acylglucosylceramides appear to be preferentially utilized for CLE-bound ceramide production rather than free (CLE-unbound) lipid production in the SC. Exact mechanisms for CLE formation have not been elucidated yet and it remains to be resolved whether preferential utilization of acylglucosylceramide for CLE formation occurs only in the present case or also in other SLS patients. Moreover, it is unknown how decrease in Cer 1, 6, 7 occur and whether barrier lipid abnormality in the patient was a primary event or a secondary phenomenon in the pathogenesis of SLS skin lesions. Cer 1 is essential lipid species to form epidermal permeability barrier formation. Thus, not only accumulation of free fatty acids, but also deficiency of specific ceramide species might contribute to formation of ichthyotic phenotype in SLS.

Acknowledgements

We greatly thank Dr. Sumiko Hamanaka (Lab. for Molecular Membrane Neuroscience, RIKEN Brain Science Institute) and Dr. Ken Hashimoto (Department of Dermatology, Wayne State University School of Medicine) for helpful discussion.

References

[1] Rizzo WB, Carney G. Sjögren-Larsson syndrome: diversity of mutations and polymorphisms in the fatty aldehyde dehydrogenese gene (ALDH3A2). Hum Mutant 2005;26:1–10.
[2] Aoki N, Suzuki H, Ito K, Ito M. A novel point mutation of the *FALDH* gene in a Japanese family with Sjögren-Larsson syndrome. J Invest Dermatol 2000;114:1065–6.
[3] Shitake A, Akiyama M, Shimizu H. Novel ALDH3A2 heterozygous mutations are associated with defective lamellar granule formation in a Japanese family of Sjögren-Larsson syndrome. J Invest Dermatol 2004;123:1197–9.
[4] Sakai K, Akiyama M, Watanabe T, Sanayama K, Sugita K, Takahashi M, et al. Novel ALDH3A2 heterozygous mutations in a Japanese family with Sjögren-Larsson syndrome. J Invest Dermatol 2006;126:2545–7.
[5] Schreiner V, Gooris GS, Pfeiffer S, Lanzendörfer G, Wenck HW, Diembeck W, et al. Barrier characteristics of different human skin types investigated with X-ray diffraction, lipid analysis, and electron microscopy imaging. J Invest Dermatol 2000;114:654–60.
[6] Uhida Y, Cho Y, Moravian S, Kim J, Nakajima K, Crumbing D, et al. Neutral lipid storage leads to acylceramide deficiency, likely cobtributing to the pathogenesis of Dorfman-Chanarin syndrome. J Invest Dermatol 2010;130:2497–9.
[7] Rizzo WB, S'Aulis D, Jennings MA, Crumbing DA, Williams ML, Elias PM. Ichthyoisis in Sjögren-Larsson syndrome reflects defective barrier function due to abnormal lamellar body structure and secretion. Arch Dermatol Res 2010;302:443–51.
[8] Elias PM, Williams ML, Holleran WM, Jiang YJ, Schmuth M. Pathogenesis of permeability barrier abnormalities in the ichthyoses: inherited disorders of lipid metabolism. J Lipid Res 2008;49:694–714.
[9] Rizzo WB, Craft DA, Somer T, Carney G, Trafrova J, Simon M. Abnormal fatty alcohol metabolism in cultured keratinocytes from patients with Sjögren-Larsson syndrome. J Lipid Res 2008;49:410–9.
[10] Paige DG, Morse-Fisher N, Harper JI. Quantification of stratum corneum ceramides and lipid envelop ceramides in the hereditary ichthyoses. Br J Dermatol 1994;131:23–7.

Kimiko Nakajima*
Shigetoshi Sano
Department of Dermatology, Kochi Medical School, Kochi University, Nankoku, Japan

Yoshikazu Uchida
Department of Dermatology, School of Medicine, University of California San Francisco, CA, USA

Masashi Akiyama
Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Yukari Morita
Department of Geriatrics, Cardiology and Neurology, Kochi Medical School, Kochi University, Nankoku, Japan

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

*Corresponding author. Tel.: +81 88 880 2363
E-mail address: nakajimk@kochi-u.ac.jp (K. Nakajima)

2 December 2010

doi:10.1016/j.jdermsci.2011.03.009

Letter to the Editor

BMP-4 down-regulates the expression of Ret in murine melanocyte precursors

Bone morphogenetic proteins (BMPs) have been implicated in a diverse array of biological processes including development and apoptosis [1]. Ret is involved in the physiological mechanisms of melanocyte activation and melanin production [2]. Ret expression in enteric neural precursors is initiated shortly after they emigrate from the neural plate.

We established three distinct cell populations of mouse neural crest (NC) cells, NCCmelb4, NCCmelb4M5 and NCCmelan5. NCCmelb4 cells have the potential to differentiate into mature melanocytes, but since they express melanocyte markers such as tyrosinase-related protein 1, DOPAchrome tautomerase and Kit, we consider them to be immature melanocytes, not multipotent precursors that can differentiate into neurons, as well as glia [3]. NCCmelb4M5 cells belong to the melanocyte lineage, but are less differentiated than NCCmelb4 cells [4]. NCCmelb4M5 cells do not express Kit and grow independently of the Kit ligand; these cells have the potential to differentiate into NCCmelb4 cells, which are Kit-positive melanocyte precursors. NCCmelan5 cells demonstrate the characteristics of differentiated melanocytes. We have also established an oncogene Ret-transgenic mouse line, line 304/B6, in which skin melanosis, benign melanocytic tumors and malignant melanomas develop in a stepwise fashion [2]. A malignant melanoma cell line, Mel-Ret, was established from the Ret-transgenic mouse. We found that all four cell lines express BMP receptors using Western blotting analysis (data not shown).

Western blotting revealed expression of the Ret protein in NCCmelb4M5 and in Mel-Ret cells, but in contrast, there was no expression of the Ret protein in NCCmelb4 or NCCmelan5 cells (Fig. 1A). Immunostaining also revealed that NCCmelb4M5 (Fig. 1B) and Mel-Ret cells are positive for Ret, but NCCmelb4 and NCCmelan5 cells are negative for Ret. Thus, Ret protein is expressed in most immature melanoblasts, while melanocytes are negative for Ret. We then analyzed Ret protein expression in BMP-4-treated NCCmelb4M5 cells by Western blotting (Fig. 1C–F). BMP-4 was added to the medium and incubated for 3 days at varying concentrations. After incubation with 10 ng/ml BMP-4 for 3 days, Ret protein expression was decreased, and disappeared completely

REVIEW

Dermato-Endocrinology 3:2, 107-112; April/May/June 2011; © 2011 Landes Bioscience

The roles of ABCA12 in keratinocyte differentiation and lipid barrier formation in the epidermis

Masashi Akiyama

Department of Dermatology; Nagoya University Graduate School of Medicine; Nagoya, Japan

Key words: ABCA12, congenital ichthyosiform erythroderma, harlequin ichthyosis, lamellar granules, lamellar ichthyosis

Abbreviations: ABC, ATP-binding cassette; ABCA12, ATP-binding cassette transporter sub-family A member 12; CIE, congenital ichthyosiform erythroderma; HDL, high-density lipoprotein; HI, harlequin ichthyosis; LG, lamellar granule; LI, lamellar ichthyosis; PPAR, peroxisome proliferator-activated receptor

ABCA12 is a member of the large superfamily of ATP-binding cassette (ABC) transporters, which bind and hydrolyze ATP to transport various molecules across limiting membranes or into vesicles. The ABCA subfamily members are thought to be lipid transporters. ABCA12 is a keratinocyte transmembrane lipid transporter protein associated with the transport of lipids in lamellar granules to the apical surface of granular layer keratinocytes. Extracellular lipids, including ceramide, are thought to be essential for skin barrier function. *ABCA12* mutations are known to underlie the three main types of autosomal recessive congenital ichthyoses: harlequin ichthyosis, lamellar ichthyosis and congenital ichthyosiform erythroderma. *ABCA12* mutations lead to defective lipid transport via lamellar granules in the keratinocytes, resulting in malformation of the epidermal lipid barrier and ichthyosis phenotypes. Studies of ABCA12-deficient model mice indicate that lipid transport by ABCA12 is also indispensable for intact differentiation of keratinocytes.

Introduction

ABCA12 is a member of the large superfamily of ATP-binding cassette (ABC) transporters,[1] which bind and hydrolyze ATP to transport various molecules across limiting membranes or into vesicles.[2] The ABCA subfamily members are thought to be lipid transporters.[3] The ABC transporter A12 (ABCA12) is known to be a key molecule in keratinocyte lipid transport (**Fig. 1**).[4-6] ABCA12 is a keratinocyte transmembrane lipid transporter protein associated with the transport of lipids in lamellar granules to the apical surface of granular layer keratinocytes.[4] This article reviews the importance of *ABCA12* as a keratinocyte lipid transporter in the context of keratinocyte differentiation and skin lipid barrier formation.

Correspondence to: Masashi Akiyama; Email: makiyama@med.nagoya-u.ac.jp
Submitted: 12/08/10; Revised: 01/26/11; Accepted: 02/14/11
DOI: 10.4161/derm.3.2.15136

ABCA12 and Other ABCA Transporters

Several genetic diseases have been shown to be caused by mutations in ABCA subfamily genes. The ABCA subfamily, of which the *ABCA12* gene is a member, comprises 12 full transporters and one pseudogene (ABCA11) that are essential for lipid transport and secretion.[7] Three ABCA genes of the same subfamily as ABCA12 have been also implicated in the development of genetic diseases affecting cellular lipid transport. In the phylogenetic tree of ABCA subfamily proteins, ABCA3 is very close ABC12.[1] ABCA3 is known to aid lipid secretion from alveolar type II cells via lamellar granules,[8] and an ABCA3 deficiency recently was reported to underlie a fatal lung surfactant deficiency in newborns,[9] a condition that often leads to death shortly after birth.

