15. Gan SQ, McBride OW, Idler WW, Markova N, Steinert PM. Organization, structure, and polymorphisms of the human profilaggrin gene. Biochemistry 1990; 29(40), 9432-9440.

16. Rawlings AV, Harding CR. Moisturization and skin barrier function. Dermatol Ther 2004; 17 Suppl 1, 43-48 .

17. Smith FJ, Irvine AD, Terron-Kwiatkowski A et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006; 38(3), 337-342.

18. Fleckman P, Brumbaugh S. Absence of the granular layer and keratohyalin define a morphologically distinct subset of individuals with ichthyosis vulgaris. Exp Dermatol 2002; 11(4), 327-336.

19. O'Regan GM, Sandilands A, McLean WH, Irvine AD. Filaggrin in atopic dermatitis. J Allergy Clin Immunol 2009; 124(3 Suppl 2), R2-6.

20. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H. Flg mutation p.Lys4021x in the c-terminal imperfect filaggrin repeat in Japanese patients with atopic eczema. Br J Dermatol 2009; 161(6), 1387-1390.

21. Palmer CN, Irvine AD, Terron-Kwiatkowski A et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet 2006; 38(4), 441-446.

22. Sandilands A, O'Regan GM, Liao H et al. Prevalent and rare mutations in the gene encoding filaggrin cause ichthyosis vulgaris and predispose individuals to atopic dermatitis. J Invest Dermatol 2006; 126(8), 1770-1775.

23. Nomura T, Sandilands A, Akiyama M et al. Unique mutations in the filaggrin gene in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Allergy Clin Immunol 2007; 119(2), 434-440.

24. Nomura T, Akiyama M, Sandilands A et al. Specific filaggrin mutations cause ichthyosis vulgaris and are significantly associated with atopic dermatitis in Japan. J Invest Dermatol 2008; 128(6), 1436-1441.

25. Nomura T, Akiyama M, Sandilands A et al. Prevalent and rare mutations in the gene encoding filaggrin in Japanese patients with ichthyosis vulgaris and atopic dermatitis. J Invest Dermatol 2009; 129(5), 1302-1305.

26. Hamada T, Sandilands A, Fukuda S et al. De novo occurrence of the filaggrin mutation p.R501x with prevalent mutation c.3321dela in a Japanese family with ichthyosis vulgaris complicated by atopic dermatitis. J Invest Dermatol 2008; 128(5), 1323-1325.

27. Chen H, Ho Jc, Sandilands A et al. Unique and recurrent mutations in the filaggrin gene in Singaporean Chinese patients with ichthyosis vulgaris. J Invest Dermatol 2008; 128(7), 1669-1675.

28. Hsu CH, Akiyama M, Nemoto-Hasebe I et al. Analysis of Taiwanese ichthyosis vulgaris families further demonstrates differences in FLG mutations between European and Asian populations. Br J Dermatol 2009; 161(2), 448-451.

29. Kang TW, Lee JS, Oh SW, Kim SC. Filaggrin mutation c.3321delA in a Korean patient with ichthyosis vulgaris and atopic dermatitis. Dermatology 2009; 218(2), 186-187.

30. Kay J, Gawkrodger DJ, Mortimer MJ, Jaron AG. The prevalence of childhood atopic eczema in a general population. J Am Acad Dermatol 1994; 30:35-9.

31. Baurecht H, Irvine AD, Novak N et al. Toward a major risk factor for atopic eczema: Meta-analysis of filaggrin polymorphism data. J Allergy Clin Immunol 2007; 120(6), 1406-1412.

32. Aalto-Korte K. Improvement of skin barrier function during treatment of atopic dermatitis. J Am Acad Dermatol 1995; 33(6):969-72.

33. Hata M, Tokura Y, Takigawa M et al. Assessment of epidermal barrier function by photoacoustic spectrometry in relation to its importance in the pathogenesis of atopic dermatitis. Lab Invest. 2002; 82(11), 1451-61.

34. Jakasa I, de Jongh CM, Verberk MM, Bos JD, Kezić S. Percutaneous penetration of sodium lauryl sulphate is increased in uninvolved skin of

patients with atopic dermatitis compared with control subjects. Br J Dermatol 2006; 155(1),104-9.

