exposures require confirmation in case-controlled studies to determine which are associated with disease onset and whether these play any role in etiology.

To our knowledge, this is the first epidemiologic study on anti-MDA-5 antibodies. Although it is difficult to draw strong conclusion from a single cohort study, epidemiologic studies play an important role in disease assessment. These studies determine the extent of disease and the natural history within a community, identify potential etiologic factors and enhance our understanding of disease pathogenesis.

Conclusions

Clinically amyopathic dermatomyositis might be growing in prevalence with the increase of anti-MDA-5 antibody-positive patients in central Japan. Regional differences in the incidences of the anti-MDA-5 antibody would suggest that environmental factors contribute to the production of autoantibodies against MDA-5. It will be important to conduct larger population-based studies through multicenter collaboration using DM-specific autoantibodies to define patient groups and clarify the disease etiology associated with environmental factors.

Abbreviations

CADM: clinically amyopathic dermatomyositis; DM: dermatomyositis; ILD: interstitial lung disease; MDA-5: melanoma differentiation-associated gene 5.

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Authors' contributions

YM, KS and KH organized the patient registry. YM and KH performed laboratory assays. KT participated in the design of the study and performed the statistical analysis. YM conceived of the study design and wrote the manuscript with input and consensus from all authors. KS and MA participated in the coordination of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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REVIEW ARTICLE

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Malignant skin tumours in patients with inherited ichthyosis

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Summary

Inherited ichthyoses are rare genodermatoses caused by mutations in the genes involved in epidermal development. Although there have been case reports on patients with ichthyosis who developed skin malignancies, it is still unknown whether or not patients with ichthyosis have an increased risk of skin malignancies. Here, we review case series of skin malignancies in patients with ichthyosis and show biological findings which might lead to cancer susceptibility. A survey of the literature revealed 28 cases of inherited ichthyoses with skin malignancy, including 12 cases of keratitis–ichthyosis–deafness (KID) syndrome, seven of autosomal recessive congenital ichthyosis, three of Netherton syndrome and six of miscellaneous ichthyosis. Twenty-four of the 28 cases developed single or multiple squamous cell carcinomas (SCCs). The age at diagnosis of the first skin malignancy ranged from 15 to 54 years. As patients with these particular subtypes of ichthyosis seem to be prone to skin malignancies, including SCC, at an unusually young age, routine cancer surveillance of these patients is strongly recommended.

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Skin cancer poses a serious problem in patients with inherited disorders, such as Gorlin syndrome, Cowden syndrome, xeroderma pigmentosum and epidermolysis bullosa. The prognosis for these patients is greatly influenced by skin malignancies, which develop at an unusually early age.

Ichthyoses are disorders characterized by skin dryness. Congenital ichthyoses are caused by mutations in the genes organizing keratinocyte differentiation and skin barrier function, although some of the causative genes are still undetermined.[1] There have been sporadic case reports of skin malignancies in patients with congenital ichthyosis. However, the epidemiology among these patients remains unknown because of the limited number of cases.

This review article summarizes skin malignancies in congenital ichthyoses described in the English language literature and discusses the biological background underlying skin barrier defects and carcinogenesis.

Skin malitgnancies in each ichthyosis subtype

Twenty-eight cases of skin malignancy in congenital ichthyoses were found in the literature: 12 cases of keratitis–ichthyosis–deafness (KID) syndrome, seven of autosomal recessive congenital ichthyosis (ARCI), three of Netherton syndrome (NS) and six of miscellaneous ichthyosis. The first malignancies were diagnosed at the ages of 15–54 years. Reported skin malignancies include squamous cell carcinoma (SCC), basal cell carcinoma (BCC), malignant proliferating trichilemmal tumour (MPTT), malignant melanoma (MM), malignant fibrous histiocytoma and cutaneous lymphoma, although single or multiple SCC was the malignancy in most of the cases (24 out of 28). Table 1 summarizes the skin malignancies in patients with ichthyosis described in the literature.

Keratitis–ichthyosis–deafness syndrome

Keratitis–ichthyosis–deafness syndrome (KID) syndrome is an autosomal dominant disease characterized by congenital erythrokeratoderma as well as sensorineural deafness and eye involvement.[2,3] Heterozygous mutations in *GJB2*, which encodes connexin 26 (Cx26), are responsible for the disease.[4,5] Mutations in *GJB6*, the gene encoding connexin 30 (Cx30), are causal in some cases which overlap with Clouston syndrome.[6,7]

There are 12 reports of patients with sporadic KID syndrome in the literature who developed skin malignancies, including SCC and MPTT (Table 1).[5,8–15] The age of onset for SCC in KID syndrome is 15–43 years, which is earlier than that for SCC in the normal population (around the age of 70 years).[16,17] p.Asp50Asn in Cx26, the most prevalent muta-

Table 1 Skin malignancies in patients with ichthyosis

Ichthyosis subtype	Age at the diagnosis of first skin malignancy (years)	Skin malignancy	Causative gene	Reference
KID	35	SCC	NE	8
KID	28	Multiple SCC	NE	9
KID	43	SCC	NE	10
KID	38	SCC	GJB2	5
KID	31	Multiple SCC	GJB2	12
KID	31	Multiple MPTT	NE	11
KID	15	SCC	NE	13
KID	28	Multiple SCC/MPTT	GJB2	15
KID	24	Multiple MPTT	ND	15
KID	30	Multiple SCC	GJB2	14
KID	38	SCC	GJB2	14
KID	40	SCC	GJB2	14
CIE	44	SCC, MM	*ABCA12*	23, 32
CIE	37	MM, cutaneous lymphoma	*ABCA12*	23
CIE	43	Multiple SCC/BCC	NE	31
CIE	51	Multiple SCC/BCC	NE	31
CIE	25	SCC, MFH	NE	29
LI	27	Multiple SCC/BCC	NE	33
LI	33	Multiple BCC	NE	30
NS	23	Multiple SCC/BCC	NE	52
NS	29	Multiple SCC	NE	53
NS	29	Multiple SCC/BCC	NE	54
ICM	54	Multiple SCC	NE	63
ICM	40	Multiple SCC	NE	62
MAUIE	21?	SCC	NE	33
MAUIE	26	Multiple SCC	NE	64
EI	49?	Multiple SCC/BCC	NE	68
CHILD	29	SCC	NE	71

