Role of fatty acid transporters in epidermis: Implications for health and disease

Denis Khnykin  
*Oslo University Hospital-Rikshospitalet*

Jeffrey H. Miner  
*Washington University School of Medicine in St. Louis*

Frode Jahnsen  
*Oslo University Hospital-Rikshospitalet*

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Role of fatty acid transporters in epidermis
Implications for health and disease

Denis Khnykin,¹* Jeffrey H. Miner² and Frode Jahnsen¹

¹Laboratory for Immunohistochemistry and Immunopathology (LIPAT); Department of Pathology; Oslo University Hospital-Rikshospitalet; Oslo, Norway; ²Department of Medicine/Renal Division and Department of Cell Biology and Physiology; Washington University School of Medicine; St. Louis, MO USA

Key words: skin barrier, fatty acids transport, fatty acid activation, FATP4, ichthyosis prematurity syndrome, inflammation

Abbreviations: FA, fatty acids; LCFA, very long chain fatty acids; VLCFA, very long chain fatty acids; SC, stratum corneum; FAS, fatty acid synthase; CD36/FAT, fatty acid translocase; FATP, fatty acid transport proteins; FABP, fatty acid binding proteins; ACSL, long chain fatty acid-CoA synthetics; ACBP, acyl-CoA binding proteins; ARCI, autosomal recessive congenital ichthyosis; IPS, ichthyosis prematurity syndrome

Skin epidermis is an active site of lipid synthesis. The intercellular lipids of human stratum corneum (SC) are unique in composition and quite different from the lipids found in most biological membranes. The three major lipids in the SC are free fatty acids, cholesterol and ceramides. Fatty acids can be synthesized by keratinocytes de novo and, in addition, need to be taken up from the circulation. The latter process has been shown to be protein mediated, and several fatty acid transporters are expressed in skin. Recent studies of transgenic and knockout animal models for fatty acid transporters and the identification of fatty acid transport protein 4 (FATP4 or SLC27A4) mutations as causative for ichthyosis prematurity syndrome highlight the vital roles of fatty acid transport and metabolism in skin homeostasis. This review provides an overview of our current understanding of the role of fatty acids and their transporters in cutaneous biology, including their involvement in epidermal barrier generation and skin inflammation.

Introduction

The skin is a complex and highly specialized organ serving multiple functions in the body, the main of which is to provide a barrier that prevents water loss and protects the body from adverse environmental agents.¹ This function is mediated exclusively by the stratum corneum (SC)—the outermost layer of the epidermis, consisting of dead flattened keratinocytes embedded in a lipid matrix, acting together as a “brick and mortar” system that is difficult to penetrate. The lipids that constitute the extracellular lamellar matrix of the stratum corneum have a unique composition and exhibit distinctive properties.² The major lipids of the human SC are ceramides, cholesterol and fatty acids (FAs), comprising approximately 50, 25 and 15% of the total lipid mass, respectively.³ Interestingly, this mass composition reflects nearly equimolar quantities of ceramides, cholesterol and fatty acids, a ratio that is imperative for normal lamellar membrane organization and epidermal barrier homeostasis.⁴⁻⁵ Both essential and non-essential fatty acids play separate and critical roles in proper skin function. In this review we will focus on the role of fatty acids and their transporters in skin epidermal barrier functions and disease.

Origin of Fatty Acids in the Epidermis

The epidermis is a very active site of lipid synthesis, exceeding even the liver, kidney and gastrointestinal epithelia on a per weight basis.⁶ Most of the FAs can be synthesized by keratinocytes de novo. FAs with carbon chains of up to C16 are synthesized by cytosolic fatty acid synthase (FAS). In normal human epidermis, FAS is strongly expressed in the stratum granulosum and moderately in the uppermost layer of the stratum spinosum, suggesting that FA synthesis may increase during normal epidermal differentiation.⁷ Acute disruption of the epidermal barrier stimulates FA synthesis, which is required for full recovery of the barrier.⁸ A significant amount of the FAs produced by FAS, as well as FAs taken up from diet, are further elongated into very long chain FAs (C ≥18).⁹ The role of FA elongation in epidermal function will be discussed in detail in the review by Dr. Y. Uchida in this series. We will only mention that during cornification, short-chain FAs are replaced by long-chain, highly saturated species, ranging from 14 to 28 carbons in length. The majority are 20 carbons and longer, with 22–24 carbon lengths being the most abundant.³

