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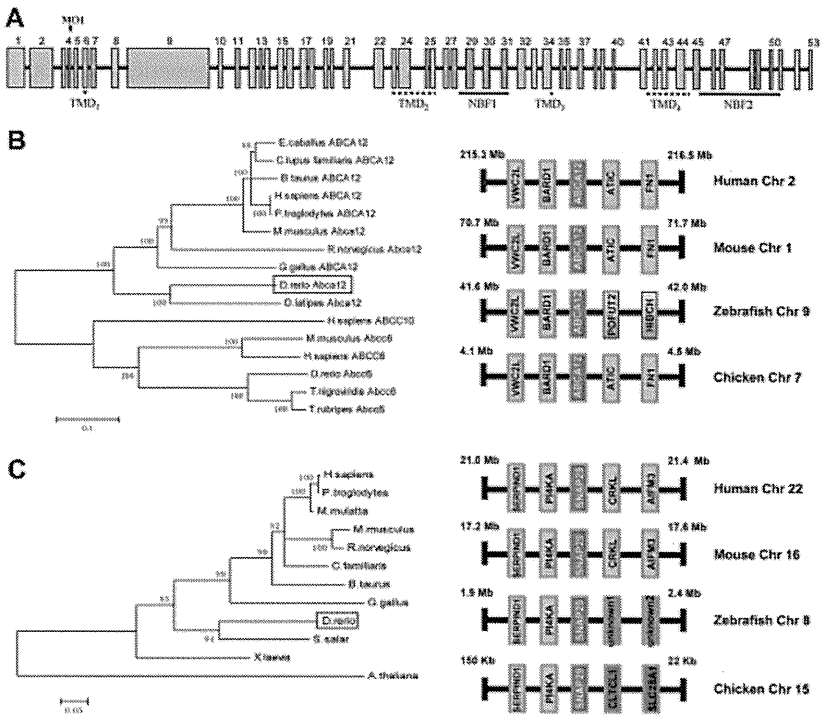
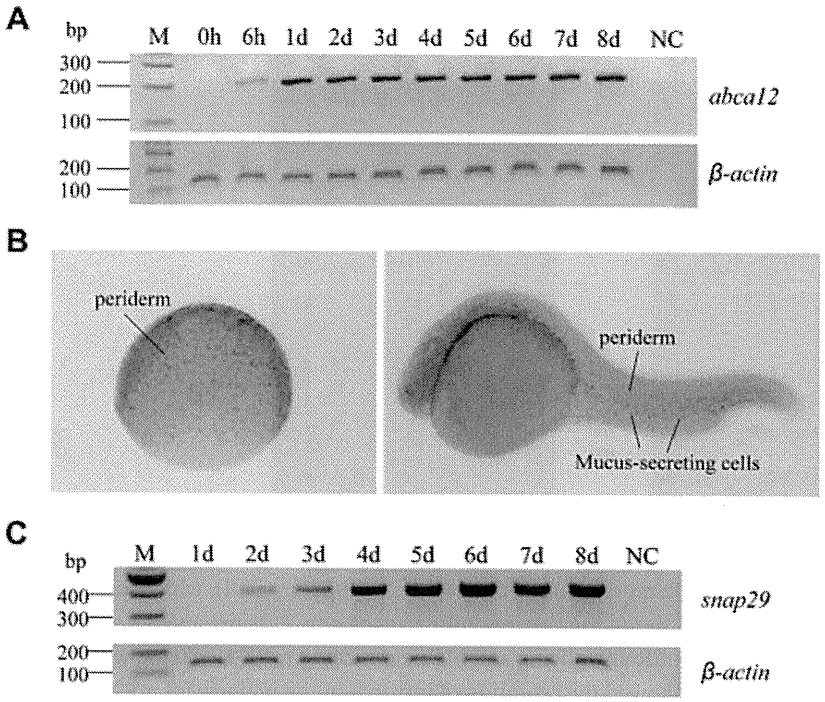
Figures and Tables

Fig. 1.



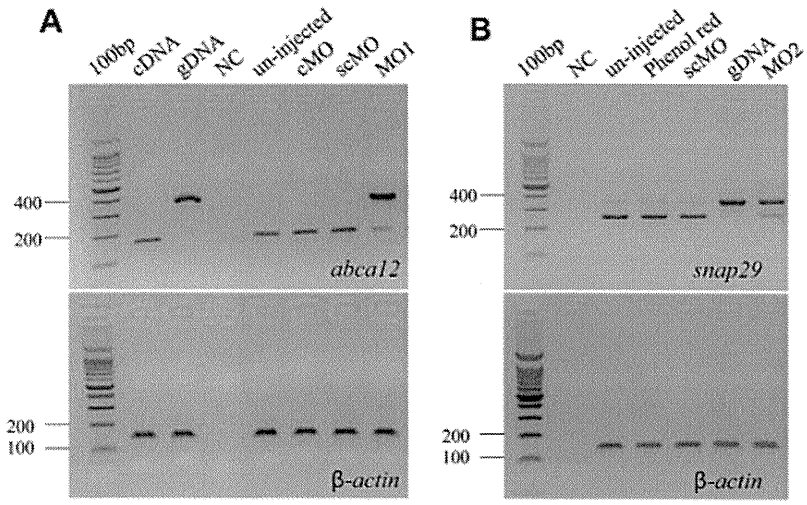
Schematic representation of the zebrafish *abca12* gene, and the phylogenetic trees of the protein sequences of Abca12 and Snap29, together with syntenic analysis of the corresponding genes. (A) The *abca12* gene consists of 53 exons, which are numbered on the top, and the coding segments for transmembrane domains (TMDs) and nucleotide binding folds (NBFs; green) are underlined. Note the location corresponding to the morpholino (MO1) at the exon-4–intron-4 junction. (B) The phylogenetic relationship between zebrafish Abca12 and the other members of the ABC family of transporters estimated by the neighbor-joining method (left panel). The syntenic analysis of the *abca12* and flanking genes in human, mouse, zebrafish and chicken chromosomes is shown on right. (C) Cladogram and syntenic analysis of *snap29*. The unknown genes 1 and 2 in zebrafish chromosome 8 have been designated as si:dkey-178e17.1 and si:dkeyp-117b11.1, respectively.