KID, keratitis–ichthyosis–deafness syndrome; CIE, congenital ichthyosiform erythroderma; LI, lamellar ichthyosis; NS, Netherton syndrome; ICM, ichthyosis Curth–Macklin; MAUIE, micropinnae, alopecia universalis, congenital ichthyosis and ectropion; EI, epidermolytic ichthyosis; CHILD, congenital hemidysplasia with ichthyosiform erythroderma and limb defects; SCC, squamous cell carcinoma; MPTT, malignant proliferating trichilemmal tumour; MM, malignant melanoma; MFH, malignant fibrous histiocytoma; BCC, basal cell carcinoma; NE, not examined; ND, not detected.

tion in KID syndrome, was found in six patients who developed SCC or MPTT.[5,12,14,15] SCC was reported in roughly 10% of patients with KID syndrome and has been proposed as a distinguishing manifestation of the disease.[5] In a recent case series, three out of 14 (21%) patients with KID syndrome developed SCC.[14] Recurrent and chronic infection of the skin in KID syndrome has been suggested to be partly responsible for the increased risk of SCC[8,13] or to be one of the many factors involved in multiple-step carcinogenesis.[15] Also, alteration of E-cadherin expression due to dysfunctional Cx26 is hypothesized to lead to cancer susceptibility.[5] Mutated Cx26 might lead to tumorigenesis through a decrease in gap junction communication, a possibility that is supported by a mouse carcinogenesis model.[18] Overexpression of Cx26 has been shown to suppress tumour growth and induce apoptosis in prostate cancer cells through Bcl-2 downregulation.[19]

In a mouse model for KID syndrome in which Cx26 harbouring the p.Ser17Phe mutation was introduced as a heterozygous mutation under control of the endogenous Cx26 promoter, the basal layer showed increased cell proliferation.[20] However, progressive skin growth and increased susceptibility to SCC were not observed.[20]

Autosomal recessive congenital ichthyosis

Congenital ichthyosiform erythroderma (CIE) and lamellar ichthyosis (LI) are two major types of ARCI.[1] CIE is characterized by fine, white scaling with erythroderma. In contrast, the typical manifestation of LI is coarse brown/dark scaling. Their causative genes are *ALOXE3*,[21] *ALOX12B*,[21] *ABCA12*,[22,23] *CYP4F22*,[24] *NIPAL4*[25] and *TGM1*.[26,27] CIE and LI have been proposed as representing variations of a single group of disorders, although the typical cases of each type have distinct clinical features.[28]

In the literature, five patients have been reported with CIE and two with LI who developed skin malignancies (Table 1).[23,29–33] They began to suffer from SCC between the ages of 25 and 51 years.[29,31–33] There is the possibility that chronic inflammation due to skin barrier defects is associated with skin carcinogenesis in CIE/LI patients,[23] as discussed in the section on KID syndrome. Scarring from chronic inflammation was suggested to underlie SCC in one CIE case,[32] although scar formation was not histologically evident in SCC specimens from two other patients with CIE.[31] The increased proliferation observed in CIE keratinocytes *in vitro*[34] might account for the early onset of SCC. It is notable that the long-term administration of systemic retinoids did not prevent SCC development in some patients with CIE,[31,32] although the retinoids might have reduced the number or severity of the SCCs.

Genetic analysis was performed on only two of the patients with CIE, both of whom had missense mutations in *ABCA12*.[23] The patients developed MM at the ages of 47 and 37, respectively. It is unclear why the ABCA12-deficient patients with CIE developed skin malignancies at those early ages. ABCA12 is an ATP-binding cassette (ABC) transporter that is thought to play a pivotal role in keratinocyte lipid transport.[35,36] ABCA12 is expressed mainly in keratinocytes, and not in melanocytes or lymphocytes.[35,37,38] A recent study also confirmed that ABCA12 is only weakly expressed in normal melanocytes and is largely absent in melanoma cells.[39] ABCA transporters are involved in regulating lipid transport and metabolism, and cholesterol levels may be a limiting factor in membrane maintenance in rapidly dividing cancer cells.[40] From these facts, it is unlikely that ABCA12 deficiency directly promotes skin tumorigenesis including that of MM. Other ABCA members that compensate for ABCA12 dysfunction might be related to tumorigenesis in patients with CIE.

Abca12-deficient mouse models have been developed, all of which showed neonatal lethality,[41–44] and these models

reproduce the severest subtype of ARCI: harlequin ichthyosis.[35,45] In one mouse model (*Abca12*-null mice), epidermal proliferation was not altered at E18.5 compared with wild-type mice.[43] From this finding, it is unlikely that loss of ABCA12 function directly causes proliferation of keratinocytes and leads to SCC development.

No patients with CIE or LI who developed skin malignancies have been reported to have mutations in *TGM1*, although TGM1 is thought to be the most prevalent causative gene for CIE/LI.[46,47] *TGM1* encodes tranglutaminase-1, which forms the cornified envelope (CE) in the cornified layer through cross-linking of CE precursor proteins.[47] Increased proliferation in the epidermis of the *Tgm1*-null neonate skin grafted onto athymic nude mice was observed,[48] which might imply that patients with CIE/LI with *TGM1* mutations might be susceptible to skin SCC.