However, not all fatty acids can be synthesized by keratinocytes, and not all that can be synthesized are produced in sufficient quantities. Research performed more than 80 years ago show that essential fatty acid (EFA) deficiency leads to abnormalities in skin function, resulting in scaly dermatosis, permeability of skin to water and hair loss.¹⁰ The human body can produce all but two of the fatty acids it needs: linoleic acid (C18:2, n6) and alpha-linolenic acid (C18:3, n3). In addition to these, nutritional studies have revealed the existence of so called conditional EFAs, such that the term EFA may be applied to over thirty omega-6,

¹Correspondence to: Denis Khnykin; Email: denis.khnykin@labmed.uio.no
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and omega-3 polyunsaturated fatty acids. Skin is devoid of 
delta-5 desaturase and so cannot directly convert gamma-linole-
nic acid to arachidonic acid. Thus, arachidonic acid (C20:4, n6) 
in the epidermis is not of keratinocyte origin, but must be syn-
thesized in the liver and transported into keratinocytes therefore 
being an essential fatty acid for the skin. In addition to linoleic 
and arachidonic acids, several long chain- and very long chain-
fatty acids (LCFA and VLCFA) were identified as EFAs for the 
skin, and they also have to be translocated across the keratinocyte 
plasma membrane.

Role of Fatty Acids in the Epidermis

FAs have multiple roles in the epidermis. They are found in 
bound form in triglycerides, phospholipids, glycosylceramides 
and ceramides, which are all playing a vital role in formation 
of the epidermal permeability barrier. However, FAs in kerati-
nocytes do not function only as building blocks. In addition to 
their well known role in energy generation and storage, FAs can 
be potent signaling molecules, activating the nuclear hormone 
receptors peroxisome proliferators-activated receptors (Pars).13-15 
Moreover, by acylation FAs may modify other signaling mol-
ecules, including Sonic hedgehog and Wnt,16 though the role 
of such modifications in epidermal biology is not yet studied. 
There are a few recent studies concerning the role of protein pal-
mitylation in skin.17,18

EFAs are integral for normal stratum corneum structure 
and function. Three acylceramide species unique to epidermis 
contain linoleic acid esterifies to the terminal omega-hydroxyl 
group of very long chain fatty acids (C ≥28–34). These cerami-
des are central to barrier function and essential for mammalian 
survival.12 Substitution of the linoleic acid with non-essential 
oleic acid, as in the case of EFA deficiency, will lead to bar-
rier defects accompanied by proliferative epidermal changes.19 
Atopic dermatitis and psoriasis, well known inflammatory skin 
diseases, are characterized by changes in FA composition in 
kernatinocytes, indicating their possible involvement in modu-
lating of inflammatory processes.20-25 Moreover, free FAs are 
one of the major contributors to the acidic pH at the SC sur-
face regulating permeability and antimicrobial barrier, inflam-
mation and desquamation.26-28 And last, but not least, there is 
an accumulating evidence for the existence of an epidermis-specific 
hepoxilin pathway for arachidonic acid utilization in keratino-
cyes, which is important for formation of functional epider-
mis.29-34 These genes include two lipoxigenases (ALOX12B, 
ALOX13), an ATP binding cassette transporter (ABCA12), a 
potential receptor (ICHTHYIN) and a gene coding for a protein 
of the cytochrome P450 family (CYP4C22).29,31-34 Mutations in 
these genes, as well as mutations in transglutaminase 1 (TG1), 
have been reported as the most common cause of autosomal 
recessive congenital ichthyosis (ARCI). However, 20–40% of 
the patients have no mutations in the six known ARCI genes 
suggesting that more genes are involved in this type of ichthyosi-
ses.30,35 The role of lipoxygenases in formation of the epidermal 
barrier will be reviewed in details by Alan Brash in this series of 
reviews.

