Journal of Investigative Dermatology
MS# JID-2005-0730.R2 2nd Revised Version

Mutation Report

Compound heterozygous mutations including a *de novo* missense mutation in *ABCA12* led to a case of harlequin ichthyosis with moderate clinical severity

Masashi Akiyama, Kaori Sakai, Yoriko Sugiyama-Nakagiri, Yasuko Yamanaka, James R. McMillan*, Daisuke Sawamura, Hironori Niizeki†, Sachiko Miyagawa†and Hiroshi Shimizu

Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan;
*Creative Research Initiative Sousei, Hokkaido University, Sapporo, Japan;
†Department of Dermatology, Nara Medical University School of Medicine, Kashihara, Japan

Correspondence and reprint requests to:
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 *de novo* ABCA12 mutation

Abbreviations: ATP-binding cassette, ABC; HI, harlequin ichthyosis; LG, lamellar granule

Abstract

Harlequin ichthyosis (HI) is one of the most devastating genodermatoses. Recently, *ABCA12* mutations were identified as the cause of HI. A newborn Japanese male demonstrated the typical features of HI. The patient was treated with oral etretinate and his general condition has been good (now aged 1.5 years). This patient with moderate clinical severity was compound heterozygous for a novel *de novo* missense mutation 1160G>A (S387N) in exon 10 and a maternal deletion mutation 4158_4160delTAC (T1387del) in exon 28 of *ABCA12*. T1387del was a deletion of a highly conserved threonine residue within the first ATP-binding domain and is thought to seriously affect the function of the ABCA12 protein. Conversely, the residue 387 is located outside the known active sites of ABCA12 and S387N is predicted not to lead to a serious functional deficiency in ABCA12. Electron microscopy revealed abnormal lamellar granules in the granular layer cells and a moderate number of lipid vacuoles in the cornified cells. Disturbed glucosylceramide transport was confirmed in the cultured keratinocytes from the patient. No *de novo* mutation in *ABCA12* has yet been reported either in HI or lamellar ichthyosis. The present case suggested that a *de novo ABCA12* mutation might underlie HI.

Abstract Word Count: 199 words

Key words barrier/ epidermis/ glucosylceramide/ lamellar granule/ lipid transporter

Harlequin ichthyosis (HI) (MIM #242500) is a severe and often fatal congenital ichthyosis with an autosomal recessive inheritance pattern (Williams and Elias, 1987; Akiyama, 1999; Judge *et al*, 2004).

In 2005, ABCA12 mutations were identified in HI families (Akiyama *et al*, 2005; Kelsell *et al*, 2005). The pathomechanisms of HI became known when functional defects in the lipid transporter ABCA12 were shown to cause abnormal lipid transport via lamellar granules (LGs) in the keratinocytes, resulting in malformation of the patient's intercellular lipid layers of the stratum corneum (Akiyama *et al*, 2005). However, genotype/phenotype correlations in HI cases with ABCA12 mutations have yet to be fully elucidated (Akiyama, in press a).

In the study of this case, we have found a compound heterozygous ABCA12 combination of mutations, a novel *de novo* missense mutation, S387N, in exon 10 and a maternal deletion mutation T1387del in exon 28 in a newborn HI baby. In the majority of HI patients, deletion or truncation mutations seriously affecting ABCA12 function were found. However, in the present case, one mutation was a missense mutation located in the cytoplasmic region of ABCA12 polypeptide, not in the transmembrane domain or the ATP binding cassettes. The nature and site of mutations might be the key to the relatively moderate phenotype of this HI patient.

In addition, the novel mutation S387N was the first reported *de novo* mutation in *ABCA12*. Our case suggested the possibility that *de novo* mutations can cause the occurrence of HI in non-consanguineous families.

RESULTS

Clinico-pathological features of the patient

The patient was a newborn Japanese male. He was born at 33 weeks and 3 days pregnancy by premature, vaginal delivery (body weight 1876g). He was the first child of non-consanguineous healthy parents. There was no family history of congenital ichthyosis. At birth, the patient had presented with severe hyperkeratosis over his entire body, severe ectropion, eclabium and malformed pinnae (**Fig 1**). The patient's clinical features at birth were characteristic of typical HI. He was admitted into a neonatal intensive care unit and had oral etretinate treatment (1 mg/kg/day). At this time, light microscopy of the lesional skin sample from the patient's trunk showed marked hyperkeratosis with only a small number of parakeratotic cells. At the age of one year, the patient showed dark brownish, thick scales over the entire body surface including the face, palms and the soles.

ABCA12 mutation analysis

Mutation analysis of the 53 exons including the intron-exon boundaries of the entire ABCA12 gene revealed a 1160G>A transition in exon 10 and one previously reported deletion mutation 4158_4160delTAC in exon 28 in each allele in a compound heterozygous fashion in the patient [sequence according to Lefèvre *et al*. (2003)] (GenBank accession NM 173076) (**Fig 2**). The mutation 1160G>A transition was a novel missense mutation that changed a serine residue of codon 387 to an asparagine residue (S387N). This missense mutation S387N was not found in either in the parent's (father or mother), although the patient's mother was heterozygous for the deletion mutation 4158_4160delTAC (**Fig 2**). Thus, the missense mutation S387N was thought to be a *de novo* mutation and the deletion mutation was a maternal mutation. Paternity testing by microsatellite marker analysis proved the father was the patient's true genetic father (data not shown). These mutations were not found in 200 normal, unrelated Japanese alleles (100 normal unrelated Japanese individuals) by sequence analysis, and were unlikely to be a polymorphism (data not shown).

The deletion mutation 4158_4160delTAC led to an in-frame deletion of a highly conserved threonine residue at codon 1387 (T1387del) within the first ATP-binding domain of ABCA12. Thus, the deletion mutation is thought to seriously affect either the function or specific critical structures of the ABCA12 protein (Akiyama *et al*, 2005).

