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Author(s)	Akiyama, M.; Sakai, K.; Wolff, G.; Hausser, I.; McMillan, J.R.; Sawamura, D.; Shimizu, H.
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Gene Corner

A novel ABCA12 mutation 3270delT causes harlequin ichthyosis

M. Akiyama,* K. Sakai,* G. Wolff,[†] I. Hausser,[‡] J. R. McMillan,[§] D. Sawamura* and H. Shimizu*

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

[†]Institut für Humangenetik und Anthropologie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany;

[‡]Department of Dermatology, University Clinic Heidelberg, Germany;

[§]Creative Research Institute Sousei, Hokkaido University, Sapporo, Japan.

Correspondence:

Masashi Akiyama, M.D., Ph.D.

Department of Dermatology

Hokkaido University Graduate School of Medicine

North 15 West 7, Kita-ku, Sapporo 060-8638, Japan

Telephone: +81-11-716-1161, ext. 5962

Fax: +81-11-706-7820

e-mail <akiyama@med.hokudai.ac.jp>

Short title: Harlequin ichthyosis and *ABCA12* Mutation

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Harlequin ichthyosis (HI) is a severe and often fatal congenital ichthyosis with an autosomal recessive inheritance pattern.¹ The clinical features include thick, plate-like scales with ectropion, eclabium, and flattened ears. The skin development is altered in utero.² *ABCA12* mutations were reported to underlie HI^{3,4} and it was clarified that HI is caused by severe functional defects in the keratinocyte lipid transporter ABCA12.³ The pathomechanism of HI lies in the defective function of the lipid transporter ABCA12 which causes abnormal lipid lamellar granule transport in the keratinocytes, and results in a malformation of the epidermal lipid barrier.³ Until the 1980s, newborns affected with HI rarely survived beyond the neonatal period. However, recently, HI babies have often had a better prognosis.⁵⁻⁷ It is still unclear whether the good prognosis in HI is due to some remnant ABCA12 protein transporter function in the patients or not. Here, we report a HI female patient with a novel homozygous ABCA12 deletion mutation leading to a complete loss of function of ABCA12, who has survived, despite suffering from severe ichthyosis showing the clinical features of non-bullous congenital ichthyosiform erythroderma.

Case and methods

The patient is the first child of healthy, unrelated German parents. There was no positive family history of any related disorders. The female child was born after premature rupture of the amnion in the 34th week of pregnancy (weight 1930 g, length 43 cm, head circumference 32 cm). The skin of the newborn was covered with large, thick, white, diamond-shaped plaques, partly bordered by irregular, bleeding fissures (Fig. 1). Hands and feet were edematous, and the tips of the fingers and toes were white with tight skin, and mobility in all joints was reduced.

After therapy with oral retinoids and local application of carbamide and emollient ointment, in a humid incubator, the hyperkeratosis detached within 2

to 6 weeks and passive and spontaneous mobility increased. The edema and skin tightness also decreased. During infancy, the patient showed white to gray-colored scales on her erythematous trunk skin, extremities and face (Fig. 2). Now, at age 8 years, the patient is doing well with continuous therapy with emollient ointment, vitamin D₃, and iodide and the avoidance of sunlight, although she is still suffering from severe ichthyosis sharing several features of non-bullous congenital ichthyosiform erythroderma. Her mental status is normal, and the patient attends the second grade of a normal school. At age 8 years the patient is 114 cm (<3rd percentile), demonstrating that growth development is delayed.

Mutational analysis of *ABCA12* was performed in the affected baby, her parents and her healthy sister. Briefly, genomic DNA isolated from peripheral blood cells was subjected to PCR amplification, followed by direct automated sequencing using an ABI PRISM 3100 genetic analyzer (ABI Advanced Biotechnologies, Columbia, MD). Oligonucleotide primers and PCR conditions used for amplification of all exons 1-53 of *ABCA12* were originally derived from the report by Lefèvre *et al.*⁸ and were partially modified for the present study. The entire coding region including the exon/intron boundaries for both forward and reverse strands from the patient, family members and 100 healthy controls were sequenced. No mutations were found in 200 normal alleles from the healthy population.