35. Wells RS, Kerr CB. Genetic classification of ichthyosis. Arch Dermatol 1965; 92(1), 1-6.

36. Kuokkanen K. Ichthyosis vulgaris. A clinical and histopathological study of patients and their close relatives in the autosomal dominant and sex-linked forms of the disease. Acta Derm Venereol Suppl (Stockh) 1969; 62, 1-72.

37. Tay YK, Khoo BP, Goh CL. The epidemiology of atopic dermatitis at a tertiary referral skin center in Singapore. Asian Pac J Allergy Immunol 1999; 17(3), 137-141.

38. Compton JG, Digiovanna JJ, Johnston KA, Fleckman P, Bale SJ. Mapping of the associated phenotype of an absent granular layer in ichthyosis vulgaris to the epidermal differentiation complex on chromosome 1. Exp Dermatol 2002; 11(6), 518-526.

39. Sugiura H, Ebise H, Tazawa T et al. Large-scale DNA microarray analysis of atopic skin lesions shows overexpression of an epidermal differentiation gene cluster in the alternative pathway and lack of protective gene expression in the cornified envelope. Br J Dermatol 2005; 152(1), 146-149.

40. Seguchi T, Cui CY, Kusuda S, Takahashi M, Aisu K, Tezuka T. Decreased expression of filaggrin in atopic skin. Arch Dermatol Res 1996; 288(8), 442-446.

41. Morar N, Cookson WO, Harper JI, Moffatt MF. Filaggrin mutations in children with severe atopic dermatitis. J Invest Dermatol 2007; 127(7), 1667-1672.

42. Marenholz I, Nickel R, Ruschendorf F et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. J Allergy Clin Immunol 2006; 118(4), 866-871.

43. Ruether A, Stoll M, Schwarz T, Schreiber S, Folster-Holst R. Filaggrin loss-of-function variant contributes to atopic dermatitis risk in the population of Northern Germany. Br J Dermatol 2006; 155(5), 1093-1094.

44. Weidinger S, Illig T, Baurecht H et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. J Allergy Clin Immunol 2006; 118(1), 214-219 .

45. Barker JN, Palmer CN, Zhao Y et al. Null mutations in the filaggrin gene (FLG) determine major susceptibility to early-onset atopic dermatitis that persists into adulthood. J Invest Dermatol 2007; 127(3), 564-567.

46. Stemmler S, Parwez Q, Petrasch-Parwez E, Epplen JT, Hoffjan S. Two common loss-of-function mutations within the filaggrin gene predispose

for early onset of atopic dermatitis. J Invest Dermatol 2007; 127(3), 722-724.

47. Weidinger S, Rodriguez E, Stahl C et al. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. J Invest Dermatol 2007; 127(3), 724-726.

48. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol 2003; 112(6 Suppl), S118-127.

49. Rodriguez E, Baurecht H, Herberich E et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: Robust risk factors in atopic disease. J Allergy Clin Immunol 2009; 123(6), 1361-1370 e1367.

50. Osawa R, Konno S, Akiyama M et al. Japanese-Specific Filaggrin Gene Mutations in Japanese Patients Suffering from Atopic Eczema and Asthma. J Invest Dermatol. 2010; 5.

51. Presland RB, Dale BA. Epithelial structural proteins of the skin and oral cavity: function in health and disease. Crit Rev Oral Biol Med 2000; 11,383-408.

52.Ying S, Meng Q, Corrigan CJ, Lee TH. Lack of filaggrin expression in the human bronchial mucosa. J Allergy Clin Immunol 2006; 118, 1386-8

53. Hudson TJ. Skin barrier function and allergic risk. Nat Genet 2006; 38(4), 399-400.

54. Callard RE, Harper JI. The skin barrier, atopic dermatitis and allergy: A role for Langerhans cells? Trends Immunol 2007; 28(7), 294-298.

55. Brown SJ, Relton CL, Liao H et al. Filaggrin null mutations and childhood atopic eczema: a population-based case-control study. J Allergy Clin Immunol. 2008; 121(4):940-46.

56. Weidinger S, O'Sullivan M, Illig T et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol 2008; 121(5):1203-1209.

57. Elias PM, Hatano Y, Williams ML. Basis for the barrier abnormality in atopic dermatitis: Outside-inside-outside pathogenic mechanisms. J Allergy Clin Immunol 2008; 121(6), 1337-1343.