Netherton syndrome

Netherton syndrome (NS) is an autosomal recessive disorder characterized by trichorrhexis invaginata (bamboo hair), congenital ichthyosis and atopic diathesis.[49,50] NS is caused by mutations in *SPINK5*, which encodes the serine protease inhibitor LEKTI.[51]

Three NS cases have been reported who developed skin malignancies (Table 1).[52–54] Surprisingly, multiple SCCs (or multiple BCCs) were observed for these patients in their twenties. In one patient, epidermodysplasia verruciformis-associated human papillomavirus (HPV) DNA (HPV-19, -23, -38 and HPV-RTRX9) was preferentially detected in malignant lesions.[52] The authors speculated that impaired epidermal defence mechanisms could have promoted latent HPV DNA persistence in the patient's skin.[52] However, polymerase chain reaction amplification using HPV universal primers failed to detect HPV DNA in tumour specimens of another patient.[54] This shows that HPV infection is not always responsible for skin carcinogenesis in patients with NS at an early age. Patients with NS show recurrent infections other than HPV.[55] From the findings that several immunological abnormalities including those of memory B cells and natural killer cells are common in NS and that the patients respond well to intravenous immunoglobulin therapy,[55] it is possible to conclude that cognate and innate immunodeficiency might be associated with skin carcinogenesis in NS.

Although other serine protease inhibitors are implicated in skin carcinogenesis,[56,57] the role of LEKTI in skin cancers is unclear. NS mouse models in which LEKTI is deficient have been reported.[58–60] In one model, increased proliferation of the epidermis was observed,[59] which might underlie a susceptibility to SCC.

Miscellaneous

In each other form of ichthyosis, only a few cases have been described as having skin cancers. Ichthyosis Curth–Macklin (ICM) is a very rare form of keratinopathic ichthyosis that is characterized by massive spiky hyperkeratosis.[1,61] Mutations in the V2 domain of keratin 1 have been reported in patients with ICM. Two patients developed multiple SCC at the ages of 54 and 40 years, respectively (Table 1).[62,63] However, one patient had a history of whole-skin X-ray therapy, which might have led to the multiple skin cancers.[63]

Micropinnae, alopecia universalis, congenital ichthyosis and ectropion (MAUIE) syndrome is a syndromic form of ichthyosis that was not included in the revised nomenclature and classification of inherited ichthyoses.[1] Causative genes of MAUIE syndrome have not been reported. Two patients with MAUIE syndrome were found to have developed SCC in their twenties (Table 1).[33,64]

Epidermolytic ichthyosis (EI), formerly called bullous CIE, is a major subtype of keratinopathic ichthyosis[1] that is caused by mutations in the genes encoding keratin 1 or keratin 10 (*KRT1* or *KRT10*, respectively).[65–67] One patient with EI was reported to have multiple SCC/BCC (Table 1), although the patient had a history of whole-skin X-ray therapy.[68]

Congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome is a rare X-linked dominant disorder[69] that is caused by mutations in *NSDHL*.[70] One patient with CHILD syndrome developed SCC in the affected skin.[71]

Ichthyosis vulgaris, the most prevalent type of inherited ichthyosis, is caused by mutations in *FLG*, the gene encoding filaggrin.[72] To our knowledge, there have been no reports on the incidence of skin malignancies in ichthyosis vulgaris. Several cohort studies have reported cancer incidence in patients with atopic dermatitis (AD), in which loss-of-function mutations in *FLG* are a major predisposing factor.[73] Although many studies have confirmed that AD is associated with an increased risk of lymphoma, the estimated risk of nonmelanoma skin cancer (NMSC) in patients with AD differs among studies. Some studies reported an increased risk of NMSC in patients with AD,[74,75] whereas others demonstrated no association between NMSC and AD.[76,77] Further studies are needed to evaluate precisely the cancer risk in patients with ichthyosis vulgaris.

Future directions

Because of the limited number of patients with inherited ichthyoses, it is still almost impossible to calculate accurately the incidence of skin malignancies in these patients. However, our review of the literature shows that patients with ichthyosis can develop skin malignancies, mostly SCC, at an early age, although the literature may be biased in favour of describing only 'interesting' cases.

Generally, impaired barrier function in patients with ichthyosis might permit breech of the stratum corneum by contact chemical carcinogens. However, epithelial desquamation has been suggested as protecting against natural chemicals.[78,79] If this is true, one might guess that more rapid epidermal turnover in ichthyosis skin would be protective against, rather than contributory to, skin carcinogenesis. There are common

types of ichthyosis, such as ichthyosis vulgaris and recessive X-linked ichthyosis, which do not seem to be associated with skin cancer at a young age. On the other hand, patients with KID syndrome, ARCI and NS have been reported to develop SCC at an early age. These differences might be explained by causative genetic defects in each ichthyosis subtype.

Recent developments in bioengineering techniques have resulted in many animal models of inherited ichthyosis.[80] Experiments on ichthyosis skin carcinogenesis, including two-stage carcinogenesis assay, might provide clues to understanding the pathomechanisms underlying skin cancer in inherited ichthyosis, although neonatal lethality will prevent these experiments in several mouse models.

In the future, a worldwide registry on ichthyoses with follow-up information would be desirable towards enabling a full evaluation of skin malignancies in patients with ichthyosis. At present, routine surveillance for skin malignant changes is strongly recommended for patients with KID syndrome and inflammatory types of congenital ichthyosis such as CIE/LI and NS, even if the patients are taking systemic retinoids.

What's already known about this topic?

- There have been sporadic case reports of malignant skin tumours in patients with congenital ichthyosis.
- The frequency of skin malignancies in patients with ichthyosis is unknown.

What does this study add?

- Patients with congenital ichthyosis, especially those with KID syndrome, congenital ichthyosiform erythroderma, lamellar ichthyosis and Netherton syndrome, can develop cutaneous squamous cell carcinoma at unusually young ages.