Biopsy samples from the patient's ichthyotic skin were fixed in 5 % glutaraldehyde solution, post-fixed in 1% OsO₄, dehydrated, and embedded in Epon 812. The samples were sectioned at 1 µm thickness for light microscopy and thin sectioned for electron microscopy (70 nm thick). The thin sections were stained with uranyl acetate and lead citrate and examined in a transmission electron microscope.

Results and discussion

Mutation analysis of the 53 exons including the intron-exon boundaries of the entire ABCA12 gene revealed a homozygous deletion mutation 3270delT in exon 23 in the patient [sequence according to Lefèvre et al.⁸] (GenBank accession NM 173076) (Fig. 3). The mutation was verified as present in a heterozygous fashion in her parents and a sister (Fig. 3). Another sister carried no mutations. The mutation was not found in sequence analysis of 100 alleles from 50 normal, unrelated individuals, and therefore is unlikely to be a polymorphism (data not shown). The deletion mutation 3270delT leads to a frameshift and introduces a stop codon at codon 1090, within the first transmembrane domain complex of the ABCA12 protein.⁹ Thus, the homozygous deletion mutation results in a serious truncation of ABCA12 peptide losing both ATP-binding cassettes, and is thought to seriously affect the function of the ABCA12 protein.

Skin biopsy specimen showed compact, severe hyperkeratosis. Electron microscopy revealed numerous abnormal lamellar granules in the granular layer keratinocytes and accumulation of extruded irregular lamellar granules as vesicular structures, either empty or filled with glycogen-like particles between the epidermal cornified cells.

The ATP-binding cassette (ABC) transporter superfamily is one of the largest gene families, encoding a highly conserved group of proteins involved in energy-dependent (active) transport of a variety of substrates across membranes.^{10, 11} The ABCA subfamily, of which the *ABCA12* gene is a member, is assumed to be involved in lipid transport.¹² Mutations in these genes underlie several human genetic diseases.^{7, 13} In 2005, it was reported that ABCA12 mutations underlie HI,^{3, 4} and we showed that serious ABCA12 mutations cause

abnormal lipid transport via lamellar granules in keratinocytes, resulting in the malformation or improper assembly of the epidermal keratinocyte surface lipid barrier and the HI phenotype.³ Recently, the long term survival of HI patients has been more frequently observed and documented,^{3-7, 14} despite HI having been thought of as an almost fatal disorder until as recently as the 1980s. In the present HI case, the underlying *ABCA12* mutation, 3270delT, results in the truncation of the ABCA12 peptide at the codon 1090 within the first transmembrane domain complex. Thus, the causative mutation leads to a loss of both ATP-binding cassettes and is thought to lead to a loss of ABCA12 function. Therefore, our homozygous patient is predicted to have an absence of ABCA12 transporter activity. The present patient, however, survived beyond the perinatal and neonatal period, probably in part due to the oral retinoid treatment. The patient is now 8 years old and her general condition is good without the need for retinoid treatment, although she has the clinical features of non-bullous congenital ichthyosiform erythroderma over her entire body. This case may suggest that an HI patient can survive beyond the perinatal and neonatal periods with oral retinoid treatment, even if the causative ABCA12 mutation leads to a complete loss of function of ABCA12. Further accumulation of the similar cases is needed to confirm the effect of systemic retinoid on the prognosis of HI.

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**Department of Dermatology,
Hokkaido University Graduate School of Medicine,
North 15 West 7, Sapporo 060-8638, Japan*

*†Institut für Humangenetik und Anthropologie,
Albert-Ludwigs-Universität Freiburg, Freiburg, Germany*

*‡Department of Dermatology,
University Clinic Heidelberg, Germany*

*§Creative Research Institute Sousei,
Hokkaido University, Sapporo, Japan*

Correspondence Masashi Akiyama.

E-mail: akiyama@med.hokudai.ac.jp

M. Akiyama*

K. Sakai*

G. Wolff†

I. Hausser‡

J. R. McMillan§

D. Sawamura*

H. Shimizu*

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FIGURE LEGENDS

Fig 1. The patient showed the typical clinical phenotype of HI in the neonatal period.

The entire body surface was covered with thick plate-like scales and fissures. Her auricles were malformed.