Ultrastructure of the patient's skin

Electron microscopy revealed that, in the keratinocyte cytoplasm of the granular layer, no normal LGs were apparent. Lipid droplets, vacuoles and multivesiculated bodies were seen in keratinocytes in the keratinized and granular cell layers (**Figs 3a,b**). The number of abnormal lipid droplets in the keratinized stratum corneum cells was smaller than that in skin samples from HI patients harboring the homozygous splice acceptor site mutation IVS23-2A>G of ABCA12 (Akiyama *et al*, 2005) (**Fig 3c**), although the number of lipid vacuoles was larger than those in lamellar ichthyosis patients with ABCA12 mutations (data not shown). Cornified cell envelope formation was normal in the granular and cornified layer cells (**Fig 3e**).

Distribution of ABCA12 and glucosylceramide in patient's skin

Immunofluorescence studies revealed that ABCA12 was positive in the upper epidermal layers, mainly in the granular layers, of normal human skin (Fig 4c). In the epidermis of the present patient, reduced ABCA12 immunostaining was seen in the upper epidermis (Fig 4a), compared with seriously reduced immunolabeling in epidermal keratinocytes from the HI patient harboring a homozygous splice acceptor site mutation IVS23-2A>G (Fig 4b) (Akiyama *et al*, 2005). These findings confirmed that the present patient expresses a significant amount of mutated ABCA12 protein in his epidermis.

Immunofluorescent staining showed that glucosylceramide, a major lipid component of LGs (Vielhaber *et al*, 2001; Ishida-Yamamoto *et al*, 2004) and an

essential component of the epidermal permeability barrier (Holleran *et al*, 1993), was sparsely distributed in the patient's upper epidermis (Fig 4a), compared with a restricted, intense distribution in the granular layers of normal skin (Fig 4c). The condensed glucosylceramide staining was not seen in the patient's granular layers (Fig 4a).

Abnormal glucosylceramide transport in patient's cultured keratinocytes

Culture of the patient's keratinocytes under high-Ca^{2+} conditions (2.0 mM) induced a large number of cells to express condensed glucosylceramide staining around the nuclei, and this glucosylceramide failed to become localized to the periphery of the keratinocyte cytoplasm (Fig 5a). Culture of normal human keratinocytes in high-Ca^{2+} conditions exhibited a more diffuse glucosylceramide staining throughout the cytoplasm (Fig 5b).

DISCUSSION

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 (Higgins, 1992; Allikmets *et al*, 1996; Dean *et al*, 2001; Borst and Elferink, 2002) and, recently, this superfamily has been highlighted in the dermatology fields (Uitto, 2005). The ABCA subfamily is suggested to work in lipid transport (Hayden *et al*, 2000; Orso *et al*, 2000; Schmitz and Langmann, 2001; Weng *et al*, 1999; Peelman *et al*, 2003) and has received considerable attention (Klein *et al*, 1999) because mutations in these genes have been implicated in several human genetic diseases (Allikmets *et al*, 1997a; Allikmets *et al*, 1997b; Brooks-Wilson *et al*, 1999; Rust *et al*, 1999; Oram, 2002). Keratinocyte LGs are known lipid transporting organelles and LG contents are secreted into the intercellular space, forming an intercellular lipid layer between the granular layer cells and keratinized cells in the stratum corneum. Our previous study (Akiyama *et al*, 2005) has clearly demonstrated

that ABCA12 functions in the transport of endogenous lipid to the keratinocyte cell periphery via LGs.

The abnormal LGs in the granular layer keratinocytes and a lack of extracellular lipid lamellae reflect the defective lipid transport via LGs and the malformation of intercellular stratum corneum lipid layer in HI (Akiyama, in press a). In 2005, *ABCA12* mutations that seriously affect its function were shown to cause a loss of the skin lipid barrier, leading to HI (Akiyama *et al*, 2005). In addition to HI with defective lipid layers in the stratum corneum, ichthyosis syndromes are also thought to share similar pathomechanisms (Akiyama, in press b). For example, Dorfman-Chanarin syndrome (neutral lipid storage disease) showed malformation of LGs and defective lipid production in LGs caused by a deficiency in the CGI-58 protein that is thought to be involved in the pathogenesis of this form of ichthyosis (Akiyama *et al*, 2003). In Sjögren-Larsson syndrome harboring fatty aldehyde dehydrogenase (FALDH) gene (ALDH3A2) mutations, defective LG formation was reported as one sign of a putative pathogenetic mechanism (Shibaki *et al*, 2004). Recently, mutations in a new gene, FLJ39501, encoding a cytochrome P450, family 4, subfamily F, polypeptide 2 homolog of the leukotriene B4-ω-hydroxylase (CYP4F2) were reported to underlie lamellar ichthyosis cases linked to chromosome 19p12-q12 (Lefèvre *et al*, 2006). These facts further support the idea that abnormal LG lipid contents and defective intercellular lipid are prevailing concepts of pathogenetic mechanisms in the ichthyoses.

In the present case based on the reported amino acids sequence (Annilo *et al*, 2002), the deletion mutation 4158_4160delTAC led to an in-frame deletion of a threonine residue at codon 1387 (T1387del) within the first ATP-binding domain of ABCA12 protein (**Fig 6**). This threonine residue is a highly conserved residue between diverse species and is thought to be important in the

function of ABCA12 (Akiyama *et al*, 2005). Indeed, a compound heterozygous patient with this deletion mutation and a separate ABCA12 truncation mutation was previously reported as a typical HI newborn who died 15 days after birth (Akiyama *et al*, 2005).