58. Cork MJ, Britton J, Butler L, Young S, Murphy R, Keohane SG. Comparison of parent knowledge, therapy utilization and severity of atopic eczema before and after explanation and demonstration of topical therapies by a specialist dermatology nurse. Br J Dermatol 2003; 149(3), 582-589.

59. Chamlin SL, Kao J, Frieden IJ et al. Ceramide-dominant barrier repair lipids alleviate childhood atopic dermatitis: Changes in barrier function provide a sensitive indicator of disease activity. J Am Acad Dermatol 2002; 47(2), 198-208.

60. Henderson J, Northstone K, Lee SP et al. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. J Allergy Clin Immunol 2008; 121(4), 872-7.

61. Nemoto-Hasebe I, Akiyama M, Nomura T, Sandilands A, McLean WH, Shimizu H. Clinical severity correlates with impaired barrier in filaggrin-related eczema. J Invest Dermatol 2009; 129(3), 682-9.

Journal of the American Academy of Dermatology
Ms. No. JAAD-D-09-00015 2nd Revised Version

CASE LETTER

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

Rinko Osawa, MD, Masashi Akiyama, MD, PhD, Kentaro Izumi, MD, Hideyuki Ujiie, MD, Kaori Sakai, MS, Ikue Nemoto-Hasebe, MD, Teruki Yanagi, MD, Hiroko Koizumi, MD, PhD* and Hiroshi Shimizu, MD, PhD

Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
*Koizumi Dermatology Clinic, Sapporo, Japan

Correspondence to:
Rinko Osawa, M.D.
Department of Dermatology,
Hokkaido University Graduate School of Medicine
N15 W7, Kita-ku, Sapporo 060-8638, Japan
Tel: +81-11-716-1161 ex.5962
Fax: +81-11-706-7820
e-mail: r-osawa@fm2.seikyou.ne.jp

Word count: 583 words in the main text

Key words
bullous congenital ichthyosiform erythroderma, epidermolytic ichthyosis, keratinopathic ichthyosis, KRT1, palmoplantar keratoderma

To the Editor:

Epidermolytic hyperkeratosis (EHK) (OMIM#113800), also termed as bullous congenital ichthyosiform erythroderma, is a rare genetic disorder of keratinization. We report a generalized EHK case showing extremely severe palmoplantar hyperkeratosis with digital contractures.

A 45-year-old Japanese man visiting our hospital reported that he had been born with erythroderma. He had exhibited skin blistering, erosions and hyperkeratosis on the erythrodermic skin since infancy. The blistering and erosion gradually diminished with age. He had developed severe palmoplantar hyperkeratosis and digital contractures at the age of 7 years. At the age of 24 years, surgical operation was performed to improve the contraction of his toes. Physical examination revealed hyperkeratosis on the whole body, especially at the ankles, elbows and knees, and erosions were observed on the inner side of the elbows and knees (Fig. 1a-d). Palmoplantar hyperkeratosis was severe with digital contracture. The morphology of his hair, nails and teeth were normal. There was no known family history of skin disease. Skin biopsy from the left upper arm showed severe granular degeneration in all the suprabasal layers (Fig. 1e). Ultrastructural analysis revealed clumping of the intermediate filaments within keratinocytes of the suprabasal layers (Fig. 1f).

Direct sequencing of the whole coding regions of *KRT1* and *KRT10* (GenBank accession numbers: NT029419.11, 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) by sequence analysis (data not shown).

The present novel *KRT1* mutation p.Leu486Arg is in the 2B segment of keratin 1 (Fig. 2a, 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 position of K1, p.Leu486Pro, was reported in EHK with severe palmoplantar hyperkeratosis (Fig. 2c) and digital contracture, and the affected individuals exhibited clinical features similar to our patient's.[2] Thus, our data further suggest that a non-conservative amino-acid change at codon 486 of K1 results in a severe form of generalized EHK.

The rod domains consist of four alpha-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. 2d).[3-5] The residues at positions ***a,d,e,g*** are considered to be highly sensitive to mutations.[6]

The present patient with generalized EHK had some of the most severe palmoplantar hyperkeratosis of 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 alters the character of amino acid seriously. Thus, it is 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.