Another important member of the ABCA subfamily is ABCA1. Mutations in the human ABCA1 gene underlie familial alpha-lipoprotein deficiency syndrome (Tangier disease), which suggests that ABCA1 is a major regulator of high-density lipoprotein metabolism.[10-12]

ABCA2, ABCA3 and ABCA7 mRNA levels were reported to be upregulated after sustained cholesterol influx,[13,14] suggesting that ABCA transporters are involved in the transmembrane transport of endogenous lipids.[15] From these facts, transporters in the ABCA subfamily are thought to be involved in the transmembrane transport of cholesterol.[16-18] Interestingly, ABCA3, a member of the same protein superfamily as ABCA12, functions in pulmonary surfactant lipid secretion through the production of similar lamellar-type granules within lung alveolar type II cells.[8,9]

The Role of ABCA12 in the Transport of Lipids into Lamellar Granules

Extracellular lipids, including ceramide, are thought to be essential for skin barrier function.[19] Mutations in the ABCA12 gene (*ABCA12*) were reported to underlie the devastating phenotype seen in harlequin ichthyosis (HI) patients,[4,20] the most severe keratinization disorder. ABCA12 mutations underlying HI are thought to have major disruptive defects on ABCA12 lipid

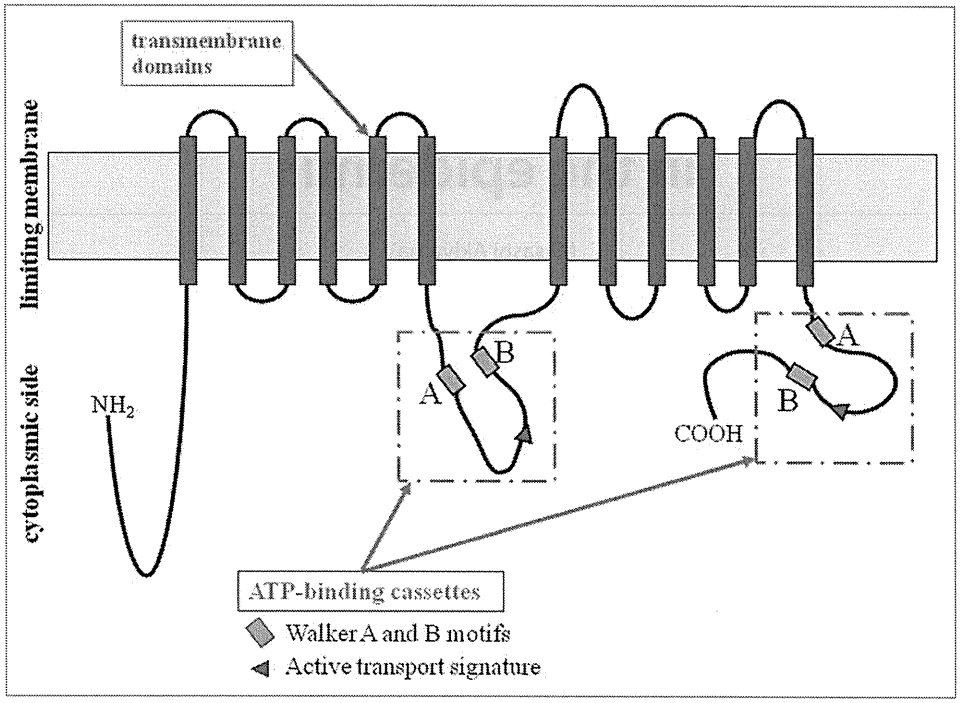

Figure 1. ABCA12 protein structure and domains. Analysis of the predicted structure of the ABCA12 protein reveals features typical of ABCA transporters.[1]

transporter function, resulting in the HI phenotype.[4] We reported that ABCA12 is localized in lamellar granules (LGs) in the granular layer keratinocytes and might work in lipid transport through LGs to form the intercellular lipid layers in the stratum corneum.[4] We have analyzed the epidermal localization of ABCA12 in comparison with the localization of Golgi apparatus markers and LG-associated proteins together with transglutaminase 1, because LGs are thought to be a part of the continuous tubular network that originates from the Golgi apparatus and extends to the cell membrane.[5] We employed antibodies to well-established marker molecules of each part of the Golgi apparatus-LG-cell membrane network, i.e., the GM130, anti-TGN-46 and anti-transglutaminase 1 antibodies (B.C1), as markers for cis-Golgi, trans-Golgi and cell membrane, respectively. Our results show that ABCA12 localizes throughout the entire Golgi apparatus to LGs at the cell periphery, mainly in the granular layer keratinocytes. These results suggest that ABCA12 works in the transport of lipids from the Golgi apparatus to LGs in the granular layer cells.[5] Double-labeling immunofluorescence staining of cultured keratinocytes clearly indicates that ABCA12 is localized from the Golgi apparatus (colocalized with cis-Golgi marker GM130 and trans-Golgi marker TGN-46) to the cell periphery (close to the plasma membrane stained with transglutaminase 1). ABCA12 fails to colocalize with TGase1, a cell membrane-bounding protein, both in vivo and in cultured keratinocytes and ABCA12 is thought to distribute only very sparsely on the cell membrane.[5]

In normal human epidermis, ABCA12 is expressed throughout, but mainly in the upper spinous and granular layers.[5] Immunofluorecent double labeling reveals that the majority of ABCA12 colocalizes with glucosylceramide in the cytoplasm within the upper spinous and granular cells (Fig. 2).[5] Immunofluorescence labeling on ultrathin cryosections clearly reveals localization of ABCA12 and glucosylceramide. In immunofluorescence labeling under light microscopy, ABCA12 and glucosylceramide staining almost completely overlap within the granular layer keratinocytes.[5] Post-embedding immunoelectron microscopy reveals both ABCA12 and glucosylceramide in the LGs of the uppermost granular layer keratinocytes.[5] Under immunoelectron microscopy using ultrathin cryosections, glucosylceramide labeling is seen with the lamellar structures in the LGs. ABCA12 immunogold labeling is observed on or close to the membrane surrounding LGs in the uppermost granular layer cells.[5]

We can hypothesize that ABCA12 is likely to be a membrane lipid transporter that functions in the transport of lipids from the trans-Golgi network to LGs at the keratinocyte periphery (Fig. 3).[4,5] Recently, it was confirmed biochemically that ABCA12 deficiency impairs glucosylceramide accumulation in lamellar granules and that ABCA12 transports glucosylceramide to the inner side of lamellar granules.[6] In addition, ceramide was reported to upregulate ABCA12 expression via PPAR delta-mediated signaling pathway, providing a substrate-driven, feed-forward mechanism for regulation of this key lipid transporter.[21] More recently, studies using *Abca12*$^{-/-}$ mice suggested that ABCA12 plays an important role in the normal differentiation of epidermal keratinocytes.[22]

ABCA12 Mutations and Ichthyoses

ABCA12 mutations are known to underlie the three main types of autosomal recessive congenital ichthyoses: harlequin ichthyosis

(HI), lamellar ichthyosis (LI) and congenital ichthyosiform erythroderma (CIE). Harlequin ichthyosis is the most severe ichthyosis subtype. Affected patients show plate-like scales over the whole body, severe eclabium and ectropion.

In 2010, a review of the literature was performed to identify all known *ABCA12* mutations in patients with ARCI and 56 *ABCA12* mutations were described (online database: www.derm-hokudai.jp/ABCA12/) in 66 unrelated families, including 48 HI, 10 LI and 8 CIE families.[23] Mutations have been reported among autosomal recessive congenital ichthyosis patients with African, European, Pakistani/Indian and Japanese backgrounds in most parts of the world. Of the 56 mutations, 36% (20) are nonsense, 25% (14) are missense, 20% (11) comprise small deletions, 11% (6) are splice site, 5% (3) are large deletions and 4% (2) are insertion mutations. At least 62.5% (35) of all the reported mutations are predicted to result in truncated proteins. There is no apparent mutation hot spot in *ABCA12*, although mutations underlying the LI phenotype are clustered in the region of the first ATP-binding cassette.[24]

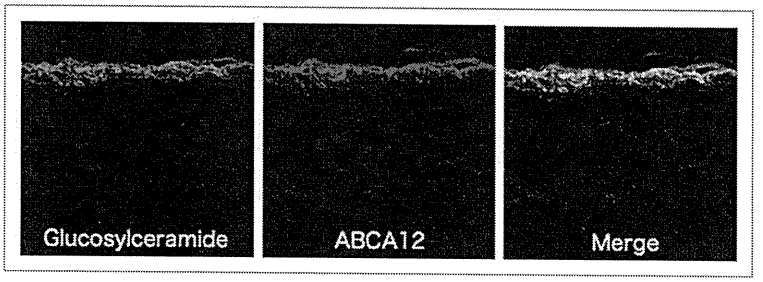

Figure 2. Immunofluorescence labeling using ultrathin cryosections as substrates reveal that glucosylceramide (green) and ABCA12 (red) overlap in the granular layers (derived from ref. 5).

In HI-affected epidermis, several morphologic abnormalities have been reported, including abnormal lamellar granules in the keratinocyte granular layer and a lack of extracellular lipid lamellae within the stratum corneum.[25-28] Lack of ABCA12 function subsequently leads to disruption of lamellar granule lipid transport in the upper keratinizing epidermal cells, resulting in malformation of the intercellular lipid layers of the stratum corneum in HI.[4] Cultured epidermal keratinocytes from an HI patient carrying *ABCA12* mutations demonstrate defective glucosylceramide transport, and this phenotype is recoverable by in vitro *ABCA12* corrective gene transfer.[4] Intracytoplasmic glucosylceramide transport has been studied using cultured keratinocytes from a total of three patients harboring *ABCA12* mutations. One patient was homozygous for the splice site mutation c.3295-2A>G[4] and another was compound heterozygous for p.Ser387Asn and p.Thr1387del.[29] Only one heterozygous mutation, p.Ile1494Thr, was identified in the other patient.[30] Cultured keratinocytes from all three patients showed apparently disturbed glucosylceramide transport, although this assay is not quantitative.

In addition, defective lamellar granule formation was observed in the skin of two CIE patients with *ABCA12* mutations.[30] Electron microscopy revealed that, in the cytoplasm of granular layer keratinocytes, abnormal, defective lamellar granules are assembled with some normal-appearing lamellar granules.[30]

Formation of the intercellular lipid layers is essential for epidermal barrier function. In ichthyotic skin with ABCA12 deficiency, defective formation of the lipid layers is thought to result in a serious loss of barrier function and a likely extensive compensatory hyperkeratosis.[31]

One hypothetical pathomechanism for ABCA12 deficiency in autosomal recessive congenital ichthyosis is explained by the "differentiation defect theory," which is derived from the clinical features of HI patients. Fetuses affected with HI start developing the ichthyotic phenotype while they are in the amniotic fluid, where stratum corneum barrier function is not required. According to this theory, barrier defects cannot be involved directly in the pathogenesis of the HI phenotype, at least during the in utero fetal period. In light of this, disturbed keratinocyte differentiation is speculated to play an important role in the pathogenesis of the HI phenotype. In fact, three-dimensional culture studies reveal that HI keratinocytes differentiate poorly according to morphologic criteria and show reduced expression of keratin 1 and defective conversion from profilaggrin to filaggrin.[32]

In an *ABCA12*-ablated organotypic co-culture system, which is an in vitro model of HI skin, the expression of keratinocyte late differentiation-specific molecules is dysregulated.[33] The expression of specific proteases associated with desquamation (kallikrein 5 and cathepsin D) is dramatically reduced in the ABCA12-ablated organotypic co-culture system.[33] In this model system, *ABCA12* ablation results in a premature terminal differentiation phenotype.[33] Furthermore, in mutant mice carrying a homozygous spontaneous missense mutation, loss of Abca12 function leads to the premature differentiation of basal keratinocytes.[34] In contrast, in our *Abca12*$^{-/-}$ HI model mice, immunofluorescence and immunoblotting of *Abca12*$^{-/-}$ neonatal epidermis revealed defective profilaggrin/filaggrin conversion and reduced expression of the differentiation-specific molecules (loricrin, kallikrein 5 and transglutaminase 1), although their mRNA expression is upregulated.[22] These data suggest that ABCA12 deficiency may lead to disturbances in keratinocyte differentiation during fetal development, resulting in an ichthyotic phenotype at birth. These observations suggest that ABCA12 deficiency might have global effects on keratinocyte differentiation, resulting in both impaired terminal differentiation and premature differentiation of the epidermis.