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INVITED REVIEW ARTICLE

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UPDATED MOLECULAR GENETICS AND PATHOGENESIS OF ICHTHYOSES

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ABSTRACT

Research into the molecular genetics and pathomechanisms of ichthyoses have advanced considerably, resulting in the identification of several causative genes and molecules underlying the disease. In 2009, the First Ichthyosis Consensus Conference was held to establish a consensus for the nomenclature and classification of inherited ichthyoses, by which an international consensus for the classification of inherited ichthyosis was achieved. In this review, the pathogeneses of various ichthyoses are summarized based on their revised classification and terminology. Skin barrier defects are involved in the pathogenesis of various types of ichthyosis. The known causative molecules underlying ichthyosis include ABCA12, lipoxygenase-3, 12*R*-lipoxygenase, CYP4F22, ichthyin and steroid sulfatase, all of which are thought to be related to the intercellular lipid layers. ABCA12 is a known keratinocyte lipid transporter associated with lipid transport in lamellar granules and a loss of ABCA12 function leads to defective lipid transport in the keratinocytes, resulting in the most severe, harlequin ichthyosis phenotype. Other causative molecules for ichthyoses are transglutaminase 1, keratins and filaggrin. Transglutaminase 1 plays a role in cornified cell envelope formation. Keratins 1, 10 and 2 are involved in the keratin network of suprabasal keratinocytes and filaggrin is essential for the formation of keratohyalin granules. It is important to obtain information concerning genetic defects and to elucidate ichthyotic disease pathomechanisms for the establishment of an effective therapy and beneficial genetic counseling, including a prenatal diagnosis for families affected by ichthyotic disease.

Key Words: ABCA12; Congenital ichthyosiform erythroderma; Epidermolytic ichthyosis; Harlequin ichthyosis; Lamellar ichthyosis; Prenatal diagnosis

INTRODUCTION

The ichthyoses form a large, clinically and etiologically heterogeneous group of cornification disorders that typically affect all or most of the skin surface.[1] Six major distinct clinical subtypes are known in hereditary non-syndromic ichthyoses. Starting with the most severe form, they are: harlequin ichthyosis (HI, MIM#242500); lamellar ichthyosis (LI, MIM#242300); congenital ichthyosiform erythroderma (CIE, MIM#242100); epidermolytic ichthyosis (EI, MIM #113800); recessive X-linked ichthyosis (RXLI, MIM#308100); to the mildest form of ichthyosis vulgaris (IV, MIM#146700).[2] Superficial epidermolytic ichthyosis (SEI, MIM #146800) is an additional subtype similar to EI. For a long time, the pathomechanisms and underlying genetic defects of ichthyoses were unknown, although significant progress has recently been made in our understand-

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ing of the molecular basis of human epidermal keratinization processes.

In 1978, the causative abnormality underlying RXLI was identified as a steroid sulfatase deficiency caused by genetic defects in the steroid sulfatase gene (*STS*).[3,4] In 1992, mutations in the keratin 1 gene (*KRT1*) and keratin 10 gene (*KRT10*) were detected as a cause of EI.[5-7] Since transglutaminase (TGase) 1 gene (*TGM1*) mutations were identified as the cause of LI in 1995,[8,9] mutations in several other genes have also been identified in severe autosomal recessive congenital ichthyoses (ARCI).[10]

In 2005, a loss-of-function mutations in the ABCA12 gene were reported to underlie HI, the most severe type of ichthyosis.[11,12] In 2006, null mutations in the gene coding filaggrin (*FLG*) were detected as the causative defects leading to IV.[13]

To date, the number of genes identified and demonstrated to cause ichthyosis in human patients has reached eleven; they are shown in Table 1, i.e., *FLG*,[13] *KRT1*, *KRT10*,[5-7] *KRT2*,[14-16] *TGM1*,[8,9] *ABCA12*,[11,17,18] two lipoxygenase genes, *ALOXE3* and *ALOX12B*,[19] *NIPAL4*[20] and *FLJ39501*.[21] Most ichthyosis phenotypes mentioned above exhibit a primary abnormality associated with a barrier function in the stratum corneum as their underlying pathogenetic mechanisms.[1] The skin barrier of the stratum corneum has three major components, i.e., intercellular lipid layers, a cornified cell envelope and keratin/filaggrin degradation products (Fig. 1)

In 2009, the First Ichthyosis Consensus Conference was held to establish a consensus for the nomenclature and classification of inherited ichthyoses and an international consensus for the classification of inherited ichthyosis was successfully achieved.[2] The new classification and nomenclature should prove useful to all clinicians and can serve as a reference point for future research. In this updated review, based on the revised classification and terminology of ichthyoses, the pathogeneses of various ichthyoses are described in association with descriptions of specific defects in the essential components comprising the epidermal skin barrier, highlighting a few crucial diseases and mechanisms. In addition, I briefly mentioned the prenatal diagnosis of severe congenital ichthyosis at the end of the present review.

Table 1 Essential components of stratum cornuem barrier and causative molecules/genes for ichthyoses (modified from Ref. No. 1)

Stratum corneum barrier components	Molecule	Gene (locus)	Mode of inheritance	Type of Mutations	Phenotype
Intercellular lipid layers	ABCA12	*ABCA12* (2q34)	AR	truncation/deletion (rarely missense)	HI
	ABCA12	*ABCA12* (2q34)	AR	missense/missense or missense/truncation	LI or CIE
	lipoxygenase-3	*ALOXE3* (17p13.1)	AR	missense/truncation	LI or CIE
	12*R*-lipoxygenase	*ALOX12B* (17p13.1)	AR	missense/truncation	LI or CIE
	CYP4F22	*FLJ39501* (19P12)	AR	missense/truncation	LI
	NIPAL4	*NIPAL4* (5q33)	AR	missense/truncation	CIE or LI
	Steroid sulfatase	*STS* (Xp22.32)	X-LR	mostly large deletion	RXLI
Cornified cell envelope	TGase 1	*TGM1* (14q11.2)	AR	missense/truncation/ deletion/insertion	LI or CIE
Keratin network and keratohyalin granules	keratin 1	*KRT1* (12q12-q13)	AD	missense	EI
	keratin 10	*KRT10* (17q21)	AD (rarely AR)	missense (rarely nonsense)	EI
	keratin 2	*KRT2* (12q11-q13)	AD	missense	SEI
	Filaggrin (profilaggrin)	*FLG* (1q21.3)	ASD	truncation	IV