The novel *de novo* mutation S387N in the present patient is located outside all of the known ABCA12 active transporter sites, within the cytoplasmic domain between the transmembrane domains and the first ATP binding cassette (**Fig 6**). Both serine and asparagine are neutral amino acids with small side chains and this missense mutation would be expected to not significantly affect the conformation of ABCA12 molecule. Thus, one would predict the *de novo* missense mutation S387N not to lead to a serious ABCA12 functional loss. Considering the nature and site of the *ABCA12* mutations in this case, we suggest that a combination of the deletion mutation and the missense mutation leads to the current patient's HI phenotype with a moderate clinical severity.

In type 2 lamellar ichthyosis, a relatively mild form of congenital ichthyosis, all five reported *ABCA12* mutations were missense mutations that resulted in only one amino acid alteration (Lefèvre *et al*, 2003). Conversely, in our previous study on HI families, no *ABCA12* missense mutations were identified and most of the defects led to severe truncation of ABCA12 peptide, affecting important nucleotide binding fold domains and/or transmembrane domains (Akiyama *et al*, 2005). The other, non-truncation mutations in HI were deletion mutations affecting highly conserved *ABCA12* sequences (Akiyama *et al*, 2005). Thus, it was thought that only truncation or deletion mutations in conserved regions, which seriously affect the function of the ABCA12 transporter protein, can lead to the HI phenotype.

In an additional series of HI patients, most ABCA12 mutations were homozygous truncation mutations (Kelsell *et al*, 2005). Only one mutation in one HI patient was a missense mutation and the patient was heterozygous for a truncation mutation and the missense mutation.

Recently, the prognosis of newborns affected with HI has improved, owing to better targeted oral retinoid treatment. More than half of HI newborns including cases with a serious functional loss of ABCA12 survive beyond the perinatal period (Akiyama *et al*, 2005; Akiyama, in press a). Thus, it is difficult to discuss genotype/phenotype correlations and quality of life prognosis for ABCA12 mutations in HI.

Most families with HI or lamellar ichthyosis caused by *ABCA12* mutations were consanguineous and, in those families, causative *ABCA12* mutations were homozygous mutations inherited with an autosomal recessive trait. No *de novo* mutation has yet been reported in *ABCA12*, as far as we know (Lefèvre *et al*, 2003; Akiyama et al, 2005; Kelsell *et al*, 2005). The present novel missense mutation S387N in our case was the first *de novo* mutation reported in ABCA12. The genetic information of whether the causative mutations are inherited or *de novo* is very important for genetic counseling in severe genetic disorders such as HI. Indeed, in the present family, the parents had requested a prenatal diagnosis of HI for their subsequent pregnancy. However, we have demonstrated that the missense mutation was a *de novo* mutation and that the patient's father was not a carrier of any *ABCA12* mutation. Thus, the family could be spared of the worry of an unnecessary HI prenatal diagnosis for the subsequent pregnancy almost, although we cannot completely exclude the possibility that the father has a mosaic mutation affecting his germ line. The present case suggested that, in non-consanguineous HI families, a *de novo ABCA12* mutation might be one

source of mutations and underlines that the detection of causative *ABCA12* mutations is essential for the genetic counseling for HI.

MATERIALS AND METHODS

Mutation detection Mutational analysis was performed in the affected baby and the parents. Briefly, genomic DNA isolated from peripheral blood 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.* (2003) 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 Japanese controls were sequenced. No mutations were found in 200 normal alleles from the healthy Japanese population. Paternity testing was carried out using 16 microsatellite markers.

Ultrastructural observations Skin biopsy samples were fixed in 5 % glutaraldehyde solution, post-fixed in 1% OsO_4, 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. As controls, lesional skin samles from lamellar ichthyosis patients with transglutaminase 1 gene mutations (Akiyama *et al*, 2001) or *ABCA12* mutations were also studied.

Cell culture

A skin sample from the patient was processed for primary keratinocyte culture, and cells were grown according to standard procedures in defined

keratinocyte serum-free medium (Invitrogen Corp, Carlsbad, CA). After several passages in low-Ca^{2+} (0.09 mM) conditions, cultures were grown in high-Ca^{2+} (2.0 mM) conditions.

Antibodies Polyclonal anti-ABCA12 antiserum was raised in rabbits using a 14 amino acids sequence synthetic peptide (residues 2567-2580) derived from the ABCA12 sequence (NM 173076) as the immunogen (Akiyama *et al*, 2005). The other primary antibody was mouse monoclonal anti-glucosylceramide antibody (Alexis Biochemicals, Lausanne, Switzerland).

Immunofluorescent labeling Immunofluorescent labeling was performed as previously described (Akiyama *et al*, 2000). Briefly, 6-μm-thick sections of fresh patient's skin was cut using a cryostat. The sections were incubated in primary antibody solution for 1 h at 37˚C. Antibody dilutions were as follows; 1/10 for anti-ABCA12 antiserum and 1/10 for anti-glucosylceramide antibody. The sections were then incubated in fluorescein isothiocyanate-conjugated to rabbit anti-mouse immunoglobulins and TRITC-conjugated goat anti-rabbit immunoglobulins diluted 1:100 (DAKO, Glostrup, Denmark) for 30 min at room temperature, followed by nuclear counterstain by TO-PRO-3 (Invitrogen, Carlsbad, CA, USA). The sections were extensively washed with phosphate-buffered saline between incubations. The stained sections were then mounted with a cover slip and observed using a confocal laser scanning microscope.

ACKNOWLEDGMENTS

We thank Ms. Megumi Sato and Ms. Akari Nagasaki for their fine technical assistance on this project.

This work was supported in part by Grant-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan to M. Akiyama (Kiban B 16390312).