HI patients often die in the first week or two of life. However, those that survive beyond the neonatal period phenotypically improve within several weeks after birth. To clarify the mechanisms of phenotypic recovery, we studied grafted skin and keratinocytes from *Abca12*-disrupted (*Abca12*$^{-/-}$) mice.[22] *Abca12*$^{-/-}$ skin grafts kept in a dry environment exhibited dramatic improvements in all the abnormalities seen in the model mice. Increased transepidermal water loss, a parameter of barrier defect, is remarkably decreased in grafted *Abca12*$^{-/-}$ skin. Ten-passage sub-cultured *Abca12*$^{-/-}$ keratinocytes show restoration of intact ceramide distribution, differentiation-specific protein

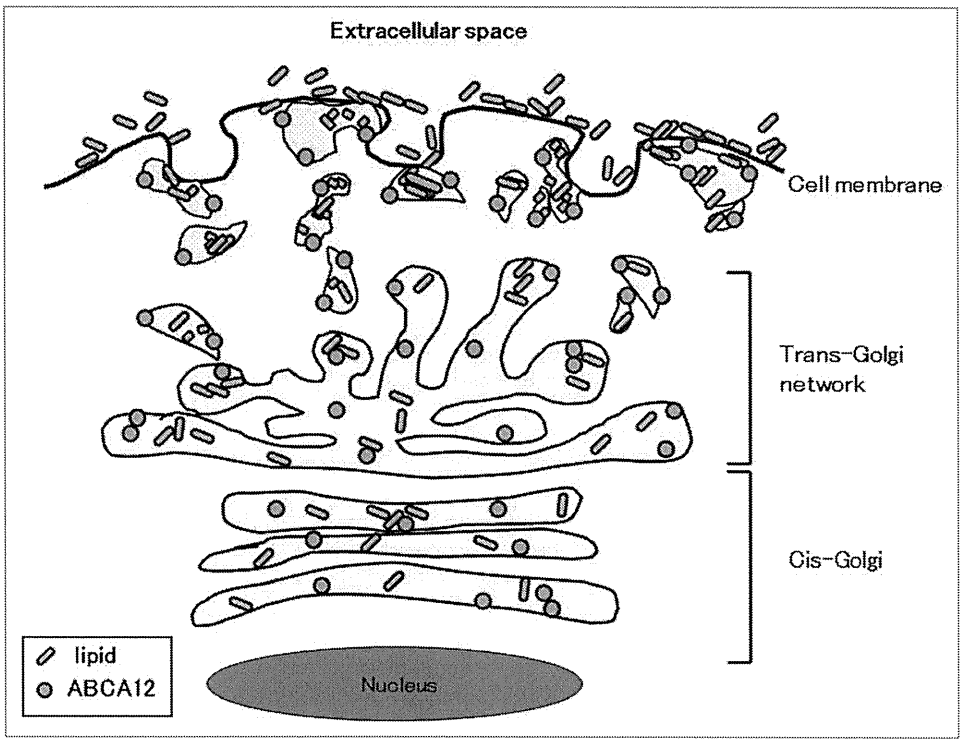

Figure 3. Scheme of ABCA12 distribution from the cis-Golgi, trans-Golgi network to lamellar granules in the upper spinous and granular layer keratinocytes (derived from ref. 5).

expression and profilaggrin/filaggrin conversion, which are defective in the primary-culture.[22] These observations suggest that, during maturation, *Abca12*$^{-/-}$ epidermal keratinocytes regain normal differentiation processes, although the exact mechanisms of this restoration remain unknown.[22]

ABCA12-deficient Animal Models

Recently, bioengineered disease models were established to investigate the ichthyotic pathomechanisms that result from defective ABCA12 function and to aid the development of innovative treatments for ichthyosis with ABCA12 deficiency.

We transplanted cultured keratinocytes from patients with HI and succeeded in reproducing HI skin lesions in immunodeficient mice.[35] These reconstituted HI lesions show similar changes to those observed in HI patients' skin. In addition, we generated *Abca12*-disrupted (*Abca12*$^{-/-}$) mice that closely reproduced the human HI phenotype, showing marked hyperkeratosis with eclabium and skin fissures.[36] Lamellar granule abnormalities and defective ceramide distribution were remarkable in the epidermis. Skin permeability assays of *Abca12*$^{-/-}$ mouse fetuses revealed severe skin barrier dysfunction after the initiation of keratinization. Surprisingly, the *Abca12*$^{-/-}$ mice also demonstrated lung alveolar collapse immediately after birth. Lamellar bodies in alveolar type II cells from *Abca12*$^{-/-}$ mice lack normal lamellar structures.[36] The level of surfactant protein B, an essential component of alveolar surfactant, is reduced in the *Abca12*$^{-/-}$ mice.[36] Another group independently developed *Abca12*$^{-/-}$ mice and these also had the clinical features of HI.[37]

A study in one *Abca12*-disrupted HI model mouse indicates that a lack of desquamation of skin cells, rather than enhanced proliferation of basal-layer keratinocytes accounts for the five-fold thickening of the *Abca12*$^{-/-}$ stratum corneum determined by in vivo skin proliferation measurements.[37] It was suggested that this lack of desquamation is associated with a profound reduction in skin linoleic esters of long-chain omega-hydroxyceramides and a corresponding increase in their glucosylceramide precursors. Omega-hydroxyceramides are required for correct skin barrier function, and these results from HI model mice establish that ABCA12 activity is required for the generation of the long-chain ceramide esters that are essential for the development of normal skin structure and function.[37]

In addition, a mouse strain carrying a homozygous spontaneous missense mutation was reported to show skin manifestations similar to ichthyosis.[34] Lipid analysis of *Abca12* mutant epidermis revealed defects in lipid homeostasis, suggesting that *Abca12* plays a crucial role in maintaining lipid balance in the skin.[34] The cells from the *Abca12* mutant mouse have severely impaired lipid efflux and intracellular accumulation of neutral lipids.[34] Abca12 was also demonstrated as a mediator of Abca1-regulated cellular cholesterol efflux.[34] Injection of a morpholino designed to target a splice site at the exon 4/intron 4 junction to block *Abca12* pre-mRNA processing induced altered skin surface contours, disorganization of the melanophore distribution, pericardial edema

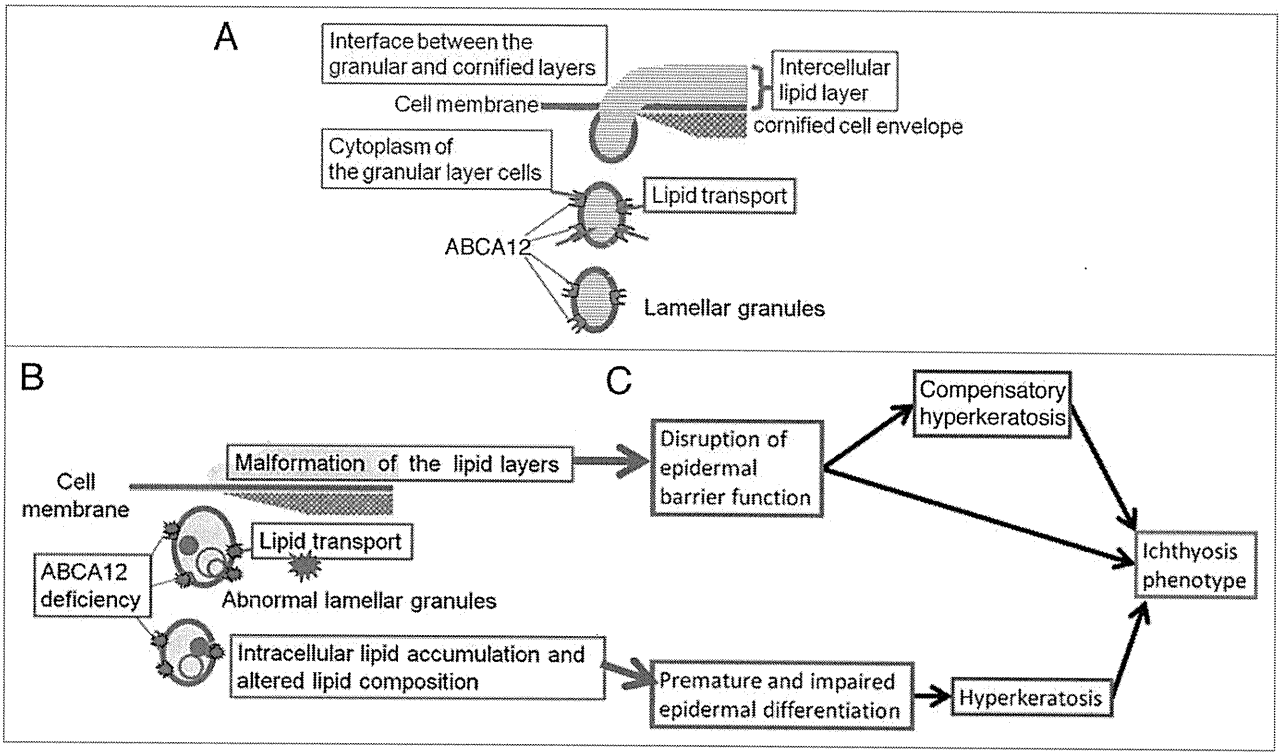

Figure 4. Physiological role(s) of ABCA12 in lipid trafficking of epidermal keratinocytes and the model of pathogenetic mechanisms in ichthyosis phenotypes caused by ABCA12 deficiency. (A) Model of how ABCA12 transports lipids in epidermal keratinocytes. (B) Model of how loss of ABCA12 function leads to lipid abnormality and lipid barrier malformation in the upper epidermis. (C) It is hypothesized that the combination of lipid barrier defects and disturbed keratinocyte differentiation cause hyperkeratosis and the ichthyosis phenotype (derived from ref. 20).

and enlargement of the yolk sac at 3 days post-fertilization in the larvae of zebrafish. It was also associated with premature death at around 6 days post-fertilization. These results suggest that *Abca12* is an essential gene for normal zebrafish skin development and provide novel insight into the function of ABCA12 (reported at the Annual Meeting of the Society for Investigative Dermatology 2010; Abstract, Frank et al. J Invest Dermatol 2010; 130:86).

Using our Abca12$^{-/-}$ HI model mice, we tried fetal therapy with systemic administration to the pregnant mother mice of retinoid or dexamethasone, which are effective treatments for neonatal HI and neonatal respiratory distress, respectively. However, neither of these improved the skin phenotype nor extended the survival period.[36] Retinoids were also ineffective in in vivo studies using cultured keratinocytes from the model mice.[22]

Conclusion

ABCA12 is apparently localized in the membrane of the trans-Golgi network and lamellar granules in the upper epidermis, mainly in the uppermost spinous and granular layer cells. Our own studies and a review of the literature suggest that ABCA12 works in the transport of lipids into the trans-Golgi network and lamellar granules, to accumulate lipids that are essential to skin barrier formation. Consequently, the lipids packed in lamellar granules are secreted to the extracellular space to form intercellular lipid layers in the stratum cornuem, which is important for skin barrier function (Fig. 4). In addition, model mouse studies indicate that lipid transport by ABCA12 is indispensable for intact differentiation of keratinocytes. To elucidate the mechanisms of ABCA12 in keratinocyte differentiation/proliferation, further accumulation of data is needed.