26 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. 2c) (Human Intermediate Filament Database, http://www.interfil.org/). Only 9 cases including the present case were diagnosed as generalized EHK with severe palmoplantar hyperkeratosis, and 7 cases out of 9 harboured 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.

Rinko Osawa, MD, Masashi Akiyama, MD, PhD, Kentaro Izumi, MD, Hideyuki Ujiie, MD, Kaori Sakai, MS, Ikue Nemoto-Hasebe, MD, Teruki Yanagi, MD, Hiroko Koizumi, MD, PhD and Hiroshi Shimizu, MD, PhD*

Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan and
**Koizumi Dermatology Clinic, Sapporo, Japan*

Funding source: This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan to M. Akiyama (Kiban 20390304).

Conflicts of interest: None declare.

REFERENCES

1 Tsubota A, Akiyama M, Sakai K, Goto M, Nomura Y, Ando S, et al. Keratin 1 gene mutation detected in epidermal nevus with epidermolytic hyperkeratosis. J Invest Dermatol 2007; 127: 1371-1374.

2 Lee DY, Ahn KS, Lee CH, Rho NK, Lee JH, Lee ES, et al. Two novel mutations in the keratin 1 gene in epidermolytic hyperkeratosis. J Invest Dermatol 2002; 119: 976-7.

3 Müller FB, Küster W, Wodecki K, Almeida H Jr, Bruckner-Tuderman L, Krieg T, et al. Novel and recurrent mutations in keratin KRT5 and KRT14 genes in epidermolysis bullosa simplex: implications for disease phenotype and keratin filament assembly.Hum Mutat 2006; 27: 719-20.

4 Lu Y, Guo C, Liu Q, Zhang X, Cheng L, Li J,et al. A novel mutation of keratin 9 in epidermolytic palmoplantar keratoderma combined with knuckle pads. Am J Med Genet A 2003; 120A: 345-9.

5 Coulombe PA, Fuch E. Elucidating the early stages of keratin filament assembly. J Cell Biol 1990; 111: 153-69.

6 Heald R, McKeon F. Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. Cell 1990; 61:579-89.

FIGURE LEGENDS

Fig. 1. Clinical, histopathological and ultrastructural features of the patient. Severe diffuse hyperkeratosis and scales are seen on the palms (a) and soles (b). Warty brown hyperkeratosis and scales are present on the margins and the dorsa of the foot (c). Generalized erythroderma and scaling on the trunk (d). Histopathological 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 of the epidermis (f).

Fig 2. Summary of mutations in the helix initiation motif (HIM) and helix termination motif (HTM) of K1 from Human Intermediate Filament Database (http://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 out 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 7 of those 9 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 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,g*** are exposed on the surface of the protein. Residues at positions ***e*** and ***g*** stabilize dimer formation through ionic and hydrogen bonds.

	IJD	4771	B	Dispatch: 11.10.10	Journal: IJD	CE: Kannan
	Journal Name	Manuscript No.		Author Received:	No. of pages: 4	PE: Prasanna

doi: 10.1111/j.1365-4632.2010.04771.x

Medical genetics

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

Hideki Nakamura, ???, Ken Natsuga, ???, Wataru Nishie, ???, James R. McMillan, ???, Hiroyuki Nakamura, ???, Daisuke Sawamura, ???, Masashi Akiyama, ???, and Hiroshi Shimizu, ???

From the Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Correspondence
Dr Hideki Nakamura
Department of Dermatology
Hokkaido University Graduate School of Medicine
North 15 West 7, Sapporo
Japan
E-mail: shimizu@med.hokudai.ac.jp

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 intra-epidermal 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. The parents sought PND for a subsequent pregnancy.