Fig. 2.



***abca12* and *snap29* gene expression in normal zebrafish.** (A–C) Zebrafish embryos were collected at 0 and 6 hpf and 1–8 dpf, and total RNA was isolated and cDNA prepared. The *abca12* (A) and *snap29* (C) mRNA expression levels were measured by RT-PCR and standardized against the mRNA expression level of the *β-actin* gene. (B) Whole-mount in situ hybridization of embryos at different stages of early development for *abca12* expression; gastrula period (left panel), 24 hpf (right panel).

Fig. 3.



Knockdown of *abca12* and *snap29* expression by morpholinos. (A) Knockdown of *abca12*. (B) Knockdown of *snap29*. MO1 and MO2 morpholinos (right lanes, the upper panel), which target the splice donor site at the exon-4–intron-4 border of the corresponding genes, prevents pre-mRNA splicing. The consequences of MO1 on *abca12* pre-mRNA splicing and MO2 on *snap29* mRNA splicing were determined by RT-PCR. The results showed the retention of intron 4 in the majority of mRNA transcripts (>90%) as compared with the normally transcribed control. The mRNA levels were normalized by the level of *β-actin* mRNA (lower panels). cDNA and gDNA represent amplification of the corresponding complementary DNA and genomic DNA, respectively. Injections with the 5-bp mismatched control morpholino for *abca12* (cMO) or global standard control morpholino (scMO) did not alter pre-mRNA processing, similar to the uninjected controls or those injected with phenol red.

Table 1.

Table 1. Survival of and development of phenotype in zebrafish injected with *abca12* morpholino

Experimental group	No. of fish	3 dpf			5 dpf		
		Survival (%)	Skin phenotype (%)	Edema (%)	Survival (%)	Skin phenotype (%)	Edema (%)
Uninjected control	152	87	0	0	87	0	0
scMO	177	81	0	0	81	0	0
abca12 MO1	180	76	92*	75*	6*	0	0
abca12 MO1+ hABCA12 mRNA	184	87†	81† (milder)	74 (milder)	62†	73† (milder)	70† (milder)

This is a representative experiment in which all groups were followed in parallel. Similar results were obtained in >ten additional experiments with the same design. Skin phenotype refers to epidermal perturbations, disruption of the chromatophore distribution and yolk sac enlargement. Edema is pericardial edema.
scMO, standard control morpholino with no biological function and no target sequence in zebrafish genome (Robu et al., 2007).
*Statistical significance between the *abca12* MO1 group and scMO group (Fisher's exact test; $P<0.0001$).
†Statistical significance between the *abca12* MO1 group and the *abca12* MO1+hABCA12 mRNA group (Fisher's exact test; $^{\dagger}P<0.05$; $^{\dagger}P<0.0001$).

Survival of and development of phenotype in zebrafish injected with *abca12* morpholino

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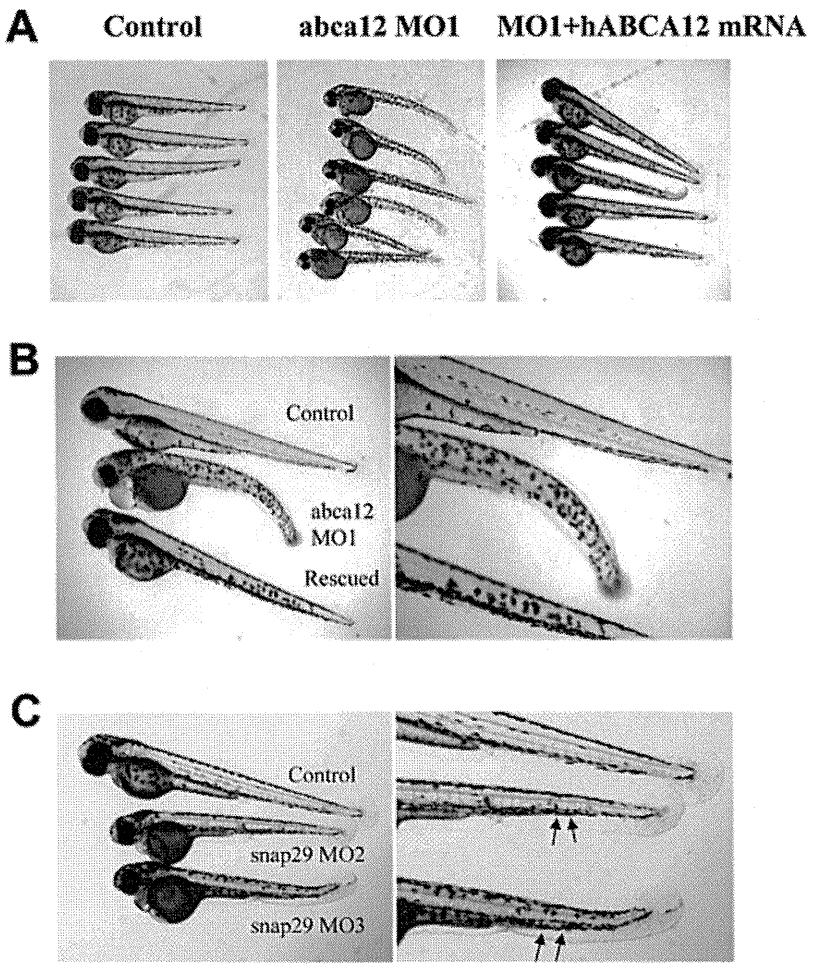
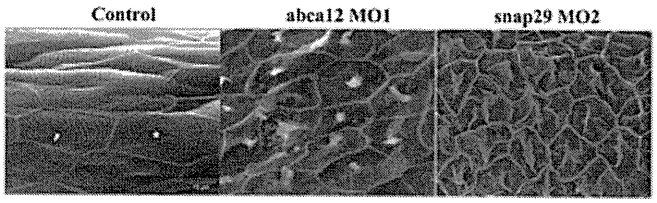
Fig. 4.