REFERENCES

Akiyama M: The pathogenesis of severe congenital ichthyosis of the neonate. J Dermatol Sci 21: 96-104, 1999

Akiyama M: Pathomechanisms of harlequin ichthyosis and ABC transporters in human diseases. Arch Dermatol (in press a)

Akiyama M: Harlequin ichthyosis and other autosomal recessive congenital ichthyoses: the underlying genetic defects and pathomechanisms. J Dermatol Sci (in press b)

Akiyama M, Sawamura D, Nomura Y, Sugawara M, Shimizu H: Truncation of CGI-58 protein causes malformation of lamellar granules resulting in ichthyosis in Dorfman-Chanarin syndrome. J Invest Dermatol 121: 1029-1034, 2003

Akiyama M, Sugiyama-Nakagiri Y, Sakai K, McMillan JR, Goto M, Arita K, Tsuji-Abe Y, Tabata N, Matsuoka K, Sasaki R, Sawamura D, Shimizu H: Mutations in ABCA12 in harlequin ichthyosis and functional rescue by corrective gene transfer. J Clin Invest 115: 1777-1784, 2005

Akiyama M, Smith LT, Shimizu H: Changing patterns of localization of putative stem cells in developing human hair follicles. J Invest Dermatol 114: 321-327, 2000

Akiyama M, Takizawa Y, Suzuki Y, Ishiko A, Matsuo I, Shimizu H: Compound heterozygous TGM1 mutations including a novel missense mutation L204Q in a mild form of lamellar ichthyosis. J Invest Dermatol 116: 992-995, 2001

Allikmets R, Singh N, Sun H, Shroyer NF, Hutchinson A, Chidambaram A, Gerrard B, Baird L, Stauffer D, Peiffer A, et al.: A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. Nat Genet **15:** 236-246, 1997a

Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Hutchinson A, et al.: Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science **277:** 1805-1807, 1997b

Allikmets R, Gerrard B, Hutchinson A, Dean M: Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. Hum Mol Genet **5:** 1649-1655, 1996

Annilo T, Shulenin S, Chen ZQ, Arnould I, Prades C, Lemoine C, Maintoux-Larois C, Devaud C, Dean M, Denefle P, et al.: Identification and characterization of a novel ABCA subfamily member, ABCA12, located in the lamellar ichthyosis region on 2q34. Cytogenet. Genome Res **98:** 169-176, 2002

Borst P, Elferink RO: Mammalian ABC transporters in health and disease. Annu Rev Biochem **71:** 537-592, 2002

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, et al.: Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet **22:**336-345, 1999

Dean M, Rzhetsky A, Allikmets R: The human ATP-binding cassette (ABC) transporter superfamily. Genome Res **11:** 1156-1166, 2001

Hayden MR, Clee SM, Brooks-Wilson A, Genest JJr, Attie A, Kastelein JJ: Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. Curr Opin Lipidol **11:** 117-122, 2000

Higgins CF: ABC transporters: from microorganisms to man. Annu Rev Cell Biol **8:** 67-113, 1992

Holleran WM, Takagi Y, Menon GK, Legler G, Feingold KR, Elias PM: Processing of epidermal glucosylceramides is required for optimal mammalian cutaneous permeability barrier function. J Clin Invest **91:** 1656-1664, 1993

Ishida-Yamamoto A, Simon M, Kishibe M, Miyauchi Y, Takahashi H, Yoshida S, O'Brien TJ, Serre G, Iizuka H: Epidermal lamellar granules transport different cargoes as distinct aggregates. J Invest Dermatol **122:** 1137-1144, 2004

Judge MR, McLean WHI, Munro CS: Disorders of keratinization. In: Rook/Wilkinson/Ebling: Textbook of Dematology. T. Burns, S. Breathnach, N. Cox, and C. Griffiths, editors. Blackwell Science, Oxford, UK. 34.1-34.111, 2004

Kelsell DP, Norgett EE, Unsworth H, The M-T, et al.: Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis. Am J Hum Genet 76: 794-803, 2005

Klein I, Sarkadi B, Varadi A: An inventory of the human ABC proteins. Biochim Biophys Acta **1461:** 237-262, 1999

Lefèvre C, Audebert S, Jobard F, Bouadjar B, Lakhdar H, Boughdene-Stambouli O, Blanchet-Bardon C, Heilig R, Foglio M, Weissenbach J, et al. Mutations in the transporter ABCA12 are associated with lamellar ichthyosis type 2. Hum Mol Genet **12:** 2369-2378, 2003

Lefèvre C, Bouadjar B, Ferrand V, Tadini G, Mégarbané A, Lathrop M, Prud'homme J-F, Fischer J: Mutations in a new cytochrome P450 gene in lamellar ichthyosis type 3. Hum Mol Genet 2006 (published on line)

Oram JF: Molecular basis of cholesterol homeostasis: lessons from Tangier disease and ABCA1. Trends Mol Med **8:** 168-173, 2002

Orso E, Broccardo C, Kaminski WE, Bottcher A, Liebisch G, Drobnik W, Gotz A, Chambenoit O, Diederich W, Langmann T, et al.: Transport of lipids from Golgi to plasma membrane is defective in tangier disease patients and Abc1-deficient mice. Nat Genet **24:** 192-196, 2000

Peelman F, Labeur C, Vanloo B, Roosbeek S, Devaud C, Duverger N, Denefle P, Rosier M, Vandekerckhove J, Rosseneu M: Characterization of the ABCA transporter subfamily: identification of prokaryotic and eukaryotic members, phylogeny and topology. J Mol Biol **325:** 259-274, 2003

Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, Assmann G: Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet

22: 352-355, 1999

Schmitz G, Langmann T: Structure, function and regulation of the ABC1 gene product. Curr Opin Lipidol **12:** 129-140, 2001

Shibaki 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 123: 1197-1199, 2004

Uitto J: The gene family of ABC transporters – novel mutations, new phenotypes. Trends Mol Med **11:** 341-343, 2005

Vielhaber G, Pfeiffer S, Brade L, Lindner B, Goldmann T, Vollmer E, Hintze U, Wittern KP, Wepf R: Localization of ceramide and glucosylceramide in human epidermis by immunogold electron microscopy. J Invest Dermatol **117:** 1126-1136, 2001

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 **98:** 13-23, 1999

Williams ML, Elias PM: Genetically transmitted, generalized disorders of cornification; the ichthyoses. Dermatol Clin **5:** 155-178, 1987

FIGURE LEGENDS

Figure 1. The patient showed a typical clinical phenotype of HI in the neonatal period. The entire body surface was covered with thick plate-like scales and fissures.

