti–CADM-140 were detected in 9 (2%), 25 (7%), and 43 (11%), respectively. These DM-specific Abs were mutually exclusive and were detected in none of 34 patients with polymyositis, 326 with systemic sclerosis, and 97 with systemic lupus erythematosus. Anti–Mi-2 was associated with classical DM without interstitial lung disease or malignancy, whereas anti–155/140 was associated with malignancy. Patients with anti–CADM-140 frequently had clinically amyopathic DM and rapidly progressive interstitial lung disease. Cumulative survival rates were more favorable in patients with anti–Mi-2 compared with those with anti–155/140 or anti–CADM-140 ($P<.01$ for both comparisons). Nearly all deaths occurred within 1 year after diagnosis in patients with anti–CADM-140.

**Conclusion:** Dermatomyositis-specific Abs define clinically distinct subsets and are useful for predicting clinical outcomes in patients with DM.

*Arch Dermatol. 2011;147(4):391-398*

POLYMYOSITIS (PM) AND DERmatomyositis (DM) represent a group of chronic inflammatory disorders characterized by myogenic changes, skin eruptions, or both. Clinical features are heterogeneous, with various degrees of skin manifestations, myositis, and pulmonary involvement, which considerably determine the severity and prognosis.[1] Although the causes of these disorders remain unclear, autoimmunity is considered to have a critical role because the presence of diagnostic autoantibodies (Abs), known as myositis-related Abs, is a prominent feature.[2] A variety of serum Abs are detected in patients with PM/DM, including Abs reactive with aminoacyltransfer RNA synthetase (ARS),[3] signal recognition particle,[4] and Mi-2.[5] These Abs are associated with clinically distinct subsets of PM/DM, that is, anti-ARS with interstitial lung disease (ILD), arthritis, Raynaud phenomenon, and mechanic hand[6,7]; anti–signal recognition particle with acute-onset severe refractory PM[8-10]; and anti–Mi-2 with typical DM with a lower risk of ILD and internal malignancy and good response to treatment.[11-13] In addition, anti–PM-Scl, anti-Ku, and anti-U1RNP Abs are associated with myositis overlap syndrome.[14] Therefore, identification of myositis-related Abs is use-

*For editorial comment see page 492*

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