Acknowledgements

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 B 20390304) and by a grant from the Ministry of Health, Labor and Welfare of Japan (Health and Labor Sciences Research Grants; Research on Intractable Disease; H22-Nanchi-Ippan-177) to M. Akiyama. The *ABCA12* mutation database is available at our site: www.derm-hokudai.jp/ABCA12/.

References

1. Annilo T, Shulenin S, Chen ZQ, Arnould I, Prades C, Lemoine C, et al. Identification and characterization of a novel ABCA subfamily member, ABCA12, located in the lamellar ichthyosis region on 2q34. Cytogenet Genome Res 2002; 98:169-76.
2. Borst P, Elferink RO. Mammalian ABC transporters in health and disease. Annu Rev Biochem 2002; 71:537-92.
3. Peelman F, Labeur C, Vanloo B, Roosbeek S, Devaud C, Duverger N, et al. Characterization of the ABCA transporter subfamily: identification of prokaryotic and eukaryotic members, phylogeny and topology. J Mol Biol 2003; 325:259-74.
4. Akiyama M, Sugiyama-Nakagiri Y, Sakai K, McMillan JR, Goto M, Arita K, et al. Mutations in ABCA12 in harlequin ichthyosis and functional rescue by corrective gene transfer. J Clin Invest 2005; 115:1777-84.
5. Sakai K, Akiyama M, Sugiyama-Nakagiri Y, McMillan JR, Sawamura D, Shimizu H. Localization of ABCA12 from Golgi apparatus to lamellar granules in human upper epidermal keratinocytes. Exp Dermatol 2007; 16:920-6.
6. Mitsutake S, Suzuki C, Akiyama M, Tsuji K, Yanagi T, Shimizu H, et al. ABCA12 dysfunction causes a disorder in glucosylceramide accumulation during keratinocyte differentiation. J Dermatol Sci 2010; 60:128-9.
7. Akiyama M. Pathomechanisms of harlequin ichthyosis and ABCA transporters in human diseases. Arch Dermatol 2006; 142:914-8.
8. Yamano G, Funahashi H, Kawanami O, Zhao LX, Ban N, Uchida Y, et al. ABCA3 is a lamellar body membrane protein in human lung alveolar type II cells. FEBS Lett 2001; 508:221-5.
9. Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M. ABCA3 gene mutations in newborns with fatal surfactant deficiency. N Engl J Med 2004; 350:1296-303.
10. Bodzioch M, Orsó E, Klucken J, Langmann T, Böttcher A, Diederich W, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 1999; 22:316-8.
11. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 1999; 22:336-45.
12. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 1999; 22:352-5.
13. Klucken J, Buchler C, Orso E, et al. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. Proc Natl Acad Sci USA 2000; 97:817-22.
14. Kaminski WE, Piehler A, Püllmann K, Porsch-Ozcürümez M, Duong C, Bared GM, et al. Complete coding sequence, promoter region and genomic structure of the human ABCA2 gene and evidence for sterol-dependent regulation in macrophages. Biochem Biophys Res Commun 2001; 281:249-58.
15. Schmitz G, Langmann T. Structure, function and regulation of the ABC1 gene product. Curr Opin Lipidol 2001; 12:129-40.
16. Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. Cell 1999; 98:13-23.
17. Hayden MR, Clee SM, Brooks-Wilson A, Genest J Jr, Attie A, Kastelein JJ. Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. Curr Opin Lipidol 2000; 11:117-22.
18. Orsó E, Broccardo C, Kaminski WE, Böttcher A, Liebisch G, Drobnik W, et al. Transport of lipids from Golgi to plasma membrane is defective in Tangier disease patients and Abc1-deficient mice. Nat Genet 2000; 24:192-6.
19. Lee HK, Nam GW, Kim SH, Lee SH. Phytocomponents of triterpenoids, oleanolic acid and ursolic acid, regulated differently the processing of epidermal keratinocytes via PPAR-alpha pathway. Exp Dermatol 2006; 15:66-73.
20. Kelsell DP, Norgett EE, Unsworth H, Teh MT, Cullup T, Mein CA, et al. Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis. Am J Hum Genet 2005; 76:794-803.
21. Jiang YJ, Uchida Y, Lu B, Kim P, Mao C, Akiyama M, et al. Ceramide stimulates ABCA12 expression via peroxisome proliferator-associated receptor delta in human keratinocytes. J Biol Chem 2009; 284:18942-52.
22. Yanagi T, Akiyama M, Nishihara H, Ishikawa J, Sakai K, Miyamura Y, et al. Self-improvement of keratinocyte differentiation defects during skin maturation in ABCA12 deficient harlequin ichthyosis model mice. Am J Pathol 2010; 177:106-18.
23. Akiyama M. *ABCA12* mutations and autosomal recessive congenital ichthyosis: A review of genotype/phenotype correlations and of pathogenic concepts. Hum Mutat 2010; 31:1090-6.
24. Lefèvre C, Audebert S, Jobard F, Bouadjar B, Lakhdar H, Boughdene-Stambouli O, et al. Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. Hum Mol Genet 2003; 12:2369-78.
25. Dale BA, Holbrook KA, Fleckman P, Kimball JR, Brumbaugh S, Sybert VP. Heterogeneity in harlequin ichthyosis, an inborn error of epidermal keratinization: variable morphology and structural protein expression and a defect in lamellar granules. J Invest Dermatol 1990; 94:6-18.
26. Milner ME, O'Guin WM, Holbrook KA, Dale BA. Abnormal lamellar granules in harlequin ichthyosis. J Invest Dermatol 1992; 99:824-9.
27. Akiyama M, Kim DK, Main DM, Otto CE, Holbrook KA. Characteristic morphologic abnormality of harlequin ichthyosis detected in amniotic fluid cells. J Invest Dermatol 1994; 102:210-3.
28. Akiyama M, Dale BA, Smith LT, Shimizu H, Holbrook KA. Regional difference in expression of characteristic abnormality of harlequin ichthyosis in affected fetuses. Prenat Diagn 1998; 18:425-36.
29. Akiyama M, Sakai K, Sugiyama-Nakagiri Y, Yamanaka Y, McMillan JR, Sawamura D, et al. Compound heterozygous mutations including a de novo missense mutation in ABCA12 led to a case of harlequin ichthyosis with moderate clinical severity. J Invest Dermatol 2006; 126:1518-23.
30. Natsuga K, Akiyama M, Kato N, Sakai K, Sugiyama-Nakagiri Y, Nishimura M, et al. Novel ABCA12 mutations identified in two cases of non-bullous congenital ichthyosiform erythroderma associated with multiple skin malignant neoplasia. J Invest Dermatol 2007; 127:2669-73.
31. Akiyama M. Harlequin ichthyosis and other autosomal recessive congenital ichthyoses: the underlying genetic defects and pathomechanisms. J Dermatol Sci 2006; 42:83-9.
32. Fleckman P, Hager B, Dale BA. Harlequin ichthyosis keratinocytes in lifted culture differentiate poorly by morphologic and biochemical criteria. J Invest Dermatol 1997; 109:36-8.
33. Thomas AC, Tattersall D, Norgett EE, O'Toole EA, Kelsell DP. Premature terminal differentiation and a reduction in specific proteases associated with loss of ABCA12 in harlequin ichthyosis. Am J Pathol 2009; 174:970-8.
34. Smyth I, Hacking DF, Hilton AA, Mukhamedova N, Meikle PJ, Ellis S, et al. A mouse model of harlequin ichthyosis delineates a key role for Abca12 in lipid homeostasis. PLoS Genet 2008; 4:1000192.
35. Yamanaka Y, Akiyama M, Sugiyama-Nakagiri Y, Sakai K, Goto M, McMillan JR, et al. Expression of the keratinocyte lipid transporter ABCA12 in developing and reconstituted human epidermis. Am J Pathol 2007; 171:43-52.
36. Yanagi T, Akiyama M, Nishihara H, Sakai K, Nishie W, Tanaka S, et al. Harlequin ichthyosis model mouse reveals alveolar collapse and severe fetal skin barrier defects. Hum Mol Genet 2008; 17:3075-83.
37. Zuo Y, Zhuang DZ, Han R, Isaac G, Tobin JJ, McKee M, et al. ABCA12 maintains the epidermal lipid permeability barrier by facilitating formation of ceramide linoleic esters. J Biol Chem 2008; 283:36624-35.

Hair Shaft Abnormalities in Localized Autosomal Recessive Hypotrichosis 2 and A Review of Other Non-syndromic Human Alopecias

Hiraku Suga[1], Yuichiro Tsunemi[1], Makoto Sugaya[1], Satoru Shinkuma[2], Masashi Akiyama[2], Hiroshi Shimizu[2] and Shinichi Sato[1]
Departments of Dermatology, [1]Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, and [2]Hokkaido University Graduate School of Medicine, Sapporo, Japan. E-mail: hiraku_s2002@yahoo.co.jp
Accepted December 16, 2010.

Localized autosomal recessive hypotrichosis (LAH) 2 is a type of non-syndromic human alopecia that is inherited as an autosomal recessive trait. We describe here a patient with LAH2 who had mutations in the *lipase H* (*LIPH*) gene. We analysed hair shaft morphology using light and scanning electron microscopy (SEM). In addition, we review the features of other non-syndromic human alopecias.

CASE REPORT

The patient was a 4-year-old boy, the firstborn of healthy and unrelated Japanese parents, born after an uneventful pregnancy. He had scant hair at birth, which grew very slowly in infancy.

Clinical examination revealed hypotrichosis of the scalp (Fig. 1a). The hairs were sparse, thin, and curly, and not easily plucked. The left eyebrow hair was sparse, but the eyelashes and other body hair were present in normal amounts. Teeth, nails, and the ability to sweat were completely normal. Clinical features of keratosis pilaris, milia, scarring, and palmoplantar keratoderma were absent. Psychomotor development was normal. The patient's younger brother also had severe hypotrichosis; since birth his hair was curly, and his eyebrow hair virtually absent (Fig. 1b). No other family members, including his parents, had similar hair abnormalities. Laboratory tests of the patient showed normal serum levels of copper and zinc, and liver and kidney function tests were all within normal ranges. Over a period of 2 years there was no improvement or exacerbation of hypotrichosis in the patient.

Light microscopy of the patient's scalp hairs revealed that approximately 10% had structural abnormalities. Abnormal hairs were composed of thick dark parts and thin light parts (Fig. 2a). SEM revealed alterations of the cuticular architecture. Cuticular cells were absent from both the thick and thin parts (Fig. 2b). Cross-sectional observation showed that thick, but not thin, sections had hair medulla (Fig. 2c, d). Light microscopy on hairs from the patient's younger brother revealed that they were composed of thin and thick parts (data not shown).

Based on the clinical features, hair microscopy and family pedigree, we suspected LAH2 or LAH3. To determine the type of LAH, we looked for gene mutations in *LIPH* and *LPAR6* (encoding lysophosphatidic acid receptor 6). Two prevalent missense mutations in *LIPH* were found (1); c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn). The mutations were carried in a compound heterozygous state. No mutations were found in *LPAR6*. The parents did not consent to genetic testing of the younger brother or themselves.