AD, autosomal dominant; AR, autosomal recessive; ASD, autosomal semidominant; X-LR, X-linked recessive; CIE, congenital ichthyosiform erythroderma; EI, epidermolytic ichthyosis; HI, harlequin ichthyosis; IV, ichthyosis vulgaris; LI, lamellar ichthyosis; RXLI, recessive X-linked ichthyosis; SEI, superficial epidermolytic ichthyosis

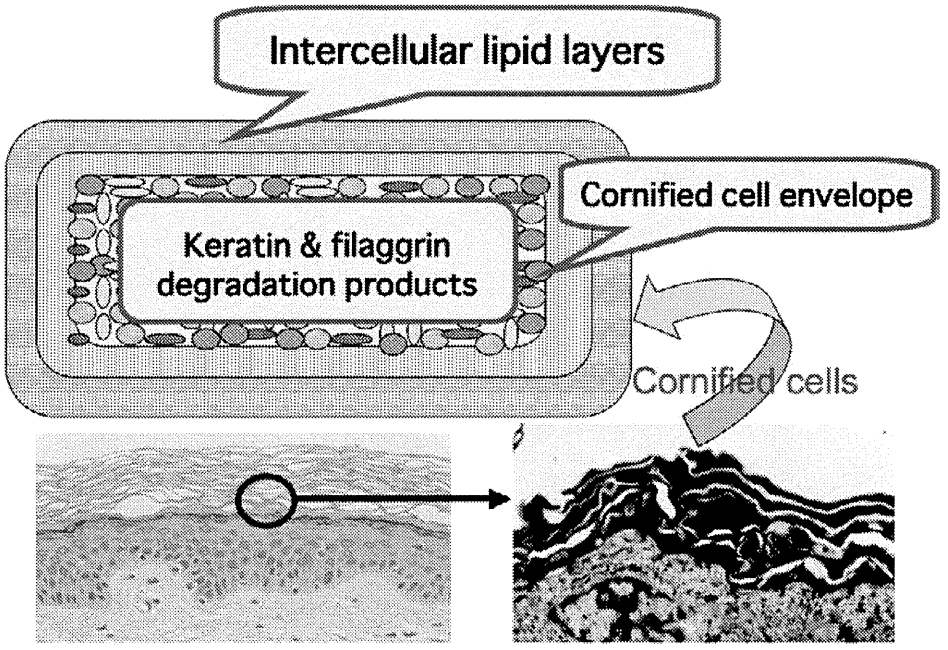

Fig. 1 Major components of skin barrier in stratum corneum consist of intercellular lipid layers, cornified cell envelope and keratin/filaggrin degradation products. Figure modified from Ref. No. 1.

MAJOR ICHTHYOSIS SUBTYPES AND THEIR CAUSATIVE MOLECULES

Harlequin ichthyosis (HI)

Formation of the intercellular lipid layers is essential for epidermal barrier function, and the defective formation of those layers is thought to result in a serious loss of barrier function, and to lead to extensive hyperkeratosis.[22] Formation of the intercellular lipid layers involves a highly complex series of processes that include the transport of lipids into the lamellar granules, and a multi-step metabolism of this lipid content within lamellar granules. ABCA12 has been highlighted, because it was recognized as a key molecule in keratinocytes lipid transport.[11,23]

Among the severe ARCI diseases, HI is the most devastating congenital ichthyosis, with affected newborns showing large, thick, plate-like scales over the whole body with severe ectropion, eclabium and flattened ears (Fig. 2A).[10] In 2005, we revealed that ABCA12 is a keratinocyte lipid transporter, and demonstrated that *ABCA12* mutations lead to the HI phenotype.[11] Another group independently reported that *ABCA12* mutations underlie HI by linkage analysis.[12] *ABCA12* mutations were also found to underlie LI and CIE cases.[17,18] ABCA12 is a member of a large superfamily of the ATP-binding cassette (ABC) transporters that bind and hydrolyze ATP to transport various molecules across a limiting membrane or into a vesicle.[24] All ABCA subfamily members are thought to be lipid transporters.[25] ABCA12 is a keratinocyte transmembrane lipid transporter protein associated with lipid transport in lamellar granules to the apical surface of granular layer keratinocytes.[11]

Ultrastructurally, lamellar granule abnormalities are apparent in HI patient epidermis.[26] Several morphologic abnormalities have been reported, e. g., abnormal lamellar granules in the granular layer keratinocytes and a lack of extracellular lipid lamellae in the stratum corneum. They reflect defective lipid transport by lamellar granules and the malformation of intercellular