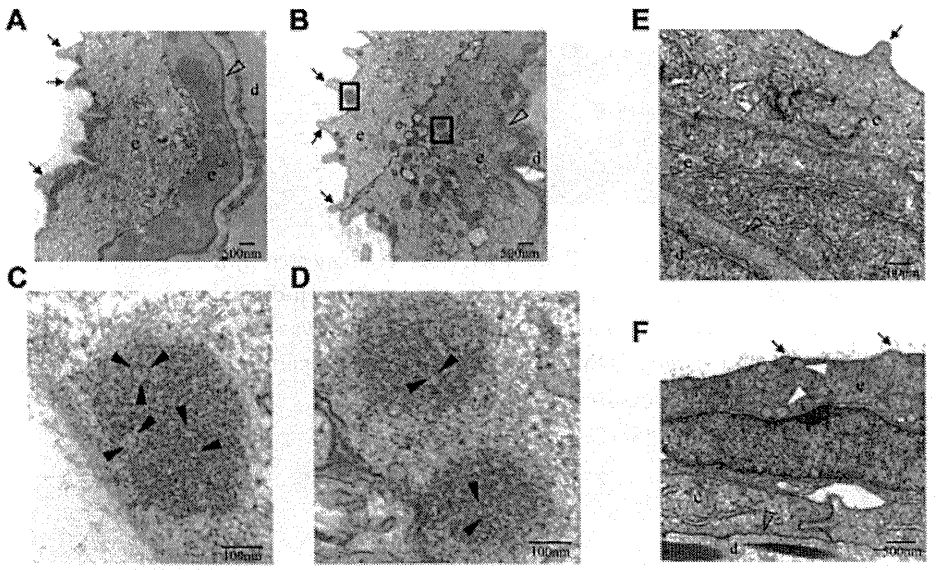
Zebrafish phenotypes and their mRNA rescue at 3 dpf. (A) Phenotypic appearance of zebrafish larvae after injection with an *abca12* MO1 morpholino (middle panel) compared with control larvae (left panel), and partial rescue with human *ABCA12* mRNA (right panel). (B) Higher magnification of the larvae shown in A. (C) Phenotype of larvae at 3 dpf injected with *snap29* morpholinos MO2 or MO3. The irregular contour of the epidermis is noted by arrows.

Fig. 5.



SEM of the skin surface. The skin of the tail of the control larvae injected with the global standard control morpholino at 3 dpf shows the presence of keratinocytes with well-demarcated cell-cell borders (arrowhead) containing microridges (star; left panel). The morphant larvae injected with MO1 morpholino for *abca12* (middle) or *snap29* (MO2; right panel) revealed perturbed microridge formation with spicules in the center of the keratinocytes.

Fig. 6.



TEM of 3-dpf larvae injected with *abca12* or *snap29* morpholinos. (A,E) Control morpholino (scMO); (B) *abca12* morpholino (MO1). Boxes surrounding electron-dense subcellular structures in B were examined at higher magnification and are shown in C and D. (F) Injection with *snap29* morpholino (MO2). (A–F) Arrows point to microridges; open arrowheads indicate basement membrane; solid black arrowheads point to the areas of accumulation of putative lipids within the electron-dense granules in C and D; solid white arrowheads in F point to apparently empty vesicles. e, epidermis; d, developing dermis.

for allergen, *S. aureus* colonization, skin barrier dysfunction and AD deterioration is recently reported [8]. The virulence factors produced by *S. aureus* have various biological characteristics to destroy epithelial barrier, inhibit opsonization, interfere neutrophil chemotaxis, inactivate neutrophil cytolysis and antimicrobial peptide [4,9,10]. Although further studies are required to examine this hypothesis, current evidence is sufficient to conclude that *S. aureus* and possibly some other skin microflora are directly related to aggravation of healthy skin conditions. Similar to diseased skin, *S. aureus* and subtle dysfunction of skin barrier in healthy skin can also be caught in a vicious circle, resulting in further breakdown of skin barrier and progress to outright deterioration.

These findings are meaningful as a first study to demonstrate that *S. aureus* is involved in skin deterioration, even in apparently healthy skin.