PND

Amniocentesis was performed at 16 weeks gestation. Genomic DNA isolated from 1-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 5 min at 94 °C for one cycle, followed by 38 cycles of 45 s at 94 °C, 30 s at 57 °C or 60 °C, and 1 min 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

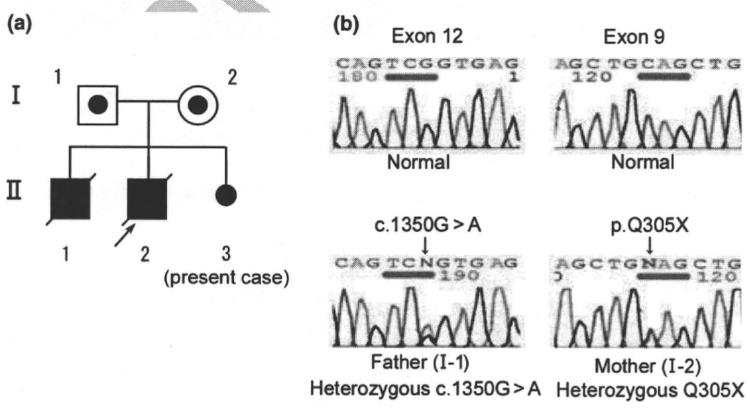

Figure 1 Family tree of the present case, and the causative *PLEC* mutations. (a) The first and second newborns exhibited clinical features typical of EBS-PA, and died shortly after birth. The proband (the second newborn) is indicated by an arrow. (b) The paternal splice-site mutation was a c.1350G>A 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)

COLOR

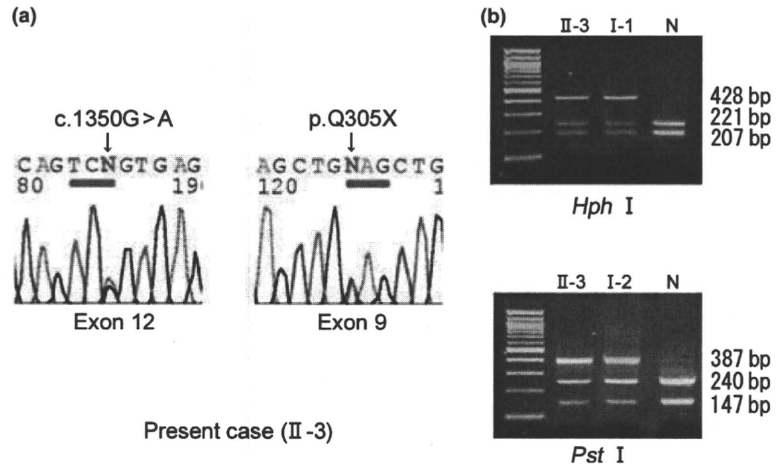

Figure 2 Analysis of the plectin gene mutations in genomic DNA from amniocytes of a fetus at risk. (a) Mutation analysis of genomic DNA from amniocytes shows both the c.1350G>A mutation in exon 12 and p.Q305X mutations in exon 9. (b) The presence of the mutations was verified by restriction enzyme digestion. 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)

COLOR

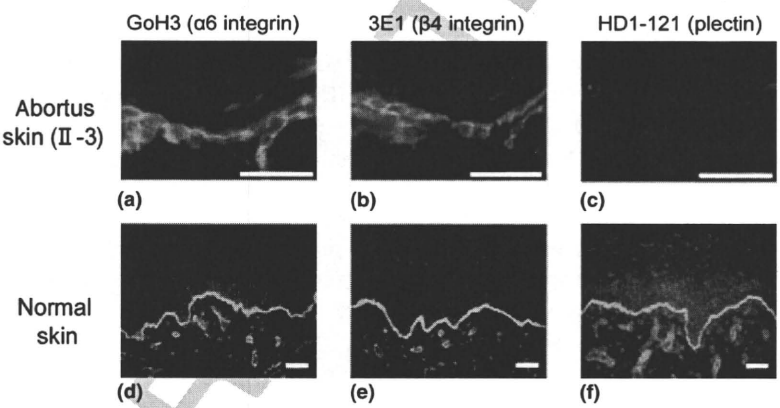

Figure 3 Absence of plectin expression in the abortus. α6 integrin (mAb GoH3) and β4 integrin (mAb 3E1) are expressed in the abortus skin (a, b) and the control skin (d, e). Staining with monoclonal antibody for plectin (mAb HD1–121) shows positive in the control skin (f), but negative in the skin of 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).