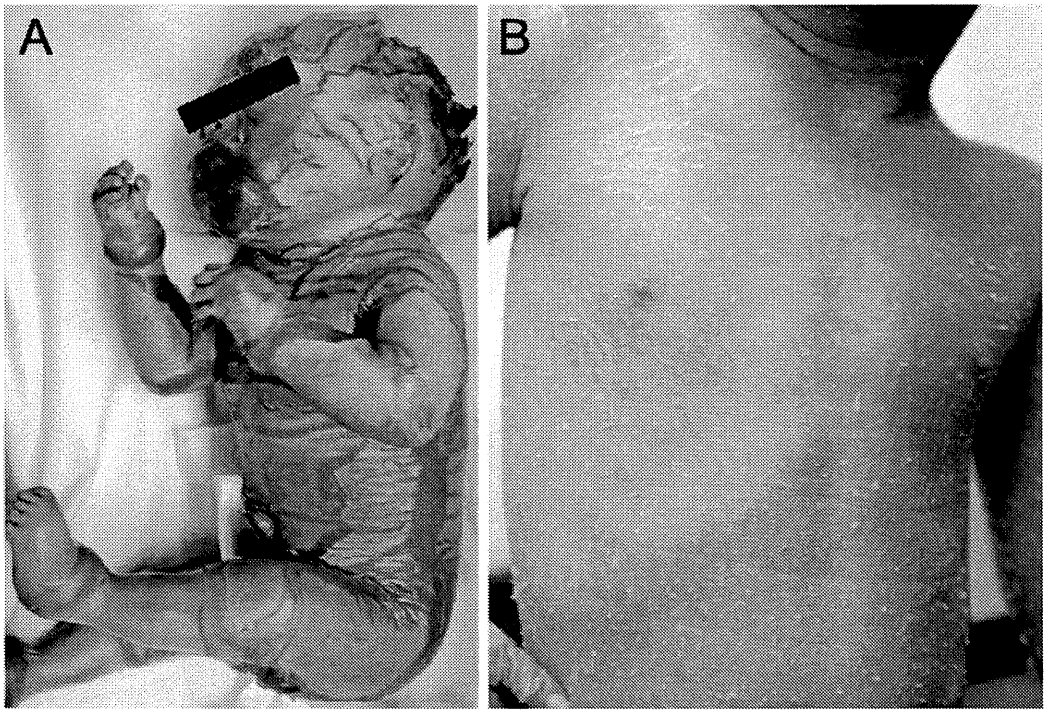

Fig. 2 Clinical features of ichthyosis. (A) An HI patient harboring a homozygous *ABCA12* splice site mutation. Thick, plate-like scales are seen on the whole body. Figure modified from Ref. No. 11. (B) A CIE patient carrying compound heterozygous *ABCA12* nonsense and missense mutations. Fine, whitish scales are observed on erythrodermic skin. Figure modified from Ref. No. 42.

lipid layers in the stratum corneum in HI.[26] In addition, cultured epidermal keratinocytes from an HI patient carrying *ABCA12* mutations demonstrated defective glucosylceramide transport, and this phenotype was recoverable by an *in vitro ABCA12* corrective gene transfer.[11] Based on these findings, we were able to shed light on the pathomechanisms of HI with the underlying *ABCA12* mutations leading to a loss of ABCA12 function. Lamellar granules are lipid transporting and secreting granules in the epidermal kerationcytes. Mutations in the lipid transporter protein ABCA12 cause defective lipid accumulations into lamellar granules,[11,27] resulting in malformation of the intercellular lipid layers of the stratum corneum.[11] The fact that ABCA3 (a member of the same protein superfamily as ABCA12) functions in pulmonary surfactant lipid secretion via the production of similar lamellar-type granules within lung alveolar type II cells[28,29] further supports this concept.

We subsequently transplanted cultured keratinocytes from patients with HI and succeeded in reconstituting HI skin lesions in immunodeficient mice.[27] These reconstructed HI lesions showed similar changes to those observed in HI patients' skin. In addition, we generated *Abca12* disrupted (*Abca12*$^{-/-}$) mice that reproduced the human HI phenotype, showing marked hyperkeratosis with eclabium and skin fissures.[30] Lamellar granule abnormalities and defective ceramide distribution were noteworthy in the epidermis. Skin permeability assays of *Abca12*$^{-/-}$ mouse fetuses revealed a severe skin barrier dysfunction after the initiation of keratinization. Another group independently developed *Abca12*$^{-/-}$ mice, which also confirmed the clinical features of HI.[31] A mouse strain carrying a homozygous spontaneous missense mutation was also reported to exhibit skin

manifestations similar to HI.[32]

HI patients often die in the first one or two weeks of life. However, once they survive beyond the neonatal period, HI survivors' phenotypes improve within several weeks after birth. In order to clarify the mechanisms of phenotype recovery, we studied grafted skin and keratinocytes from *Abca12*-disrupted (*Abca12*$^{-/-}$) mice and found that, during maturation, *Abca12*$^{-/-}$ epidermal keratinocytes regain their normal differentiation processes, although the exact mechanisms of this restoration are still unknown.[33]

Congenital ichthyosiform erythroderma (CIE) and lamellar ichthyosis (LI)

The formation of a 15-nm-thick layer of protein called the cornified cell envelope (CCE) on the inner surface of the cell membrane is essential for the skin barrier function.[1] CCE is assembled by the accumulation of several precursor proteins including involucrin, small proline-rich proteins and loricrin.[34] TGases in the epidermis are thought to be responsible at least in part, for the assembly of cornified cell envelope precursor proteins that form the cornified cell envelope.[35] TGase 1, the major subtype of the three TGases expressed in the epidermis,[36,37] is a membrane-associated TGase of about 92 kD. Since the identification of TGase 1 gene (*TGM1*) mutations in a number of families with LI in 1995,[8,9] further *TGM1* mutations have been reported in LI families. In addition, *TGM1* mutations were reported to underlie the CIE phenotype.[38,39]

There is little doubt that the defective formation of the stratum corneum intercellular lipid layers is caused by abnormal keratinocyte lipid metabolism, transport, and/or secretion, constituting one of the major pathogenetic mechanisms underlying congenital ichthyosis. Several critical molecules causing ichthyosis are thought to be involved in the formation of the stratum corneum intercellular lipid layers.[1]

In 2003, a keratinocyte lipid transporter ABCA12 was reported to be the causative molecule in type 2 LI (OMIM #601277) (see "harlequin ichthyosis" section above).[17] Type 2 LI is a subtype of LI which links to 2q33-35. Several genotype/phenotype correlations with *ABCA12* mutations have been elucidated as follows.[40] Combinations of missense mutations resulting in only one amino acid alteration underlie the LI phenotype.[17] In contrast, most mutations in HI are truncation or deletion mutations which lead to more severe changes, such as a loss of the ABCA12 peptide function affecting important nucleotide-binding fold domains and/or transmembrane domains. In HI, so far at least one mutation on each allele must be a truncation or deletion mutation within a conserved region that seriously impacts the ABCA12 function.[11,40,41] In the Japanese population, CIE patients harboring *ABCA12* mutations as the causative genetic defect are not rare (Fig. 2B).[18,42,43] Further accumulations of data on *ABCA12* mutations and their effects on the protein function/structure together with specific mutation sites are needed to better elucidate genotype/phenotype correlations that help to predict HI patient prognosis.