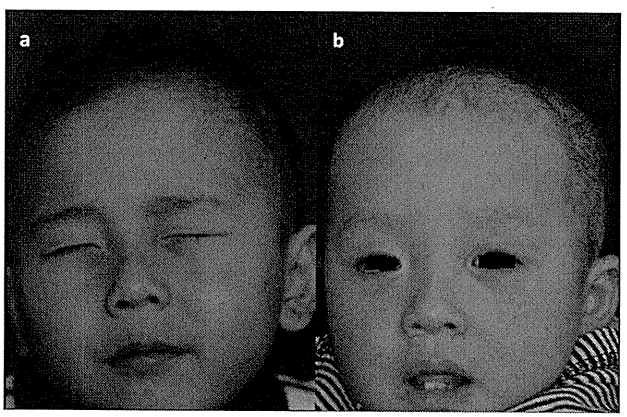

Fig. 1. (a) Clinical features of the patient at 4 years of age. (b) Clinical features of the younger brother at 1 year 4 months of age. Permission is given from the parents to publish these photos.

DISCUSSION

The different LAH subtypes map to chromosomes 18q12.1, 3q27.3 and 13q14.11–13q21.32, and are designated LAH1, LAH2 and LAH3, respectively (2–4). Mutations in *DSG4* (encoding desmoglein 4) have been found to be responsible for LAH1 (5). Kazantseva et al. (6) reported deletion mutations in *LIPH* leading to LAH2. Pasternack et al. (7) reported disruption of *LPAR6* in families affected with LAH3.

Table I summarizes of genetic, non-syndromic human alopecias. In *hypotrichosis simplex of the scalp*, hair loss

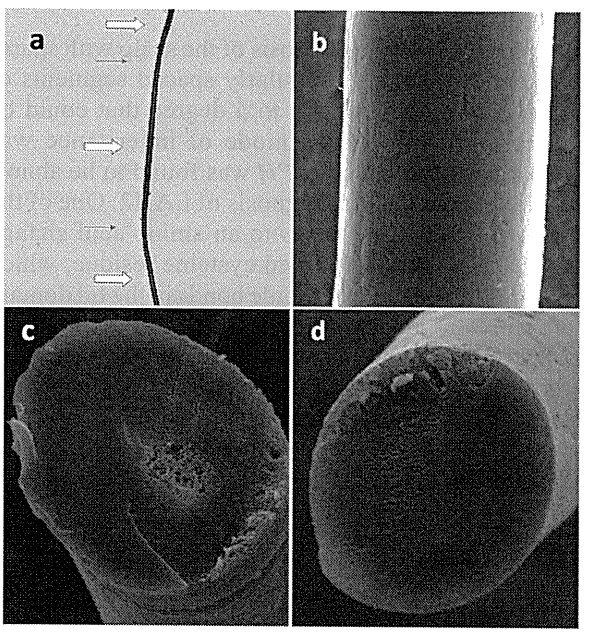

Fig. 2. (a) Light microscopy (×40). Hair was composed of thick (⇨) and thin parts (→). (b) Scanning electron microscopy (×900). Cuticular cells were absent in both thick and thin sections. (c, d) Scanning electron microscopy (cross-section, ×900). (c) Thick regions showed hair medulla, while (d) thin regions did not.

is limited to the scalp without hair shaft abnormalities. The causative gene is *CDSN* (encoding corneodesmosin) on 6p21.3 (8). The clinical presentations of *monilethrix* vary among patients. Mild cases have hair loss limited to the scalp, while severe cases show generalized alopecia. Hair shaft abnormalities are characteristic, demonstrating regularly-spaced, spindle-shaped swellings. The nodes are as thick as normal hair and the atrophic internodes represent areas where the hair is easily broken. Causative genes are *hHb1*, *hHb3* and *hHb6* (12q13) (9), which encode for basic hair keratins.

In case of *atrichia with papular lesions*, hair loss on the entire body occurs several months after birth. The gene responsible is *HR* (encoding "hairless") (10), a transcription modulating factor that influences the regression phase of the hair shaft cycle. Patients with *hypotrichosis, Marie Unna type* have hard and rough scalp hair, described as iron-wire hair. Generalized hypotrichosis is often seen. *U2HR*, an inhibitory upstream open reading frame of the human hairless gene (11), is mutated in this condition. *Hereditary hypotrichosis simplex* is characterized by hair follicle miniaturization. The defective gene is *APCDD1* (encoding adenomatosis polyposis down-regulated 1) (12). Hairs are short, thin, and easily plucked. Eyelashes and eyebrows are also affected.

As already mentioned, there are three types of *localized hereditary hypotrichosis*. LAH1 patients have hair shaft abnormalities that resemble moniliform hair (13). LAH1 can be viewed as an autosomal recessive form of monilethrix. Patients with LAH2 and LAH3 have woolly hair (14, 15), and eyelashes and eyebrows are often sparse or absent. Upper and lower limb hairs are sometimes absent too.

Our patient had hypotrichosis of the scalp with sparse left eyebrow hair and irregularly spaced segments of thick and thin hair, but not to a degree that could be labelled moniliform. The mode of inheritance was autosomal recessive and *LIPH* was found to be abnormal, thus establishing a diagnosis of LAH2. One of the mutations (c.736T>A) leads to an amino acid change (p.Cys246Ser) of a conserved cysteine residue, which forms intramolecular disulphide bonds in the lid domain in the structure model of LIPH (1). The other mutation (c.742C>A) results in alteration of one of the amino acids of the catalytic triad (Ser^{154}, Asp^{178}, and His^{248}) of LIPH (p.His248Asn) (1).

Regarding hair shaft morphology, Horev et al. (14) reported that hairs of LAH2 patients showed decreased diameter under light microscopy. This is the first report to describe hairs from an LAH2 patient by SEM. Shimomura et al. (13) observed hairs of LAH1 patients by SEM and found variable thickness of the hair shaft, resulting in nodes and internodes. Which are absent in LAH1 (our observation). Longitudinal ridges and flutes were observed at internodes, and the breaks always occurred at internodes in LAH1. These features resemble those of moniliform hair rather than LAH2. However, in the end gene analysis is probably easier to accomplish than SEM to distinguish the two types of LAH.

ACNOWLEDGEMENTS

We thank Dr Andrew Blauvelt, Department of Dermatology, Oregon Health & Science University, for many helpful comments.

REFERENCES

1. Shinkuma S, Akiyama M, Inoue A, Aoki J, Natsuga K, Nomura T, et al. LIPH prevalent founder mutations lead to loss of P2Y5 activation ability of PA-PLA1α in autosomal recessive hypotrichosis. Hum Mutat 2010; 31: 602–610.
2. Rafique MA, Ansar M, Jamai SM, Malik S, Sohail M, Faiyaz-Ul-Haque M, et al. A locus for hereditary hypotrichosis localized to human chromosome 18q21.1. Eur J Hum Genet 2003; 11: 623–628.
3. Aslam M, Chahrour MH, Razzaq A, Haque S, Yan K, Leal SM, et al. A novel locus for autosomal recessive form of hypotrichosis maps to chromosome 3q26.33-q27.3. J Med Genet 2004; 41: 849–852.
4. Wali A, Chishti MS, Ayub M, Yasinzai M, Kafaitullah, Ali G, et al. Localization of a novel autosomal recessive hypotrichosis locus (LAH3) to chromosome 13q14.11-q21.32. Clin Genet 2007; 72: 23–29.
5. Kljuic A, Bazzi H, Sundberg JP, Martinez-Mir A, O'Shaughnessy R, Mahoney MG, et al. Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotrichosis and acquired pemphigus vulgaris. Cell 2003; 113: 249–260.
6. Kazantseva A, Goltsov A, Zinchenko R, Grigorenko AP, Abrukova AV, Moliaka YK, et al. Human hair growth deficiency is linked to a genetic defect in the phospholipase gene LIPH. Science 2006; 314: 982–985.
7. Pasternack SM, von Kugelgen I, Aboud KA, Lee YA, Ruschendorf F, Voss K, et al. G protein-coupled receptor P2RY5 and its ligand LPA are involved in maintenance of human hair growth. Nat Genet 2008; 40: 329–334.

Table I. *Features of genetic, non-syndromic human alopecias*

Disease (ref)	Hair shaft shape	Eyelash/eyebrow	Causative gene	Mode of inheritance
Hypotrichosis simplex of scalp (8)	Normal	Normal	*CDSN*	Autosomal dominant
Monilethrix (9)	Regularly spaced, spindle-shaped swellings	Absent to normal	*hHb1, 3, 6*	Autosomal dominant
Atrichia with papular lesions (10)	Normal	Absent	*HR*	Autosomal recessive
Hypotrichosis, Marie Unna type (11)	Iron-wire	Sparse	*U2HR*	Autosomal dominant
Hereditary hypotrichosis simplex (12)	Short, thin, easily plucked	Absent to sparse	*APCDD1*	Autosomal dominant
Localized hereditary hypotrichosis (LAH1) (2, 5, 13)	Moniliform	Absent to normal	*DSG4*	Autosomal recessive
Localized hereditary hypotrichosis (LAH2) (3, 6, 14)	Curled	Absent to normal	*LIPH*	Autosomal recessive
Localized hereditary hypotrichosis (LAH3) (4, 7, 15)	Curled	Absent to normal	*LPAR6*	Autosomal recessive

8. Davalos NO, Garicia-Vargas A, Pforr J, Davalos IP, Picos-Cardenas VJ, Garcia-Cruz D, et al. A non-sense mutation in the corneodesmosin gene in a Mexican family with hypotrichosis simplex of the scalp. Br J Dermatol 2005; 153: 1216–1219.
9. Richard G, Itin P, Lin JP, Bon A, Bale SJ. Evidence for genetic heterogeneity in monilethrix. J Invest Dermatol 1996; 107: 812–814.
10. Ahmad W, Faiyaz ul Haque M, Brancolini V, Tsou HC, ul Haque S, Lam H, et al. Alopecia universalis associated with a mutation in the human hairless gene. Science 1998; 279: 720–724.
11. Wen Y, Liu Y, Xu Y, Zhao Y, Hua R, Wang K, et al. Loss-of-function mutations of an inhibitory upstream ORF in the human hairless transcript cause Marie Unna hereditary hypotrichosis. Nat Genet 2009; 41: 228–233.
12. Shimomura Y, Agalliu D, Vonica A, Luria V, Wajid M, Baumer A, et al. APCDD1 is a novel Wnt inhibitor mutated in hereditary hypotrichosis simplex. Nature 2010; 464: 1043–1047.
13. Shimomura Y, Sakamoto F, Kariya N, Matsunaga K, Ito M. Mutations in the desmoglein 4 gene are associated with monilethrix-like congenital hypotrichosis. J Invest Dermatol 2006; 126: 1281–1285.
14. Horev L, Tosti A, Rosen I, Hershko K, Vincenzi C, Nanova K, et al. Mutations in lipase H cause autosomal recessive hypotrichosis simplex with wooly hair. J Am Acad Dermatol 2009; 61: 813–818.
15. Horev L, Saad-Edin B, Ingber A, Zlotogorski A. A novel deletion mutation in P2RY5/LPA_6 gene cause autosomal recessive woolly hair with hypotrichosis. J Eur Acad Dermatol Venereol 2010; 24: 858–859.