References

1 Fine JD, Eady RA, Bauer EA, *et al.* The classification of inherited epidermolysis bullosa (EB): Report of the Third International Consensus Meeting on Diagnosis and Classification of EB. *J Am Acad Dermatol* 2008; 58: 931–950.
2 Shimizu H. Prenatal diagnosis of epidermolysis bullosa. *Prenat Diagn* 2006; 26: 1260–1261.
3 Pfendner EG, Nakano A, Pulkkinen L, *et al.* Prenatal diagnosis for epidermolysis bullosa: a study of 144 consecutive pregnancies at risk. *Prenat Diagn* 2003; 23: 447–456.
4 D'Alessio M, Zambruno G, Charlesworth A, *et al.* Immunofluorescence analysis of villous trophoblasts: a tool for prenatal diagnosis of inherited epidermolysis bullosa with pyloric atresia. *J Invest Dermatol* 2008; 128: 2815–2819.
5 Fassihi H, Renwick PJ, Black C, *et al.* Single cell PCR amplification of microsatellites flanking the COL7A1 gene and suitability for preimplantation genetic diagnosis of Hallopeau-Siemens recessive dystrophic epidermolysis bullosa. *J Dermatol Sci* 2006; 42: 241–248.
6 Fassihi H, Liu L, Renwick PJ, *et al.* Development and successful clinical application of preimplantation genetic haplotyping for Herlitz junctional epidermolysis bullosa. *Br J Dermatol* 2010; ???: ???–???. 4
7 Nakamura H, Sawamura D, Goto M, *et al.* Epidermolysis bullosa simplex associated with pyloric atresia is a novel clinical subtype caused by mutations in the plectin gene (PLEC1). *J Mol Diagn* 2005; 7: 28–35.
8 Pfendner E, Uitto J. Plectin gene mutations can cause epidermolysis bullosa with pyloric atresia. *J Invest Dermatol* 2005; 124: 111–115.
9 Sawamura D, Goto M, Sakai K, *et al.* Possible involvement of exon 31 alternative splicing in phenotype and severity of epidermolysis bullosa caused by mutations in PLEC1. *J Invest Dermatol* 2007; 127: 1537–1540.
10 Rezniczek GA, Walko G, Wiche G. Plectin gene defects lead to various forms of epidermolysis bullosa simplex. *Dermatol Clin* 2010; 28: 33–41.
11 Shimizu H, Takizawa Y, Pulkkinen L, *et al.* Epidermolysis bullosa simplex associated with muscular dystrophy: phenotype-genotype correlations and review of the literature. *J Am Acad Dermatol* 1999; 41: 950–956.
12 Natsuga K, Nishie W, Akiyama M, *et al.* Plectin expression patterns determine two distinct subtypes of epidermolysis bullosa simplex. *Hum Mutat* 2010; 31: 308–316.
13 Pfendner E, Rouan F, Uitto J. Progress in epidermolysis bullosa: the phenotypic spectrum of plectin mutations. *Exp Dermatol* 2005; 14: 241–249.
14 McLean WH, Pulkkinen L, Smith FJ, *et al.* Loss of plectin causes epidermolysis bullosa with muscular dystrophy: cDNA cloning and genomic organization. *Genes Dev* 1996; 10: 1724–1735.
15 Smith FJ, Eady RA, Leigh IM, *et al.* Plectin deficiency results in muscular dystrophy with epidermolysis bullosa. *Nat Genet* 1996; 13: 450–457.
16 Elliott CE, Becker B, Oehler S, *et al.* Plectin transcript diversity: identification and tissue distribution of variants with distinct first coding exons and rodless isoforms. *Genomics* 1997; 42: 115–125.
17 Koster J, van Wilpe S, Kuikman I, *et al.* Role of binding of plectin to the integrin beta4 subunit in the assembly of hemidesmosomes. *Mol Biol Cell* 2004; 15: 1211–1223.

Accepted Date : 19-Sep-2010
Article type : Item of Correspondence

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

H.-Y. Lin, T. Yanagi, M. Akiyama, M.M. Iitani, R. Moriuchi, K. Natsuga, S. Shinkuma, N. Yamane, D. Inokuma, K. Arita, H. Shimizu.

Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Correspondence: Teruki Yanagi, MD, PhD
E-mail: yanagi@med.hokudai.ac.jp

Key words: mucous membrane pemphigoid, epidermolysis bullosa acquisita, childhood, anti-laminin-332 antibody

Funding/support: None.
Conflict of interest disclosures: None.