Figure 2. Compound heterozygous mutations of ABCA12 in the patient. (a) Direct sequencing revealed a heterozygous 1160G>A transition (a missense mutation S387N) in exon 10 of *ABCA12* of the patient, but not in his parents or normal control samples. (b) A heterozygous deletion mutation 4158_4160delTAC (T1387del) was found in exon 28 of *ABCA12* of the patient and his mother, but not in his father or in normal controls.

Figure 3. Abnormal lamellar granules (LGs) in the granular layer cells and accumulation of lipid droplets in the stratum corneum cells of the patient's epidermis.

(a) Abnormal LGs in the granular layer cells of the patient. (b) Abnormal lipid droplets accumulated in the cornified cells in the patient's epidermis, although the numbers and amounts of lipid droplets was smaller than that seen in an HI patient harboring a homozygous splice acceptor site mutation IVS23-2A>G of ABCA12 (c). (d) No lipid droplets were observed in control normal human stratum corneum cells. (a,b) the present patient; (c) control typical HI patient reported previously (Akiyama et al, 2005); (d) normal control human skin. (e) Cornified cell envelope (arrows) with normal thickness was seen in the cornified layer cells in the present HI patient. Inset: the cornifed layer cell of the control lamellar ichthyosis patient with transglutaminase 1 gene mutations showed malformed, thin cornified cell envelope (arrowheads). Bars, 200 nm.

Figure 4. Abnormal ABCA12 immunostaining and disturbed distribution of glucosylceramide in the patient's epidermis.

(a) In the patient's upper epidermis, weak ABCA12 immunostaining (red: arrows) was diffusely seen in the keratinocyte cytoplasm. Glucosylceramide labeling (green: arrowheads) was also diffusely observed in the keratinocytes of upper epidermis. (b) In the control HI patient carrying a homozygous splice acceptor site mutation IVS23-2A>G of ABCA12, ABCA12 immunolabeling (red) was very weak, although diffuse glucosylceramide immunolabeling (green: arrowheads) was seen in the keratinocyte cytoplasm of upper epidermis. (c) In normal control human epidermis, both ABCA12 (red) and glucosylceramide (green) labelings overlapped, resulting in a yellow color in the granular layer (arrows). Dotted line; granular layer-cornified layer interface. ABCA12, TRITC (red); glucosylceramide, FITC (green); nuclear staining, TO-PRO (blue). Bar, 10 μm.

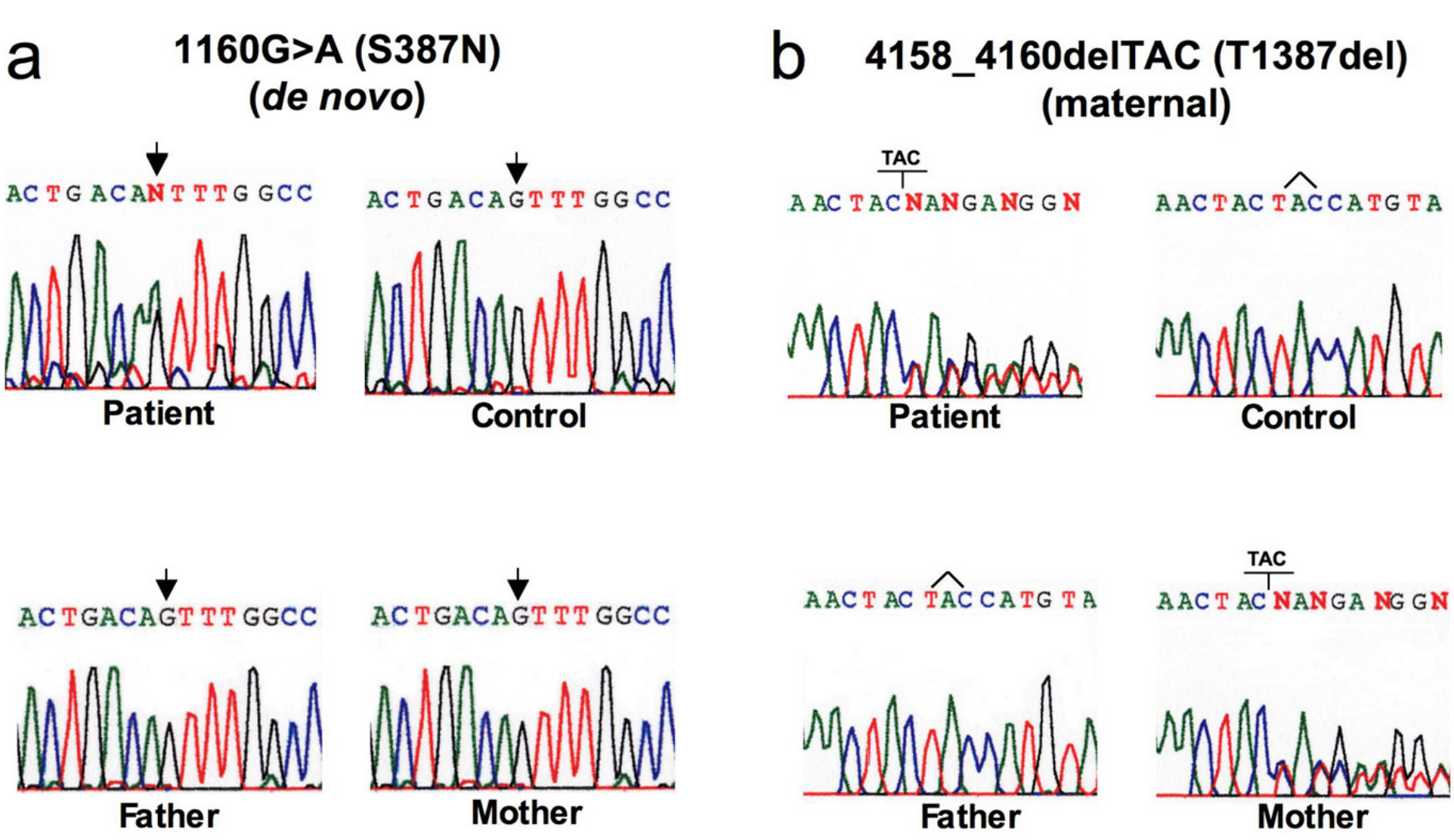
Figure 5. Altered glucosylceramide distribution in the patient's cultured keratinocytes.