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Kyeho Shin[a,b]
[a]*Skin Research Institute, R&D Center, AmorePacific Corporation, Yongin-si, Gyeonggi-do, Republic of Korea*
[b]*Biomaterials Process Engineering Lab, Department of Biotechnology, Yonsei University, Seoul, Republic of Korea*

Tae Ryong Lee
Enyoung Lee
Yoon Hyeok Jeong
Yuna Yun
Tae Hun Park
Hankon Kim
Skin Research Institute, R&D Center, AmorePacific Corporation, Yongin-si, Gyeonggi-do, Republic of Korea
Kashif Ghafoor

Jiyong Park*
Biomaterials Process Engineering Lab, Department of Biotechnology, Yonsei University, Seoul, Republic of Korea
*Corresponding author. Tel.: +82 2 2123 2888; fax: +82 2 362 7265
E-mail address: foodpro@yonsei.ac.kr (J. Park)

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Letter to the Editor

LEDGF/DFS70 activates the MK2/IL6/STAT3 pathway in HaCaT

Lens epithelium-derived growth factor (LEDGF), also known as dense fine speckles 70 kDa protein (DFS70), was isolated as a transcription cofactor, a survival factor and a target of autoantibodies in atopic dermatitis [1–3]. LEDGF/DFS70 has been implicated as a key player in cancer [4]. Psoriasis is a common skin disorder that is characterized by abnormal differentiation of the epidermal keratinocytes (KCs), inflammatory cells recruitment and changes in the endothelial vascular system. It has been suggested that psoriatic KCs have abnormal expression of various cytokines and chemokines, and deregulation of several signaling pathways. For example, psoriatic KCs are characterized by activation of the signal transducer and activator of transcription 3 (STAT3) [5]. Activated TNF-α and MAPK-activated protein kinase 2 (MK2) have also been observed in lesional psoriatic epidermis [6].

We recently found that LEDGF/DFS70 localizes to the nuclei of spinous layers as well as the basal layer of psoriatic skin, although LEDGF/DFS70 is restricted to the cytoplasm in cells of normal spinous layers [7,8]. We also generated stable cell lines which constitutively express enhanced green fluorescent protein-tagged LEDGF (EGFP-LEDGF-HaCaT) or EGFP alone (EGFP-HaCaT) as a control, and we demonstrated that LEDGF/DFS70 regulated the IL-6 via p38 phosphorylation and deregulated S100A7, S100A9 and filaggrin. In light of this, it was suggested that LEDGF/DFS70 plays a pivotal role in activating psoriatic KCs. This study aims to clarify how LEDGF/DFS70 contributes to the formation of psoriatic skin lesions.

To determine whether minichromosome maintenance 2 (MCM2) phosphorylation is increased in EGFP-LEDGF-HaCaT, we performed Western blot analyses. As shown in Fig. 1a, MCM2 (Ser53) was more phosphorylated in EGFP-LEDGF-HaCaT than in EGFP-HaCaT, although the levels of total MCM2 and MCM2 phosphorylation (Ser40/41) were the same. LEDGF/DFS70 has been shown to interact with the Cdc7-activator of S-phase kinase (ASK), which is essential for initiation of DNA replication throughout the S-phase, and LEDGF/DFS70 has been demonstrated to stimulate its enzymatic activity, increasing phosphorylation of MCM2 (Ser53) *in vitro* [9]. Our results are compatible with the previous reports and suggest that LEDGF/DFS70 may function as an S-phase regulator in the nuclei of proliferating KCs. To determine whether the Cdc7 is involved in MCM2 phosphorylation (Ser53) in EGFP-LEDGF-HaCaT, small interfering RNA (siRNA) was used to reduce Cdc7 expression (siCdc7) in EGFP-LEDGF-HaCaT, and then the amount of phosphorylated MCM2 (Ser53) was measured. Fig. 1b shows that the phosphorylation level of MCM2 (Ser53) was reduced compared to the controls (siCD4) when the Cdc7 protein level was reduced by siRNA.

We previously reported that EGFP-LEDGF-HaCaT have higher IL-6 expression and higher phosphorylation of STAT3 than EGFP-HaCaT has [7]. The gp130 receptor constitutes an essential signal transducing component of the IL-6 receptor complex, and IL-6-