In 2002, mutations in two lipoxygenase genes, *ALOXE3* and *ALOX12B*, coding lipoxygenase-3 and 12(R)-lipoxygenase, respectively, were reported to underlie ARCI.[19] Although their exact functions are unknown, lipoxygenase-3 and 12(R)-lipoxygenase are non-heme iron-containing dioxygenases expressed in the epidermis.[44,45] They may be associated with the lipid metabolism of lamellar granule contents and/or intercellular lipid layers in the epidermis. 12(R)-lipoxygenase knockout mice have exhibited a severe impairment of the skin barrier function.[46] That loss of barrier function was associated with a perturbance of the assembly/extrusion of lamellar granules. Cornified cell envelopes from the skin of 12(R)-lipoxygenase deficient mice showed increased fragility.[46] Lipid analysis revealed a disordered composition of ceramides, especially a decrease in th ester-bound ceramide species.[46] Based on these findings, the 12(R)-lipoxygenase-lipoxygenase-3 pathway was thought to play a key role in the process of epidermal barrier formation by affecting lipid metabolism.[46] In fact, partially disturbed lamellar granule secretion has been reported in

the epidermis of a CIE patient with *ALOX12B* mutations.[47]

NIPAL4 (ichthyin) defects have also been reported to underlie certain cases of LI or CIE phenotypes.[20] NIPAL4 is a protein with several transmembrane domains, which belongs to a new family of proteins with an unknown function. NIPAL4-like proteins are localized in the plasma membrane, and share homologies to both transporters and G-protein coupled receptors.[20] NIPAL4 was suggested to be a membrane receptor for certain ligands (trioxilins A3 and B3) from the hepoxilin pathway,[20] although the underlying mechanisms of exactly how *NIPAL4* mutations lead to an ichthyotic phenotype remain to be clarified.

Mutations in *FLJ39501* were identified as causative genetic defects in lamellar ichthyosis type 3 (MIM 604777).[21] *FLJ39501* encodes a cytochrome P450, family 4, subfamily F, polypeptide 2 homolog of the leukotriene B4-omega-hydroxylase (CYP4F22). The exact function of CYP4F22 has not yet been elucidated yet, but it is thought to catalyze the 20-hydroxylation of trioxilin A3 form the 12(R)-lipoxygenase pathway. Further oxidation of this substrate would lead to 20-carboxy-(R)–trioxilin A3, a compound suspected to be involved in skin hydration, and would be an essential product lacking in various forms of ARCI.

Epidermolytic ichthyosis (EI) and superficial epidermolytic ichthyosis (SEI)

A normal keratin filament network is an important structure for keratohyalin granule formation and for maintaining the integrity and dimensions of the cornified cell cytoplasm. In this context, the keratin-network would be essential to normal skin barrier formation. Mutations in differentiation-specific keratins are known to result in ichthyosis phenotypes.

EI is caused by mutations in either the keratin 1 gene (*KRT1*) or the keratin 10 gene (*KRT10*).[5-7] Most of the causative mutations are missense mutations that reside within the beginning or at the end of the rod domain segments of keratin peptides, which are called helix initiation and helix termination motifs. Those motifs are highly conserved regions of approximately 20 amino acids, which have been implicated in molecular overlapping interactions as part of the formation of 10 nm intermediate filaments from dimers comprising both type I acidic and a type II basic-neutral keratins.[48] Single amino acid alterations in these essential helix boundary motifs frequently lead to a significant disease phenotype in the majority of keratin diseases.

EI is a severe congenital ichthyosis that exhibits from birth widespread blisters and erosions on a background of erythrodermic skin.[1,2] After the perinatal period, blister formation ceases and generalized hyperkeratosis becomes apparent. Histologically, a predominant vacuolization of the cells is observed in the middle and upper spinous and granular layers of the epidermis. The vacuolated keratinocytes show large and irregularly shaped cytoplasmic granules. Ultrastructurally, irregularly shaped, abnormal, clumped keratin filaments are seen in the keratinocytes from the upper spinous to the granular layers.[49] EI generally exhibits an autosomal dominant inheritance, although only a few families showing recessive inheritance traits have been reported.[50,51] In such families, the causative mutations are nonsense mutations. As for genotype-phenotype correlations in EI, palmoplantar keratoderma exists in patients with *KRT1* mutations, but not in those with *KRT10* mutations.[52]

SEI is also an autosomal dominant ichthyosis which shows similar, but slightly milder clinical features than those of EI.[53] Keratin 2 gene (*KRT2*) mutations underlie SEI patients.[14-16] Occasionally, cases with SEI can be difficult to clinically differentiate from EI, so that molecular genetic studies would be essential for a more definite diagnosis.[54] In the human epidermis, keratin 1 and 10 expressions occur in the suprabasal layers, replacing keratins 5 and 14 as the cells differentiate. Keratin 2 is expressed somewhat later than keratins 1 and 10 in keratinocyte differentiation as the keratinocytes approach the granular layer. Thus, consistent with the restricted keratin 2 expression sites, in SEI, clumped keratin filaments were restricted to the cytoplasm of

granular layer cells and the uppermost spinous layer cells, leading to granular degeneration only in the uppermost spinous and granular layers of the patient's epidermis. Such restricted granular degeneration results in milder clinical manifestations and the presence of superficial denuded areas (the *mauserung* phenomenon) that are characteristic of SEI.