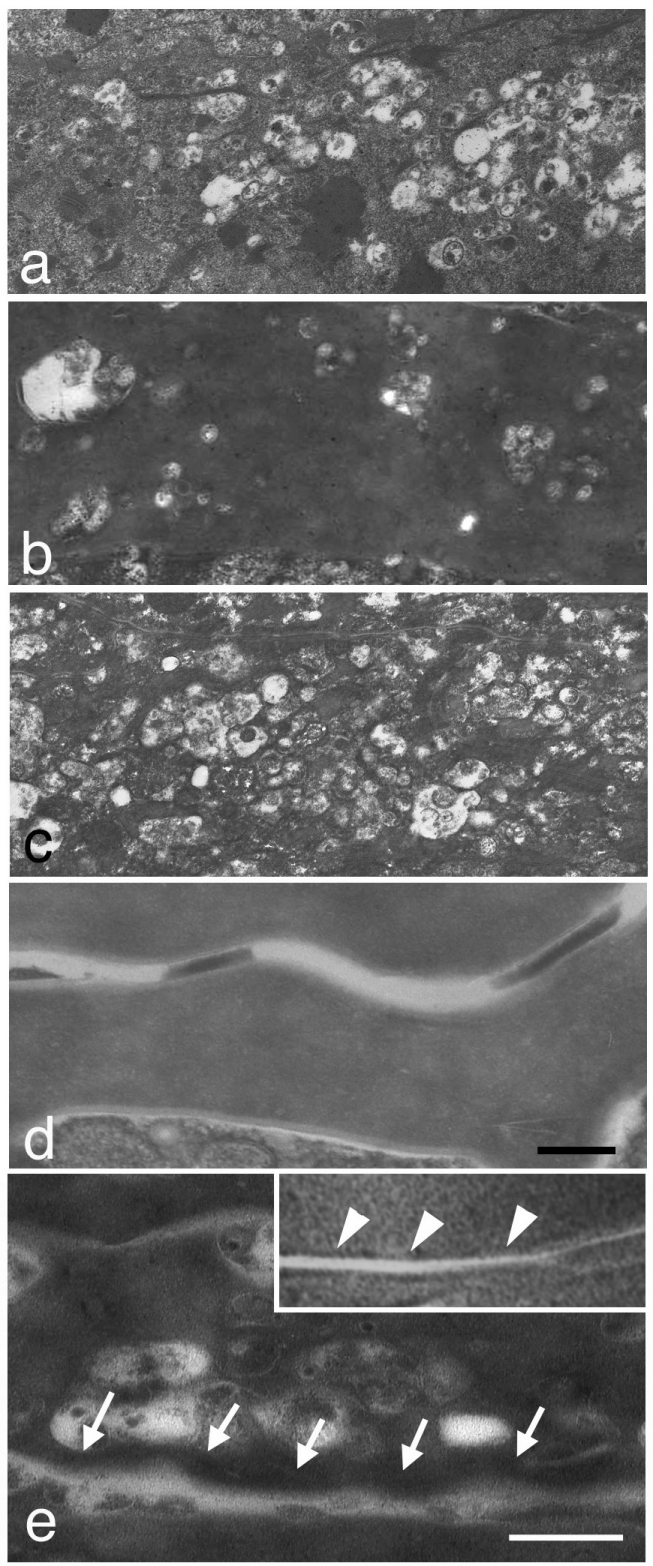
(a) In keratinocytes cultured from the patient, glucosylceramide labeling (green) was observed mainly in the perinuclear area of cytoplasm, suggesting disturbed glucosylceramide transport. (b) In normal cultured human keratinocytes, glucosylceramide (green) was seen diffusely in the cytoplasm to the cell periphery. Glucosylceramide, FITC (green); nuclear staining, propidium idodide (red). Bar, 10 μm.