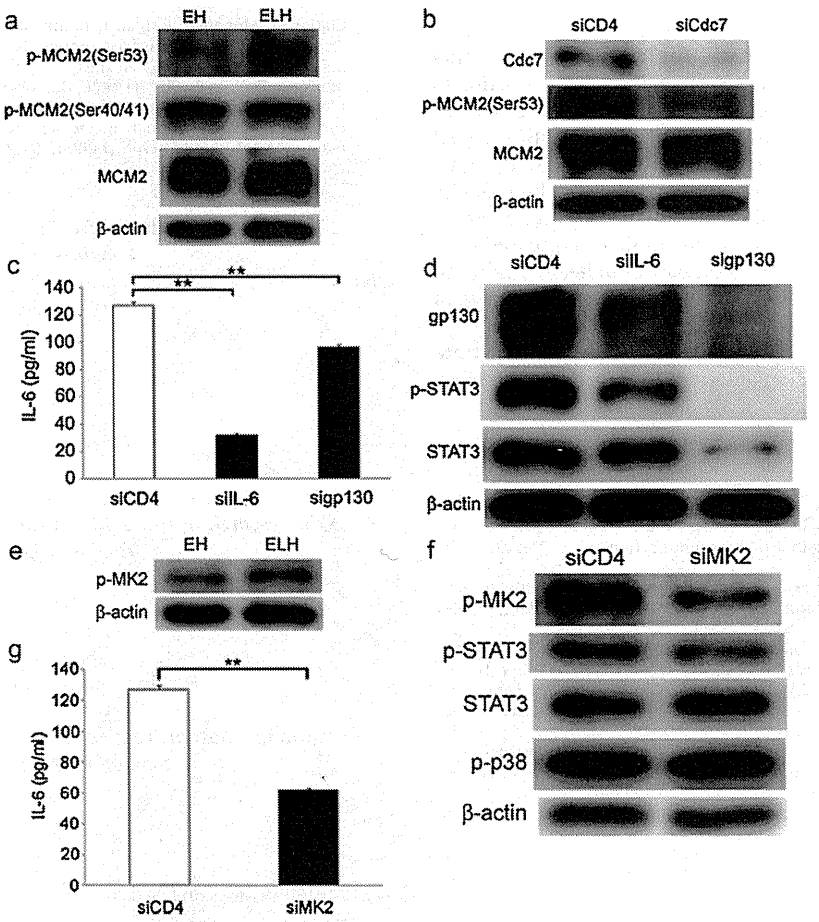

Fig. 1. MCM2 and MK2 are activated in EGFP-LEDGF-HaCaT (a, e); Western blot of lysates from EGFP-LEDGF-HaCaT transfected with several specific small interfering RNAs (b–d, f, g). (a, e) Lysates of EGFP-LEDGF-HaCaT (ELH) and EGFP-HaCaT (EH) incubated for 72 h and analyzed by Western blot for MCM2, phosphorylated MCM2 (p-MCM2), phosphorylated MK2 (p-MK2) and β-actin. (b–d, f, g) EGFP-LEDGF-HaCaT transfected with small interfering (si) RNA against Cdc7 (siCdc7), IL-6 (siIL-6), gp130 (sigp130), MK2 (siMK2) and CD4 (siCD4). After 72 h incubation of the cells, proteins of Cdc7, MCM2, p-MCM2, IL-6, gp130, STAT3, phosphorylated STAT3 (p-STAT3), p-MK2, phosphorylated p38 (p-p38) and β-actin were evaluated by ELISA or Western blot analysis. Statistical significance between groups was assessed by paired Student's *t*-test ($n = 4$). Error bars represent the SEM. $^{**}P < 0.01$.

induced tyrosine phosphorylation of gp130 leads to the activation of STAT3. To determine whether increased IL-6 is involved in phosphorylation of STAT3 by the overexpression of LEDGF/DFS70, mRNA production of IL-6 and gp130 in EGFP-LEDGF-HaCaT was suppressed by siRNA to IL-6 (siIL-6) and gp130 (sigp130), and then the protein levels of phosphorylated STAT3 in the cells were measured. As shown in Fig. 1c and d, both siIL-6 and sigp130 decreased phosphorylated STAT3 (p-STAT3) compared to the control (siCD4) under the diminution of each protein. Our findings provide evidence that IL-6 and gp130 are essential for the increased p-STAT3 expression that is observed in EGFP-LEDGF-HaCaT.

We have demonstrated that IL-6 expression is significantly upregulated via activation of the p38 pathway in EGFP-LEDGF-HaCaT [7]. The mechanism by which the regulatory effects of p38 are mediated involves several p38 downstream kinases, including the MAPK-activated protein kinase 2 (MK2). Thus, we investigated whether MK2 is also activated in EGFP-LEDGF-HaCaT, using Western blot analysis. As shown in Fig. 1e, the MK2 was more phosphorylated in EGFP-LEDGF-HaCaT than in EGFP-HaCaT. Furthermore, to determine whether MK2 activation is involved in the IL-6/STAT3 pathway in EGFP-LEDGF-HaCaT, protein levels of IL-6 and p-STAT3 were measured in the EGFP-LEDGF-HaCaT whose MK2 phosphorylation had been suppressed by siRNA to MK2 (siMK2). The increased expression of both the IL-6 and the p-STAT3 proteins was downregulated with siMK2 in the EGFP-LEDGF-HaCaT (Fig. 1f and g).

We studied whether excessive expression of LEDGF/DFS70 indeed results in up-regulated expression of the proinflammatory cytokines TNF-α and IFN-β1, the chemokines IL-8 and CCL5, and the KC differentiation-related genes keratin K1 and keratin K10, as occurs in psoriatic KCs. We compared their mRNA and protein expression levels with EGFP-LEDGF-HaCaT versus with EGFP-HaCaT, in both cases cultured for 24–72 h after incubation. These analyses showed that EGFP-LEDGF-HaCaT had higher mRNA expression for IL-8 (24, 48 and 72 h), TNF-α (24 and 72 h), CCL5 (24, 48 and 72 h) and IFN-β1 (24, 48 and 72 h) than EGFP-HaCaT had (Fig. 2a–d). In contrast, the mRNA expression of the KC-differentiating markers keratin K1 and keratin K10 was decreased by a factor of approximately 0.1–0.5 in EGFP-LEDGF-HaCaT compared with that in EGFP-HaCaT (Fig. 2e and f). Moreover, IL-8 protein expression was increased in EGFP-LEDGF-HaCaT compared to that in EGFP-HaCaT at 72 h after incubation (Fig. 2g). Similarly, TNF-α (Fig. 2h) and CCL5 (data not shown) proteins also increased. Although LEDGF/DFS70 was shown to be induced by TNF-α and the overexpression of LEDGF/DFS70 abolished the effect of TNF-α [10], there are no reports that overexpression of LEDGF/DFS70 induces TNF-α expression. Our findings suggest that LEDGF/DFS70 may regulate the expression of TNF-α in KCs, and it is a potentially attractive target for controlling the effect of TNF-α