Fig 2. Clinical features at three years of age.

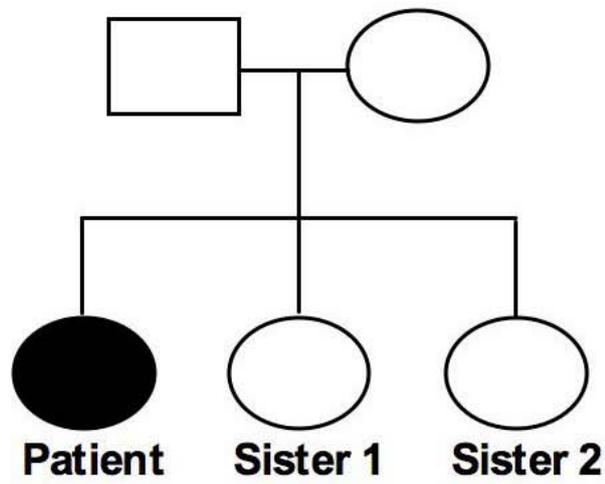
The entire skin surface (a) was erythematous and covered with gray-white to gray scales, including hands (b) and feet (c).

Fig 3. Homozygous mutation of ABCA12 in the patient.

The pedigree of the patient's family (top). Direct sequencing revealed a homozygous 3270delT (changing tyrosine residue at codon 1090 to a stop codon) in exon 23 of *ABCA12* of the patient. In both parents and one sister, the deletion mutation was found in a heterozygous state. The mutation was not present in the other sister or the control samples.

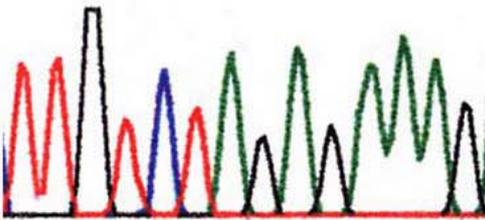






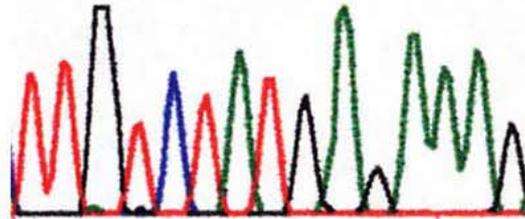
3270delT

TTGTCTAGAGAAAG



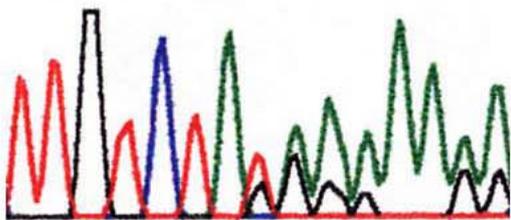
Patient

TTGTCTATGAGAAAG



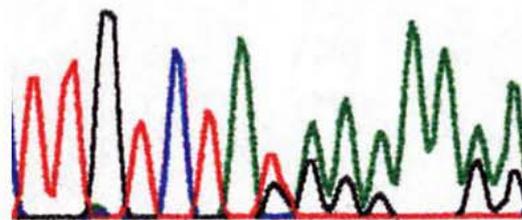
Control

TTGTCTATNAAAAAA



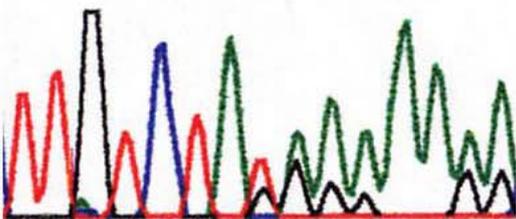
Father

TTGTCTATNAAAAAA



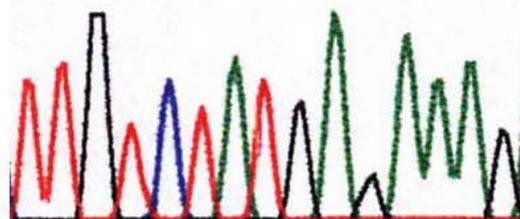
Mother

TTGTCTATNAAAAAA



Sister1

TTGTCTATGAGAAAG



Sister2