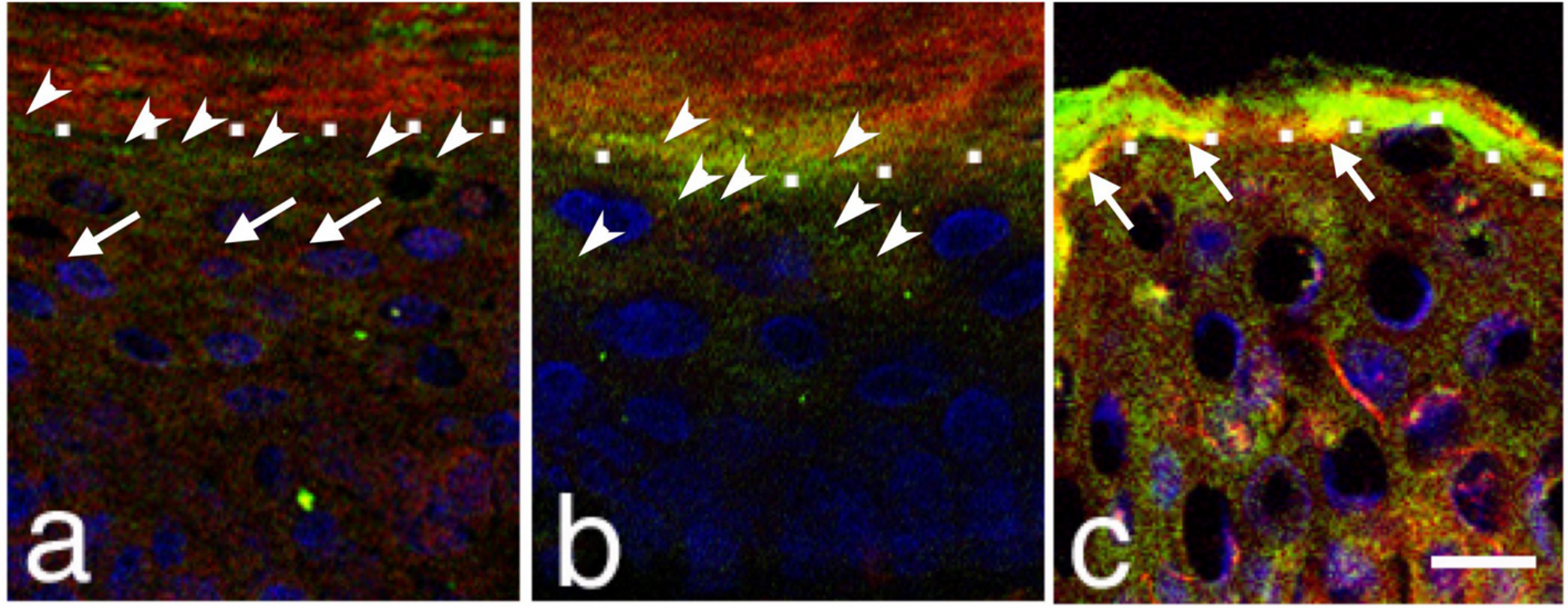
Figure 6. Schematic sequential arrangement of the domain structures of ABCA12 protein and the position of mutations in the present HI patient.

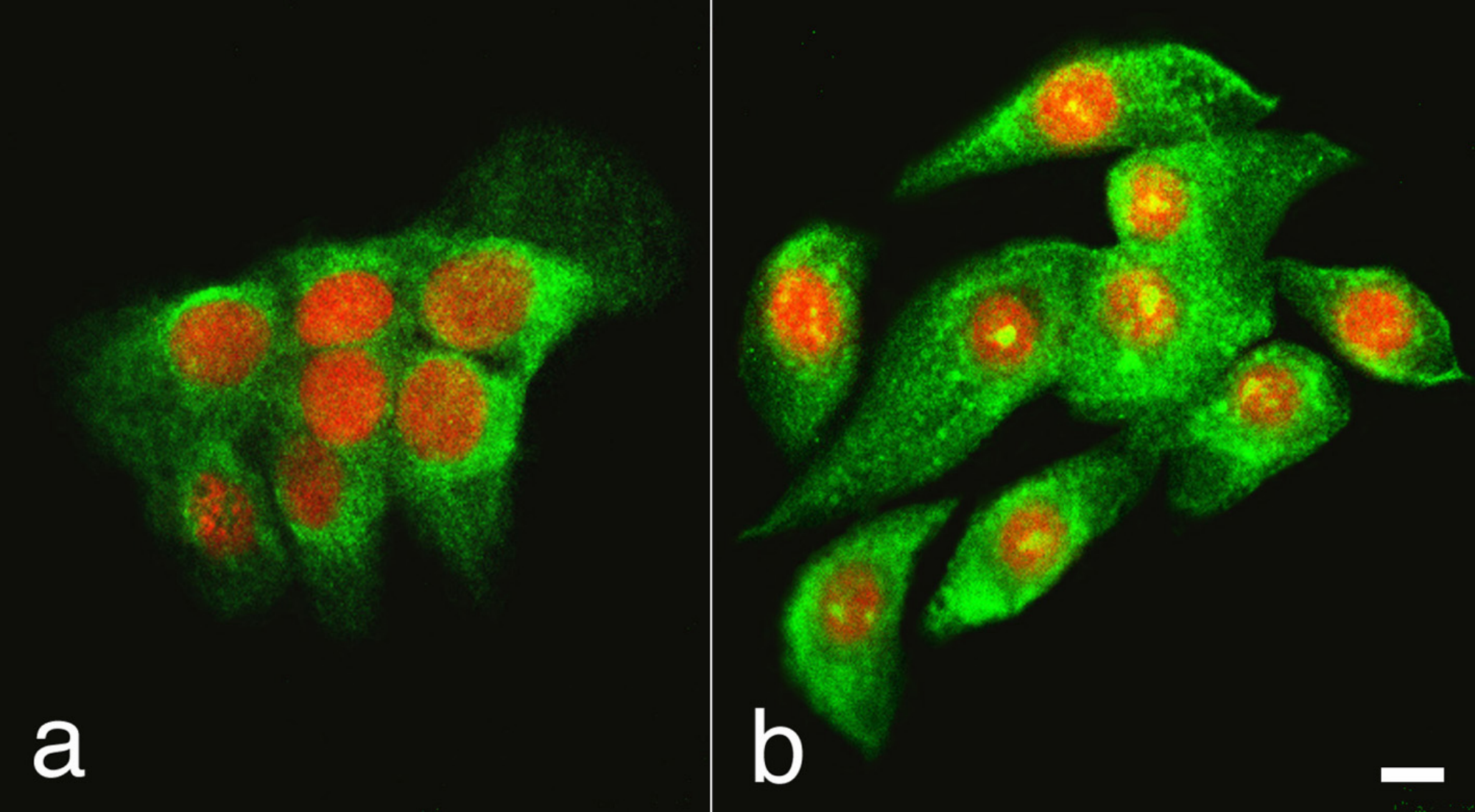
Mutations in the present HI patient are marked by red arrows. Note that the *de novo* missense mutation S387N is located in the intracytoplasmic region between the N-terminus and the first transmembrane domain, not in any active sites, and the other, deletion mutation T1387del is within the first ATP-binding cassette, which is thought to be important for ABCA12 lipid transporter activity.