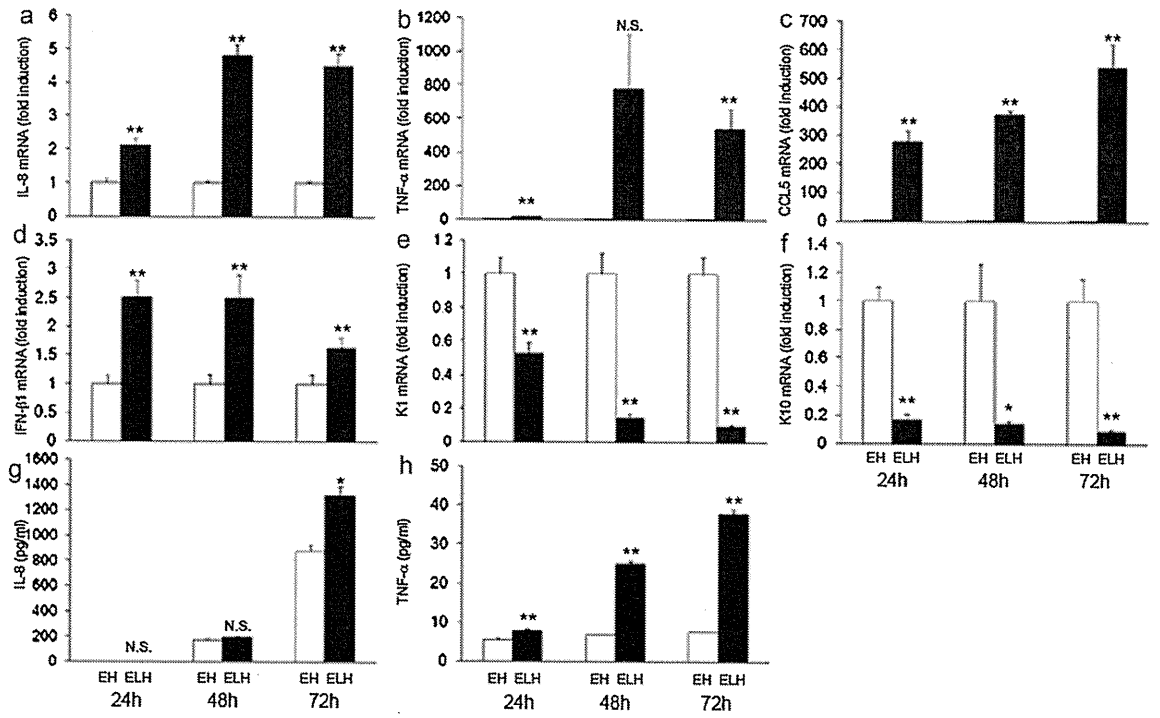

Fig. 2. qPCR and ELISA of EGFP-LEDGF-HaCaT and EGFP-HaCaT. After 24, 48 and 72 h of incubation, mRNA and protein levels in EGFP-LEDGF-HaCaT (ELH) and EGFP-HaCaT (EH) were evaluated by RT-PCR (a–f) and Western blot (g and h), respectively. RT-PCR was performed for IL-8 (a), TNF-α (b), CCL5 (c), IFN-β1 (d), K1 (e) and K10 (f). GAPDH was used to normalize the gene expression. Protein secretion levels of IL-8 (g) and TNF-α (h) were measured by ELISA. Statistical significance between groups was assessed using paired Student's *t*-test ($n = 4$). Error bars represent the SEM. N.S.: not significant. $^{*}P < 0.05$. $^{**}P < 0.01$.

In summary, we demonstrated that the MK2/IL-6/STAT3 pathway is activated in EGFP-LEDGF-HaCaT. Furthermore, we revealed that the expression of TNF-α, IL-8, CCL5 and IFN-β1 is increased and the expression of K1 and K10 is decreased in these cells. These findings suggest that LEDGF/DFS70 may play a pivotal role in activating psoriatic KCs *in vivo* via the p38/MK2/IL-6/STAT3 pathway. Because of its role as a regulator of KC differentiation, LEDGF/DFS70 is a potentially effective target for new therapies aimed at psoriasis and other proliferative diseases. We believe EGFP-LEDGF-HaCaT will be useful in the development of novel therapeutic drugs for psoriasis.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jdermsci.2011.05.004.

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Takuya Takeichi
Kazumitsu Sugiura*
Yoshinao Muro
Yasushi Ogawa
Masashi Akiyama
Department of Dermatology,
Nagoya University Graduate School of Medicine,
65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

*Corresponding author. Tel.: +81 52 744 2314;
fax: +81 52 744 2318
E-mail address: kazusugi@med.nagoya-u.ac.jp (K. Sugiura).

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REVIEW ARTICLE

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Malignant skin tumours in patients with inherited ichthyosis

K. Natsuga, M. Akiyama* and H. Shimizu

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

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.

Correspondence
Ken Natsuga.
E-mail: natsuga@med.hokudai.ac.jp

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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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Mårten C.G. Winge*
Dermatology Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden

Torborg Hoppe
Department of Medical Sciences, Dermatology and Venereology, Uppsala University, SE-75185 Uppsala, Sweden

Agne Liedén
Magnus Nordenskjöld
Department of Molecular Medicine & Surgery, Karolinska Institutet, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden

Anders Vahlquist
Department of Medical Sciences, Dermatology and Venereology, Uppsala University, SE-75185 Uppsala, Sweden

Carl-Fredrik Wahlgren
Dermatology Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden

Hans Törmä
Department of Medical Sciences, Dermatology and Venereology, Uppsala University, SE-75185 Uppsala, Sweden

Maria Bradley[a,b]
[a]*Dermatology Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden*
[b]*Department of Molecular Medicine & Surgery, Karolinska Institutet, Karolinska University Hospital Solna, SE-171 76 Stockholm, Sweden*

Berit Berne
Department of Medical Sciences, Dermatology and Venereology, Uppsala University, SE-75185 Uppsala, Sweden

*Corresponding author. Tel.: +46851776538
E-mail address: marten.winge@ki.se (M.C.G. Winge).