Ichthyosis vulgaris (IV)

IV is a common genetic keratinization disorder, clinically characterized by scaling, especially on the flexor limbs, and with palmoplantar hyperlinearity. The epidermis of IV patients shows a decrease in their size and numbers or, complete absence of keratohyalin granules.[55]

The degradation products of keratohyalin granules occupy the cytoplasm of keratinized cells in the stratum corneum and play important roles in the skin barrier function. Keratohyalin granules in the granular layer of the epidermis are predominantly composed of large (>400-kDa) profilaggrin polyproteins.[56,57] Upon the terminal differentiation of keratinocytes, profilaggrin is cleaved into 10-12 essentially identical 37-kDa filaggrin peptides. The liberated filaggrin aggregates the keratin filaments,[56] causing a collapse of the granular cells into a flattened squame-shape. In addtion, the degradation products of filaggrin contribute to moisture retention in the cornified layers. Thus, filaggrin, a major component of keratohyalin granules, is indispensable to the normal, intact, skin barrier function. In this context, a loss or reduction in filaggrin expression results in excessively dry skin and impaired barrier function, which leads to clinical features of IV.

In 2006, *FLG* mutations were identified in IV patients in European populations[13] and have been shown to be major predisposing factors for atopic dermatitis.[58] Subsequently, *FLG* mutations were identified in Japanese, Chinese, Taiwanese and Korean populations.[59,60] Based on the information about population-specific *FLG* mutations, numbers of cohort studies of atopic dermatitis for *FLG* mutations have been performed confirming that about 25–50% of patients with atopic dermatitis were demonstrated to harbor *FLG* mutations as a predisposing factor. It was demonstrated that *FLG* mutations are also strongly associated with atopic dermatitis in the Japanese population.[59,61] Skin barrier defects due to *FLG* mutations are thought to play important roles in the pathogenesis of atopic diseases including atopic dermatitis, allergic rhinitis and asthma.[62]

Recessive X-linked ichthyosis (RXLI)

Genetic defects in the steroid sulfatase gene (*STS*) were reported to underlie RXLI.[3,4] Most *STS* mutations underlying RXLI are large deletions and, nowadays, fluorescence *in situ* hybridization (FISH) techniques are a useful tool in detecting causative *STS* mutations.[63] The hyperkeratosis and scaling observed in RXLI are associated with an abnormal accumulation of cholesterol sulfate in the stratum corneum.[64] Steroid sulfatase is concentrated in lamellar granules and then secreted into the intercellular spaces of the stratum corneum, along with other lamellar granule-derived lipid hydrolases.[65] In those spaces, steroid sulfatase degrades cholesterol sulfate, generating some cholesterol for the barrier. Furthermore, the progressive decline in cholesterol sulfate permits corneodesmosome degradation leading to intact desquamation.[65] Thus, two molecular pathways contribute to disease pathogenesis in RXLI. Steroid sulfatase deficiency leads to both malformation of the intercellular lipid barrier, and a delay in corneodesmosome degradation, resulting in corneocyte retension.[65] In addition, increased Ca^{2+} in the intercellular space of the stratum corneum in X-linked ichthyosis has been reported to contribute to corneocyte retention by increasing corneodesmosomes and interlamellar cohesion.[65]

PRENATAL DIAGNOSIS OF SEVERE CONGENITAL ICHTHYOSES

The quality of life of patients with severe congenital ichthyoses is seriously affected in some cases, so that parental requests for prenatal diagnosis cannot be easily ignored. Due to the recent advances in our understanding of the genetic defects underlying severe congenital ichthyosis, it has become possible to make DNA-based prenatal diagnoses for congenital ichthyosis families by sampling chorionic villus or amniotic fluid in the earlier stages of pregnancy. That lowers the risk to fetal health and reduces the burden on mothers compared with prenatal diagnoses by fetal skin biopsy.[1)]

In cases of HI, before identification of *ABCA12* as the causative gene, prenatal diagnoses had been performed by fetal skin biopsy and electron microscopic observation at the later stages of pregnancies, 19–23 weeks estimated gestational age, for more than 20 years.[66-69)] When a fetus was diagnosed as affected, any interruption at the late stage of pregnancy posed a serious problem.

After the identification of *ABCA12* as the causative gene for HI, it became feasible to perform DNA-based prenatal diagnoses for HI by chorionic villus or amniotic fluid sampling at a much earlier stage of pregnancy. That significantly lowered risk to fetal health and reduced the burden on mothers, as in the case of other severe genetic disorders.[70)] Indeed, prenatal diagnoses and the exclusion of HI by DNA testing have been performed in our laboratory.[70,71)]

Prenatal diagnosis of LI by the ultrastructural observation of fetal skin samples involves a somewhat high-risk, since LI patients can exhibit regional, individual, and familial variability in their disease phenotypes.[72)] In LI families with *TGM1* mutations, successful prenatal DNA-base diagnoses and prenatal exclusions of LI have been reported.[73,74)] Prenatal diagnosis by mutation analysis in lipoxygenase-3, 12(R)-lipoxygenase and ABCA12, etc. is theoretically available in LI and CIE families with previously identified mutations on a case-by-case basis.

Successful prenatal diagnosis of EI by fetal skin biopsy was reported in the 1980s[75)] and, at present, prenatal diagnosis by mutation analysis has become feasible for EI in families whose causative mutations have been elucidated.[76,77)]

CONCLUSION AND REMARKS

As summarized above in this updated review, our knowledge of the molecular genetics and pathogenesis of ichthyosis has advanced dramatically in the last couple of decades. In addition, we now have several powerful tools for the treatment of genetic disorders, such as siRNA gene-silencing technology, read-through compounds to read through nonsense mutations, and improved corrective gene transfer techniques. Fortunately, the skin is the most easily accessible organ for these novel treatment approaches. Thus, based on our knowledge of the pathomechanisms of various ichthyoses described in the present review, I am sanguine about the development of novel, highly effective therapeutic methods in the near future.

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REVIEW

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

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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.

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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