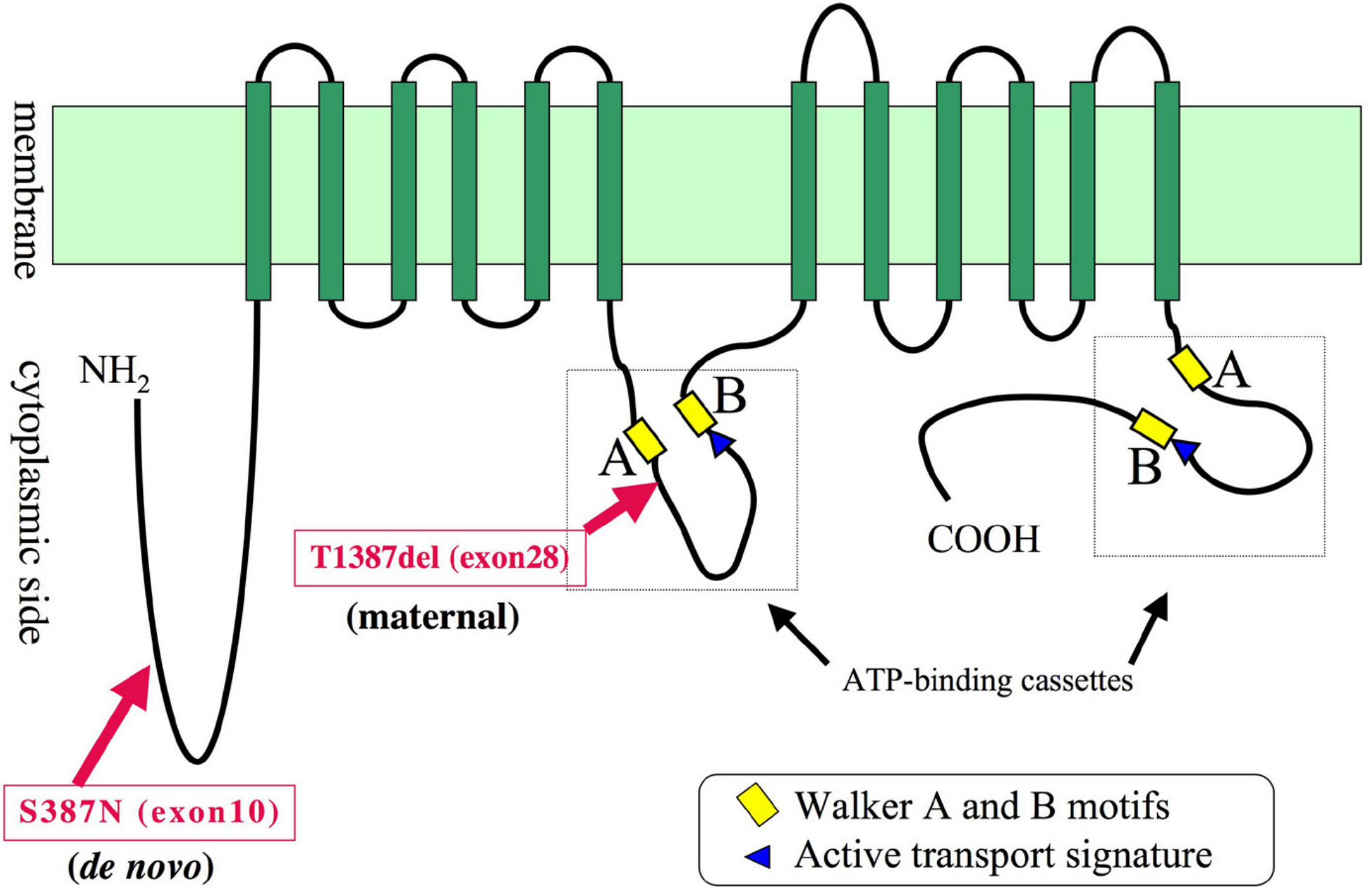
[redacted]


a
1160G>A (S387N)
(de novo)
ACTGACANTTTGGCC
Patient
ACTGACAGTTTGGCC
Control
ACTGACAGTTTGGCC
Father
ACTGACAGTTTGGCC
Mother
b
4158_4160delTAC (T1387del)
(maternal)
TAC
AACTACNANGANGGN
Patient
AACTACTACCATGTA
Control
AACTACTACCATGTA
Father
TAC
AACTACNANGANGGN
Mother


a
b
c
d
e


a
b
c


a
b


membrane
cytoplasmic side
NH2
A
B
A
B
A
B
COOH
T1387del (exon28)
(maternal)
ATP-binding cassettes
S387N (exon10)
(de novo)
Walker A and B motifs
Active transport signature