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Letter to the Editor

Altered lipid profiles in the stratum corneum of Sjögren-Larsson syndrome

Sjögren-Larsson syndrome (SLS) is a rare, autosomal recessive neurocutaneous disorder characterized by clinical triads, congenital ichthyoids, spasticity and mental retardation [1]. SLS is caused by mutations in fatty aldehyde dehydrogenase (*FALDH*) (or *ALDH3A2*) gene [1]. FALDH is a microtonal NAD-dependent enzyme, which oxidizes medium- to long-chain aliphatic aldehydes to fatty acids. Accumulation of fatty alcohol has been shown in cultured fibroblasts and in plasma from SLS patients [1]. Numbers of mutations of *FALDH* gene have been shown, although only three mutations have been identified in Japanese SLS patients [2–4]. We here report a SLS patient who is a homozygote for one of the known mutations. In addition to assessing skin phenotype, permeability barrier function and cutaneous morphology, biochemical analysis revealed novel alterations in lipid profiles in the stratum corneum associated with barrier function.

A 57-year-old Japanese woman complaining of slightly pruritic and dry skin with scaling visited our hospital. The patient has been suffering from scaly skin lesions over the entire body since her early childhood. She presented generalized dryness, widespread itchy hyperkeratosis scaly lesions with brown scaling plaques, and slight erythema on the trunk and extremities (Fig. 1a). The neurologic examination revealed severe spastic paraplegia in the lower limbs with an increased muscle tone, hyperreflexia in all limbs, and positive Babinski reflexes bilaterally. She also showed mental retardation (IQ 39). A skin biopsy specimen from the right arm revealed orthohyperkeratosis with thin granular layers and mild acanthosis with papillomatosis (Fig. 1b). Electron microscopic examination showed several lipid droplets without surrounding membrane in the cornified cells (Fig. 1c). Moreover, abnormal lamellar granules, which lacked lamellar contents, were present in the granular cells (Fig. 1d). From these clinical features and cutaneous morphology, this patient was diagnosed as SLS. Mutation analysis using a cDNA sample from the patient's peripheral white blood cells showed a homozygous point mutation c.1157A>G which results in alteration from asparagine to serine at cordon 386 (p.Asn386Ser) in the β-9 chains containing active domain of FALDH (Fig. 2a).

Transepidermal water loss (TEWL) of the ichthyosiform lesion on the extensor and flexor sides of the forearm and back (6.3, 12.2, 10.2 g h^{-1} m^{-2}, respectively) was within the normal range (0–10, very good; 10–15, good; 15–20, fair; 25–30, poor; more than 30, very poor). On the other hand, water retention capability was impaired in the lesion (25.5, normal > 60).

Major barrier lipid content of involved skin was assessed in comparison to non-ichthyotic scaly lesions from sunburn dermatitis as a control subject (note: we and others found that there is no significant difference in lipid content of sunburn scale and of non-sunburn scales from normal donors [5]). Although there was no difference in the quantity of cholesterol between the patient and control, free fatty acid (FFA) was increased by about two-fold over control (Fig. 2b). In contrast, ceramide (Cer) 1, 6, 7 were decreased in the patient's scales compared with those in control samples, while membrane-bound Cer species, Cer A, which are constituent of the corneocyte lipid envelope (CLE), were increased. We recently demonstrated that linoleate required for acylceramide synthesis is primarily derived from triglyceride (TG) [6]. However, TG content was not changed in SLS compared with that in control scales (Fig. 2b).

The identical mutation in our case was described in another Japanese patient with SLS [2]. The other mutations reported in the