Recurrence of Hydroxyurea-induced Leg Ulcer After Discontinuation of Treatment

Kazuhiro Kikuchi[1], Ken Arita[1], Yasuki Tateishi[1], Masahiro Onozawa[2], Masashi Akiyama[1] and Hiroshi Shimizu[1]
Departments of [1]Dermatology and [2]Hematology, Hokkaido University Graduate School of Medicine, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan.
E-mail: kikku@med.hokudai.ac.jp
Accepted November 1, 2010.

Hydroxyurea (HU) is a hydroxylated derivative of urea that has been recognized since 1960 as effective against cancer (1). It is an inhibitor of cellular DNA synthesis, and it promotes cell death in the S phase of the cell cycle through inhibition of the enzyme ribonucleotide reductase (2). The most common indications for HU therapy are chronic myeloid leukaemia and other myeloproliferative disorders (3, 4) such as essential thrombocythemia (5) and polycythemia vera (PV) (6). Cutaneous side-effects, such as alopecia, diffuse hyperpigmentation, scaling, lichen planus-like lesions, poikiloderma, atrophy of the skin and subcutaneous tissues, and nail changes, can occur during the treatment with HU (7–9). The occurrence of painful leg ulcers represents another rare and incompletely characterized complication that has been described in patients with myeloproliferative diseases receiving high-dose long-term HU treatment (10). While the mode of action of HU on bone marrow elements is well established, its effects on actively proliferating epithelial cells remain less described (11). Poor response to traditional local and systemic therapy is a typical feature of HU-induced leg ulcers, and discontinuation of the drug is often required to achieve complete wound healing (6, 8). Cessation of the drug usually improves the skin ulcer; although, in some cases, the ulcer remains and additional therapies, such as skin grafting, are needed (12). We report here the first case of a leg ulcer that recurred even after discontinuation of HU treatment.

CASE REPORT

The patient was an 82-year-old Japanese male who had been diagnosed with PV 9 years before and had been treated only with phlebotomy and an anti-platelet agent for several years. Due to splenomegaly and elevated blood cell counts, HU therapy was started 3 years ago at a dosage of 1 g daily for a month, followed by 1.0 or 1.5 g daily for 28 months. A good clinical response was achieved. However, the patient developed painful ulcers on the left second toe after two years of HU treatment.

He visited our outpatient clinic and was diagnosed with an HU-induced skin ulcer. HU was discontinued, topical application of sulfadiazine silver was performed, an oral antibiotic (cefdinir) was administered, and the ulcer epithelialized. However, a new ulcer appeared on the left lateral malleolar area 46 days after cessation of HU and gradually enlarged in size. The patient was admitted to our hospital for treatment of the ulcer.

Examination revealed a 48 × 56 mm ulcer with yellow necrotic tissue and marginal erythematous oedema (Fig. 1). Laboratory examination revealed a white blood cell count of $11.6 \times 10^3/\mu l$, a platelet count of $64.2 \times 10^4/l$, and a red blood cell count of $5.07 \times 10^6/\mu l$. Anti-nuclear antibody, anti-neutrophilic cytoplasmic antibodies, anti-cardiolipin antibody, and cryoglobulin were negative. A skin biopsy taken from the margin of the ulcer demonstrated leukocytoclastic vasculitis in the upper dermis (Fig. 2). A wound-healing strategy of surgical debridement, intravenous prostaglandin E1 administration, and topical application of beta-fibroblast growth factor, sulfadiazine silver and alprostadil alfadex was started, and the ulcer began to epithelialize. After 4 months, re-epithelialization was complete. The PV was treated with busulfan, achieving a good clinical response.

DISCUSSION

HU is usually well tolerated and has low toxicity (1). However, cutaneous adverse effects such as diffuse hyperpigmentation, brown discoloration of the nails, acral erythema, photosensitization, fixed drug eruption, alopecia, and oral ulceration have been reported (7–9). Stahl & Silber (10) first reported HU-induced skin ulcers in 1985. Montefusco et al. (11) reported

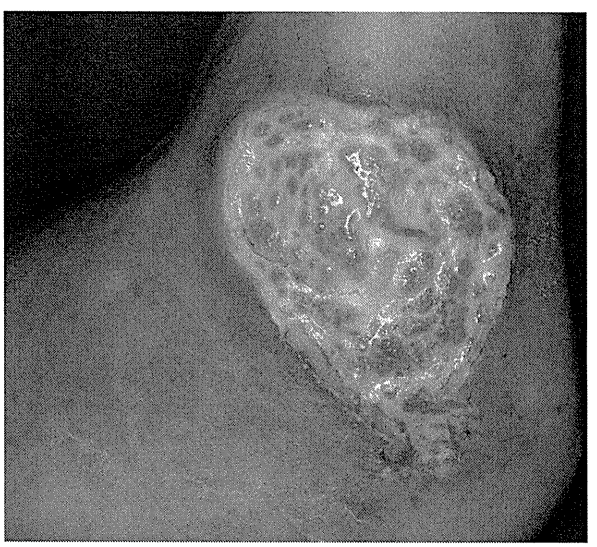

Fig. 1. Left foot with an ulcer on the lateral malleolar area after two months free of hydroxyurea administration. The ulcer was covered with yellow necrotic tissue and surrounded by oedematous erythema.

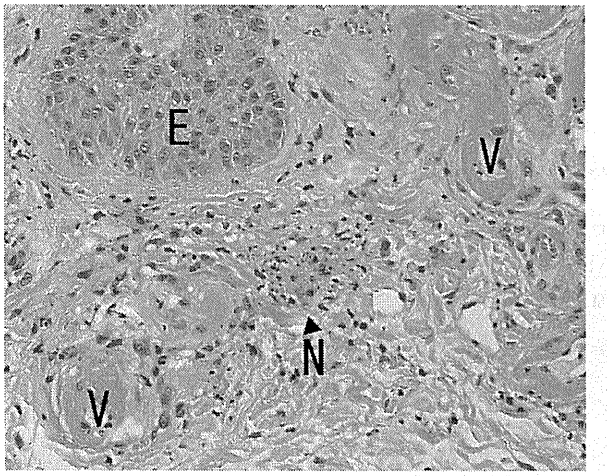

Fig. 2. Histology of erythema on the margin of the ulcer (haematoxylin-eosin staining). Fibrin deposition on the vascular wall and nucleic debris were evident around small vessels (×100). (E: epidermis; V: blood vessels; N: neutrophilic nuclear debris).

that, among 200 chronic myeloid leukaemia patients treated with HU, 17 (8.5%) developed leg ulcers. However, they achieved complete resolution or significant improvement after discontinuation of HU therapy (11). HU-induced leg ulcer and complete resolution within several months after drug discontinuation has also been reported in other myeloproliferative disorder, such as PV (6) and essential thrombocythemia (5). In those cases, as in ours, most of the patients had been treated with >1 g of HU per day for at least one year (8). In the present case, the patient was treated with >1 g of HU per day for 28 months. The ulcer occurred on his lateral malleolus, which histologically showed leukocytoclastic vasculitis. These features are consistent with previous reports of HU-induced leg ulcer.

From previous reports, the pathogenesis of HU-induced ulceration remains unclear and it may be multifactorial. It has been postulated that ulcers may be the result of: (*i*) interruption of microcirculation due to leukocytoclastic vasculitis or arterial microthrombi related to platelet dysregulation (13, 14); (*ii*) cumulative toxicity in the basal layer of the epidermis through inhibition of DNA synthesis (8); and (*iii*) repeated mechanical injury in areas subject to trauma: a perimalleolar area for instance (15).

In the case described here, a new ulcer developed even after cessation of HU administration. As for the pathogenic mechanism of recurrence, (*i*) interruption of microcirculation could result from hyperviscosity due to the elevated platelet count (as high as 100×10^4/l in one measurement) (13, 14), although no thrombi were observed histologically in the capillaries or small vessels. (*ii*) The direct cytotoxic effect of HU (8) may continue even after the withdrawal of the drug, and it may inhibit the repair of (*iii*) small injuries in the perimalleolar area: the one of the area susceptible to physical trauma (15). These assumptions can be made from the pathogenesis of HU-induced ulcer reported previously (8, 13–15).

To our knowledge, this is the first report of recurrence of HU-related leg ulcer after the discontinuation of medication. The case suggests that it is important to pay careful attention to recurrence even after cessation of HU therapy. Precise, early treatment for microtraumas and small ulcers should be administered to patients with a long history of HU medication.

REFERENCES

1. Boyd AS, Neldner KH. Hydroxyurea therapy. J Am Acad Dermatol 1991; 25: 518–524.
2. Yarbro JW. Mechanism of action of hydroxyurea. Semin Oncol 1992; 19: 1–10.
3. Goldman JM. Therapeutic strategies for chronic myeloid leukemia in the chronic (stable) phase. Semin Hematol 2003; 40: 10–17.
4. Rice L, Baker KR. Current management of the myeloproliferative disorders: a case-based review. Arch Pathol Lab Med 2006; 130: 1151–1156.
5. Demirçay Z, Cömert A, Adigüzel C. Leg ulcers and hydroxyurea: report of three cases with essential thrombocythemia. Int J Dermatol 2002; 41: 872–874.
6. Bader U, Banyai M, Böni R, Burg G, Hafner J. Leg ulcers in patients with myeloproliferative disorders: disease- or treatment-related? Dermatology 2000; 200: 45–48.
7. Najean Y, Rain JD. Treatment of polycythemia vera: the use of hydroxyurea and pipobroman in 292 patients under the age of 65 years. Blood 1997; 90: 3370–3377.
8. Weinlich G, Schuler G, Greil R, Kofler H, Fritsch P. Leg ulcers associated with long-term hydroxyurea therapy. J Am Acad Dermatol 1998; 39: 372–374.
9. Daoud MS, Gibson LE, Pittelkow MR. Hydroxyurea dermopathy: a unique lichenoid eruption complicating long-term therapy with hydroxyurea. J Am Acad Dermatol 1997; 36: 178–182.
10. Stahl RL, Silber R. Vasculitic leg ulcers in chronic myelogenous leukemia. Am J Hematol 1985; 78: 869–872.
11. Montefusco E, Alimena G, Gastaldi R, Carlesimo OA, Valesini G, Mandelli F. Unusual dermatologic toxicity of long-term therapy with hydroxyurea in chronic myelogenous leukaemia. Tumori 1986; 72: 317–321.
12. Kato N, Kimura K, Yasukawa K, Yoshida K. Hydroxyurea-related leg ulcers in a patient with chronic myelogenous leukemia: a case report and review of the literature. J Dermatol 1999; 26: 56–62.
13. Sirieix ME, Debure C, Baudot N, Dubertret L, Roux ME, Morel P, et al. Leg ulcers and hydroxyurea: forty-one cases. Arch Dermatol 1999; 135: 818–820.
14. Chaine B, Neonato MG, Girot R, Aractingi S. Cutaneous adverse reactions to hydroxyurea in patients with sickle cell disease. Arch Dermatol 2001; 137: 467–470.
15. Saravu K, Velappan P, Lakshmi N, Shastry BA, Thomas J. Hydroxyurea induced perimalleolar ulcers. J Korean Med Sci 2006; 21: 177–179.