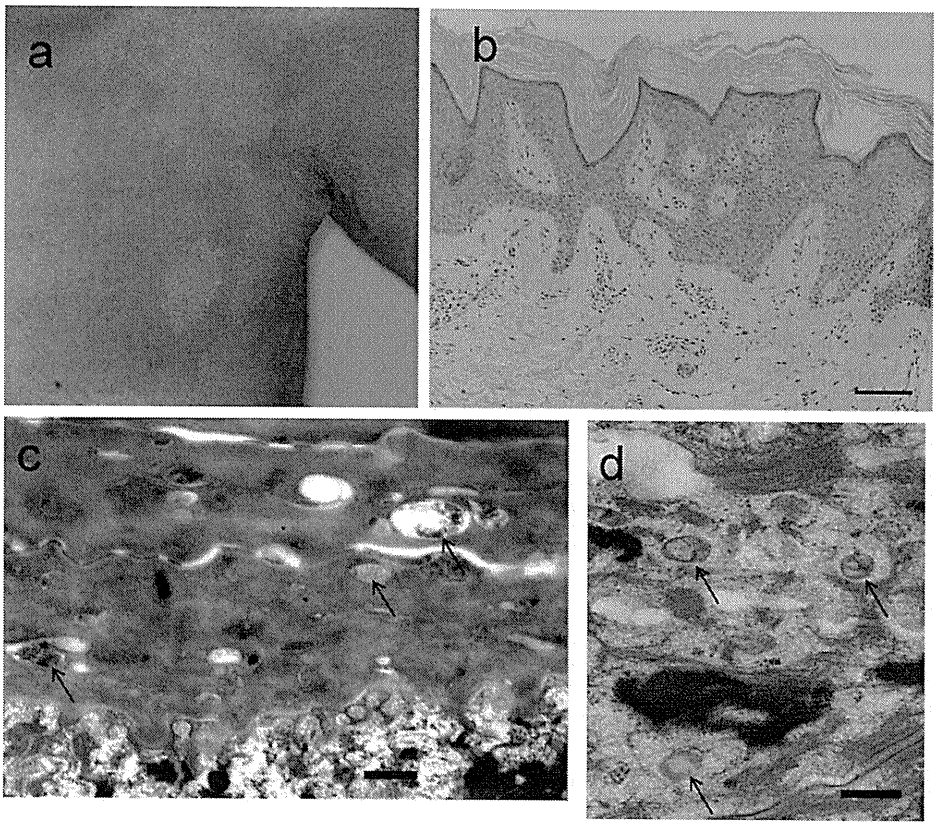

Fig. 1. Clinical appearance. (a) Scaly ichthyosiform erythema was apparent over the trunk. Morphological features of the patient's epidermis. (b) H&E staining of lesional skin from the patient's forearm. Orthohyperkeratosis, slightly thin granular layers and mild acanthosis with papillomatosis are noted, scale bar, 50 μm. (c) Ultrastructually, electron-lucent vacuoles are present within corneocytes (arrows) scale bar, 2 μm. (d) The presence of abnormal lamellar bodies lacking lamellar contents are evident in the cytoplasm of the granular cell (arrows) scale bar, 2 μm.

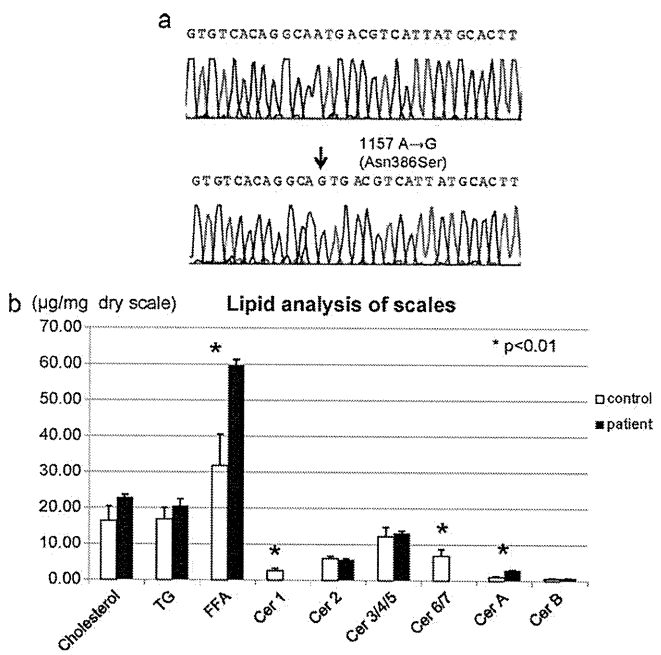

Fig. 2. (a) Sequencing analysis of FALDH gene. A homozygous point mutation (c.1157A>G) in the exon 8 that substitutes serine for asparagine at position 386 (p.Asn386Ser). (b) Lipid analysis of scales taken from sunburn lesions of a normal control individual (white bar) and from the patient's lesions (black bar) show increased FFA and Cer A level and decreased ceramide 1, 6, 7 levels in the patient's scale compared with control samples. Scales were taken from the upper back skin of the patient or control subjects. Gene and lipid analysis were performed as we described previously [4,6].

Japanese cases were c.481delA, c.1087_1089delGTA, c.332G>A (p.Trp111X) and c.636T>G (p.Ser212Arg) [3,4]. All the mutations found in Japanese families were distinct from one another and no founder effect was suggested in *ALDH3A2* mutations underlying Japanese SLS cases.

Recent studies by lanthanium perfusion assay, which is more sensitive for assessing permeability barrier function *in vitro* using skin sections than TEWL measurements employed in our study, reveals abnormal permeability barrier formation, structures, and function in SLS patients [7], while our present study is the first time for assessing both TEWL and hydration of SLS patient *in vivo*. Consistent with this prior study abnormal epidermal barrier structures [7] are evident in our patient, but alterations of TEWL were not observed. We assume that hyperkeratosis could attempt to compensate barrier dysfunction as previously suggested [8] and result in attempting to minimize barrier abnormality. Yet, decreased SC hydration in a SLS patient could alter normal SC environment, leading to abnormal epidermal homeostasis.

It remains to be resolved, however, why FFA level was high in spite of the deficient activity of FALDH, which was the enzyme catalyzing the sequential oxidation of fatty alcohol to fatty acid. It is likely that increased levels of wax esters and alkyl-diacylglycerol in scales and keratinocytes of SLS [9] derived from fatty alcohol may contribute to FFA production via hydrolysis with lipase, because the levels of these lipids were high.

Consistent with a prior study showing a deficiency of Cer 1, 6 in SLS patients' skin [10], Cer 1, 6, 7 were decreased in the epidermis of our case. We further demonstrated that the levels of CLE-bound ceramides, Cer A, which are produced from acylglucosylceramide, elevated in the scale from the patient, although Cer 1 (EOS) generated from the same precursors decreased. Therefore,