International Journal of
Dermatology

Medical genetics

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

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

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

Correspondence
Dr Hiroshi Shimizu, MD, PhD
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 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

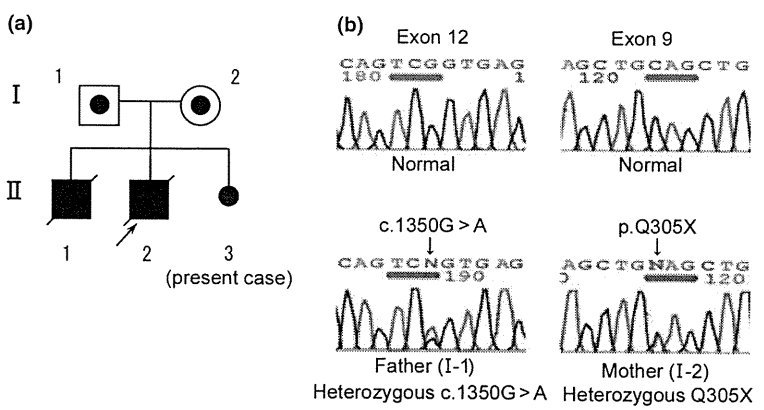

Figure 1 Family tree of the present case and the causative *PLEC* mutations. (a) The first and second newborns exhibited clinical features typical of EBS-PA and died shortly after birth. The proband (the second newborn) is indicated by an arrow. (b) The paternal splice-site mutation was a c.1350G>A transition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

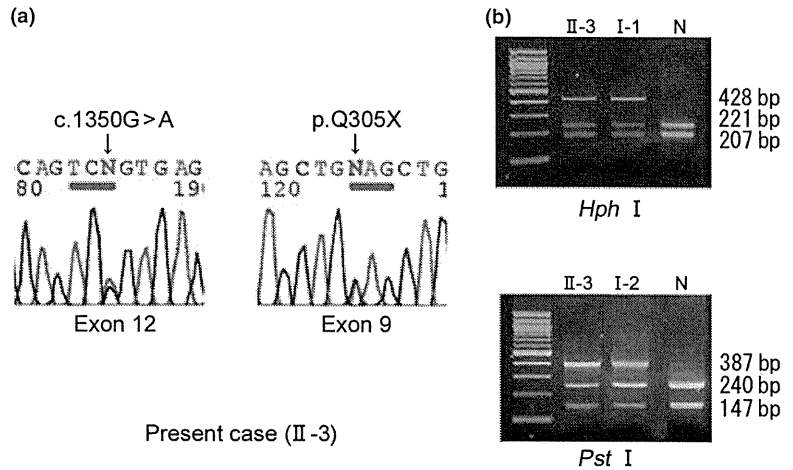

Figure 2 Analysis of the plectin gene mutations in genomic DNA from amniocytes of a fetus at risk. (a) Mutation analysis of genomic DNA from amniocytes shows both the c.1350G>A mutation in exon 12 and p.Q305X mutations in exon 9. (b) The presence of the mutations was verified by restriction enzyme digestion.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

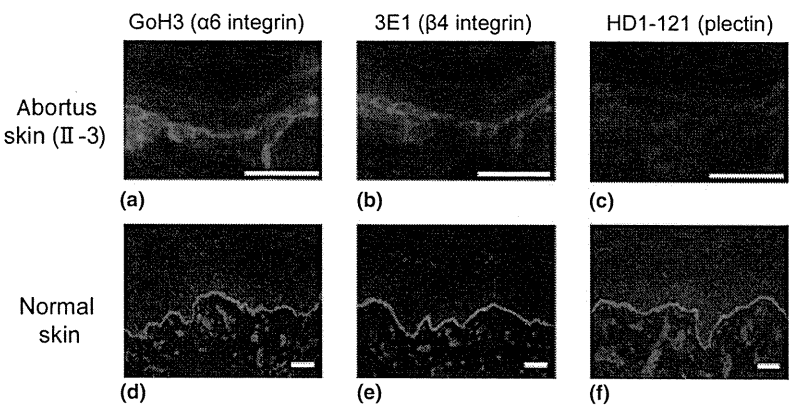

Figure 3 Absence of plectin expression in the abortus. α6 integrin (mAb GoH3) and β4 integrin (mAb 3E1) are expressed in the abortus skin (a, b) and the control skin (d, e). Staining with monoclonal antibody for plectin (mAb HD1–121) shows positive in the control skin (f) but negative in the skin of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

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; in Press.
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.

JAPANESE DERMATOLOGICAL ASSOCIATION
THE JOURNAL OF DERMATOLOGY

doi: 10.1111/j.1346-8138.2011.01209.x

Journal of Dermatology 2011; **38**: 310–320

SPECIAL REPORT

Prevalence of dermatological disorders in Japan: A nationwide, cross-sectional, seasonal, multicenter, hospital-based study

Masutaka FURUE,[1,2]* Souji YAMAZAKI,[1] Koichi JIMBOW,[1] Tetsuya TSUCHIDA,[1] Masayuki AMAGAI,[1] Toshihiro TANAKA,[1,2] Kayoko MATSUNAGA,[1,2] Masahiko MUTO,[1,2] Eishin MORITA,[1,2] Masashi AKIYAMA,[2] Yoshinao SOMA,[2] Tadashi TERUI,[2] Motomu MANABE[2]

Scientific Committee, Japanese Dermatological Association [1](2006–2007), and [2](2008–2009), Tokyo, Japan

ABSTRACT

To clarify the prevalence of skin disorders among dermatology patients in Japan, a nationwide, cross-sectional, seasonal, multicenter study was conducted in 69 university hospitals, 45 district-based pivotal hospitals, and 56 private clinics (170 clinics in total). In each clinic, information was collected on the diagnosis, age, and gender of all outpatients and inpatients who visited the clinic on any one day of the second week in each of May, August, and November 2007 and February 2008. Among 67 448 cases, the top twenty skin disorders were, in descending order of incidence, miscellaneous eczema, atopic dermatitis, tinea pedis, urticaria/angioedema, tinea unguium, viral warts, psoriasis, contact dermatitis, acne, seborrheic dermatitis, hand eczema, miscellaneous benign skin tumors, alopecia areata, herpes zoster/postherpetic neuralgia, skin ulcers (nondiabetic), prurigo, epidermal cysts, vitiligo vulgaris, seborrheic keratosis, and drug eruption/toxicoderma. Atopic dermatitis, impetigo, molluscum, warts, acne, and miscellaneous eczema shared their top-ranking position in the pediatric population, whereas the most common disorders among the geriatric population were tinea pedis, tinea unguium, psoriasis, seborrheic dermatitis, and miscellaneous eczema. For some disorders, such as atopic dermatitis, contact dermatitis, urticaria/angioedema, prurigo, insect bites, and tinea pedis, the number of patients correlated with the average high and low monthly temperatures. Males showed a greater susceptibility to some diseases (psoriasis, erythroderma, diabetic dermatoses, *inter alia*), whereas females were more susceptible to others (erythema nodosum, collagen diseases, livedo reticularis/racemosa, hand eczema, *inter alia*). In conclusion, this hospital-based study highlights the present situation regarding dermatological patients in the early 21st century in Japan.

Key words: age, Japan, prevalence, sex, skin diseases.

INTRODUCTION

Skin forms the outermost part of the human body and it acts as a vital barrier to external and internal damage. Various external and internal stimuli, which can be either short- or long-term, can affect the homeostasis of the skin, leading to a variety of disorders. The development and perpetuation of skin disorders are multifactorial in nature, and can result from genetic, environmental, mechanical, meteorological and even cultural effects. Skin disorders therefore include a vast range of diseases.

Although it is difficult to know the exact prevalence or incidence of skin diseases, several hospital-based

Correspondence: Masutaka Furue, M.D., Ph.D., Department of Dermatology, Kyushu University, Maidashi 3-1-1 Higashiku, Fukuoka 812-8582, Japan. Email: furue@dermatol.med.kyushu-u.ac.jp
*Chair.
Received 1 September 2010; accepted 1 September 2010.

studies have shown that skin diseases are very common. Of a total of 11 191 patients seen by a general practitioner in the UK, 2386 (21%) presented dermatological complaints. Among these there was a preponderance of females (1604, 67%), and the most common skin diseases seen were viral warts, eczema and benign tumors.[1] In the Netherlands, 235–460/1000 person-years of children aged 0–17 years contacted general practitioners in 1987 and 2001,[2] and these contacts frequently involved bacterial, viral, fungal, eczematous or traumatic skin diseases.[2] Tamer *et al.* reported on 6300 pediatric cases aged 0–16 years who visited dermatological clinics in Turkey; this group showed a preponderance of bacterial, viral and eczematous skin diseases.[3] In the case of Japan, there is no authentic report in the published work on any investigation of the prevalence of skin diseases; therefore, the Japanese Dermatological Association conducted a nationwide, cross-sectional, seasonal, multicenter, hospital-based study.

METHODS

A total of 190 dermatology clinics at 76 university hospitals, 55 district-based pivotal hospitals and 59 private clinics participated in this study. At each clinic,

Table 1. Numbers of patients recruited in each season

	Number of patients				
	May 2007	August 2007	November 2007	February 2008	Total
University Hospitals $n = 69$	8558	7944	7782	7778	32 062 (47.54%)
District-based Hospitals $n = 45$	3505	3450	2890	2864	12 709 (18.84%)
Private clinics $n = 56$	5779	6709	5364	4825	22 677 (33.62%)
Total	17 842	18 103	16 036	15 467	67 448 (100%)

Table 2. Age distribution and sex difference of patients

Age distribution (years old)	Number of patients	Male patients	Female patients	Sex undescribed
0–5	4192 (6.22%)	2200 (7.12%)	1983 (5.49%)	9
6–10	2099 (3.11%)	1047 (3.39%)	1047 (2.9%)	5
11–15	1711 (2.54%)	815 (2.64%)	893 (2.47%)	3
16–20	2270 (3.37%)	995 (3.22%)	1266 (3.5%)	9
21–25	3219 (4.77%)	1245 (4.03%)	1960 (5.43%)	14
26–30	3516 (5.21%)	1378 (4.46%)	2126 (5.89%)	12
31–35	4050 (6%)	1546 (5%)	2483 (6.87%)	21
36–40	3807 (5.64%)	1604 (5.19%)	2180 (6.03%)	23
41–45	3298 (4.89%)	1387 (4.49%)	1879 (5.2%)	32
46–50	3201 (4.75%)	1326 (4.29%)	1848 (5.12%)	27
51–55	4062 (6.02%)	1763 (5.71%)	2279 (6.31%)	20
56–60	5543 (8.22%)	2503 (8.1%)	3012 (8.34%)	28
61–65	5413 (8.03%)	2533 (8.2%)	2846 (7.88%)	34
66–70	5629 (8.35%)	2775 (8.98%)	2824 (7.82%)	30
71–75	6157 (9.13%)	3195 (10.34%)	2923 (8.09%)	39
76–80	4777 (7.08%)	2487 (8.05%)	2259 (6.25%)	31
81–85	2636 (3.91%)	1297 (4.2%)	1318 (3.65%)	21
86–90	1098 (1.63%)	508 (1.64%)	583 (1.61%)	7
91–100	427 (0.63%)	166 (0.54%)	259 (0.72%)	2
≥101	16 (0.02%)	3 (0.01%)	2 (0.01%)	11
Age undescribed	327 (0.48%)	126 (0.41%)	155 (0.43%)	46
Total	67 448 (100%)	30 899 (100%)	36 125 (100%)	424

information on diagnosis, age and sex was collected from all outpatients and inpatients who visited the clinics or who were hospitalized on any single day of the second week in each of May, August and November 2007 and February 2008. Reports on the monthly average values of the high and low temperatures and humidities were collected from the Meteorological Agency. The information on 67 448 cases from 170 clinics (69 university hospitals, 45 district-based pivotal hospitals and 56 private clinics) that participated in all of the four seasonal examinations was analyzed. Statistical analyses were performed by using Spearman's rank correlation coefficient. A P-value of <0.05 was considered to be statistically significant. This study was approved by the internal ethical review boards of the Japanese Dermatological Association.

Table 3. Prevalence of skin diseases in 67 448 patients

Burn	899 (1.33%)	Syphilis	24 (0.04%)
Trauma	409 (0.61%)	Miscellaneous sexually transmitted diseases	41 (0.06%)
Skin ulcer (nondiabetic)	1334 (1.98%)	Bullous pemphigoid	510 (0.76%)
Pressure ulcer	608 (0.9%)	Pemphigus	424 (0.63%)
Miscellaneous physico-chemical skin damage	681 (1.01%)	Miscellaneous bullous diseases	141 (0.21%)
Diabetic dermatoses	436 (0.65%)	Systemic sclerosis	619 (0.92%)
Atopic dermatitis	6733 (9.98%)	Systemic lupus erythematosus	525 (0.78%)
Hand eczema	2024 (3%)	Dermatomyositis	304 (0.45%)
Contact dermatitis	2643 (3.92%)	Miscellaneous collagen diseases	915 (1.36%)
Seborrheic dermatitis	2213 (3.28%)	Anaphylactoid purpura	171 (0.25%)
Miscellaneous eczema	12590 (18.67%)	Reticular/racemous livedo	81 (0.12%)
Urticaria/angioedema	3369 (4.99%)	Miscellaneous vasculitis/purpura/ circulatory disturbance	632 (0.94%)
Prurigo	1229 (1.82%)	Mycosis fungoides	427 (0.63%)
Drug eruption/toxicoderma	1018 (1.51%)	Miscellaneous lymphomas	285 (0.42%)
Psoriasis	2985 (4.43%)	Pigmented nevus	709 (1.05%)
Palmoplantar pustulosis	832 (1.23%)	Seborrheic keratosis	1095 (1.62%)
Miscellaneous pustulosis	172 (0.26%)	Soft fibroma/acrochordon	231 (0.34%)
Lichen planus	200 (0.3%)	Epidermal cyst	1194 (1.77%)
Miscellaneous inflammatory keratotic disorders	241 (0.36%)	Lipoma	173 (0.26%)
Tylosis/clavus	917 (1.36%)	Dermatofibroma	111 (0.16%)
Ichthyosis	61 (0.09%)	Miscellaneous benign skin tumors	1666 (2.47%)
Miscellaneous keratinization disorders	502 (0.74%)	Actinic keratosis	261 (0.39%)
Ingrown nail	597 (0.89%)	Basal cell carcinoma	324 (0.48%)
Miscellaneous nail disorder	397 (0.59%)	Squamous cell carcinoma/Bowen's disease	455 (0.67%)
Alopecia areata	1653 (2.45%)	Paget's disease	224 (0.33%)
Androgenic alopecia	210 (0.31%)	Malignant melanoma	808 (1.2%)
Miscellaneous skin appendage disorders	266 (0.39%)	Miscellaneous malignant skin tumors	534 (0.79%)
Scabies	98 (0.15%)	Vitiligo vulgaris	1134 (1.68%)
Insect bite	762 (1.13%)	Chloasma/senile freckle	336 (0.5%)
Tinea pedis	4379 (6.49%)	Miscellaneous pigmented disorders	154 (0.23%)
Tinea unguium	3231 (4.79%)	Erythema multiforme	197 (0.29%)
Miscellaneous tinea	610 (0.9%)	Erythema nodosum	111 (0.16%)
Candidiasis	408 (0.6%)	Miscellaneous disorders with erythematous plaques	130 (0.19%)
Miscellaneous mycosis	211 (0.31%)	Nevus/phacomatosis (other than pigmented nevus)	267 (0.4%)
Acne	2430 (3.6%)	Rosacea/rosacea-like dermatitis	150 (0.22%)
Impetigo contagiosum	507 (0.75%)	Granulomatous diseases	192 (0.28%)
Folliculitis	755 (1.12%)	Keloid/hypertrophic scar	186 (0.28%)
Erysipelas	81 (0.12%)	Cheilitis/angular cheilitis/mucous membrane diseases	95 (0.14%)
Cellulitis	594 (0.88%)	Erythroderma	63 (0.09%)
Miscellaneous bacterial infection	914 (1.36%)	Other diseases	666 (0.99%)
Molluscum contagiosum	604 (0.9%)	Total	67 448 (100%)
Herpes simplex	691 (1.02%)		
Herpes zoster/zoster-associated pain	1609 (2.39%)		
Viral wart	3028 (4.49%)		
Miscellaneous viral disorders	353 (0.52%)		

RESULTS

Demographic data for the 67 448 patients

Among the 67 448 patients, 32 062 (47.54%) cases were recruited from university hospitals, 12 709 (18.84%) from district-based hospital and 22 677 (33.62%) from private clinics (Table 1). More patients were enrolled in August 2007 (18 103) than in February 2008 (15 467) (Table 1). With regards to the age distribution, the group aged 71–75 years (6157; 9.13%) was the biggest, followed by groups aged 66–70 (5629; 8.35%), 56–60 (5543; 8.22%) and 61–65 (5413; 8.03%) (Table 2). For patients aged under 20 years, the group aged 0–5 years formed the biggest population (4192; 6.22%). Among the 67 448 patients, there were 30 899 (46.1%) males and 36 125 (53.9%) females; the sex of 424 patients was not described. Female patients aged between 16 and 60 years tended to visit dermatology clinics more frequently than their male counterparts (Table 2).

Prevalence of skin disorders

We classified skin diseases into 85 categories, as listed in Table 3, and determined the prevalence of each. The 20 most common diseases were miscellaneous eczema (12 590; 18.67%) followed, in order, by atopic dermatitis (6733; 9.98%), tinea pedis (4379; 6.49%), urticaria/angioedema (3369; 4.99%), tinea unguium (3231; 4.79%), viral warts (3028; 4.49%), psoriasis (2985; 4.43%), contact dermatitis (2643, 3.92%), acne (2430; 3.6%), seborrheic dermatitis (2213; 3.28%), hand eczema (2024; 3%), miscellaneous benign skin tumors (1666; 2.47%), alopecia areata (1653; 2.45%), herpes zoster/zoster-associated

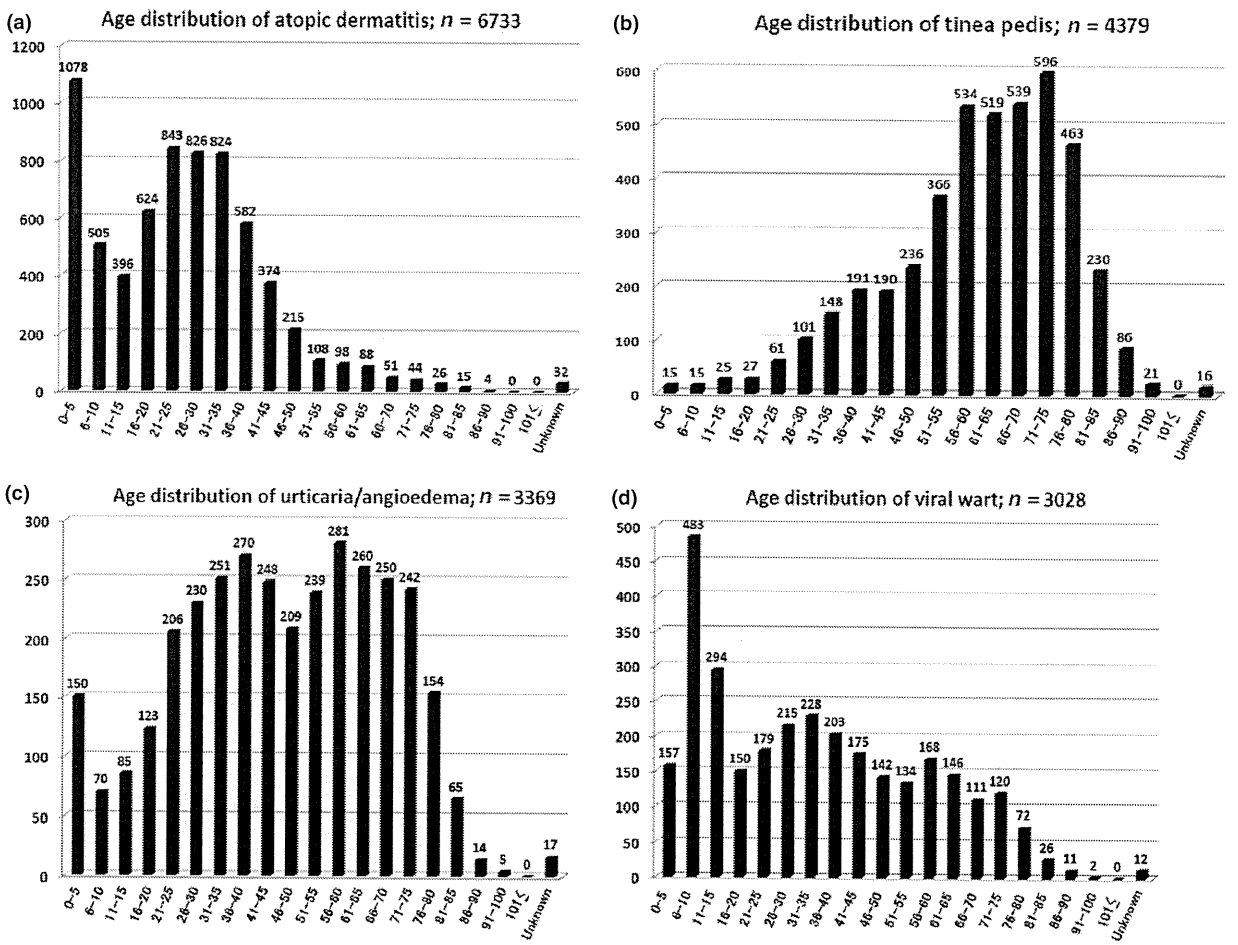

Figure 1. Age distribution of atopic dermatitis, tinea pedis, urticaria/angioedema and viral wart.