Transport of Fatty Acids to the Epidermis

There are still ongoing debates regarding the mechanisms by 
which free FAs are transported across cell membranes. On the one 
hand, due to the lipophilic nature of FAs, it has been proposed 
that they may be passively transported through the lipid bilayer 
by “flip-flop” diffusion. This mechanism does not involve any 
protein mediators.36,37 On the other hand, many studies have pro-
vided considerable support for the protein-mediated entry of long 
chain fatty acids.38 While the exact mechanism by which FAs are 
preferentially transported into cells is not yet settled, apparently 
both modes of FA uptake (diffusion or protein-dependant) may 
coexist in mammalian cells. The preferential mode of transport 
may depend on: (1) the nature of the membrane (plasma mem-
brane organelle or vesicular membrane); (2) cell type (e.g., adi-
posities, neurons and keratinocytes may have different systems 
for FA transport); and (3) functional state of the cell (e.g., rest-
ing, activated, proliferative or cancer cells of the same origin may 
evolve different mechanisms). Recently, a unified hypothesis for 
the role of the lipid bilayer and proteins was proposed for trans-
port of FA into brain cells.39 However, due to space limitations 
we will not discuss the different models for fatty acid transport in 
details, but rather refer to several comprehensive reviews which 
cover this topic.38,40-43

There are several lines of evidence suggesting that keratino-
cyes must import fatty acids from extracutaneous sites. In addi-
tion to EFAs which by definition must be obtained from the diet, 
eicosapentaenoic acid (C22:6, n3) and docosahexaenoic acid 
(C20:5, n3) derived from dietary fish oil are incorporated into 
epidermal lipids.44 Second, plant-derived fatty acids accumulate 
in the epidermis in certain diseases states, such as in Résumé’s 
disease.45 Third, studies have shown that systemically adminis-
tered 14C-labelled FAs were delivered to the epidermis.40 Fourth, 
high concentration of FA synthesis in the epidermis does not completely 
block barrier recovery after acute disruption, indicating that sev-
eral sources of fatty acids are available to the keratinocytes.3 And 
finally, studies have shown the active uptake of FAs by kerati-
nocytes, which is temperature sensitive, has saturable kinetics, 
and can be reduced by prior treatment with try sin, indicating 
protein-mediated FA uptake.46 The latter study demonstrated that 
keratinocytes transport EFAs, i.e., linoleic acid and arachi-
donic acid, with higher specificity than for non-essential FA, 
such as oleic acid (C18:1, n9). These transport preferences were 
not shared with other cell types, such as hepatocytes and dermal 
fibroblasts, which transport non-essential and essential FA with 
similar kinetics. However, identity of the protein(s) responsible 
for FA uptake in keratinocytes was not determined in this study.46

In recent years, a number of proteins have been identified 
that in some way may facilitate the LCFA transport in mam-
alian cells. They include fatty acid translocase (CD36/ 
FAT),48 fatty acid transport proteins (FATPs),49 fatty acid bind-
ing proteins (FABPs),50 long chain fatty acid-CoA synthetics 
(ACSLs),52 and acyl-CoA binding proteins (ACBP).53 Despite 
the fact that these proteins have a different tissue expression 
pattern and subcellular localization, there are evidences to sug-
gest that each of these transporters can independently increase
FA uptake (see references above). In addition, it was proposed that FA transport can occur through the combination of caveolin and CD36/FAT in conjunction with lipid rafts. Finally, an unknown protein has been reported recently to be involved in uptake of FAs by adipocytes.

How proteins contribute to FA uptake is again highly controversial. It was proposed that FATPs and CD36/FAT are the only real transporters directly involved in binding and transport of FAs across membranes. Moreover, they may interact in such a way that FA binds first to CD36, which subsequently delivers the free FA to the FATPs. However, whether CD36/FAT and FATPs are directly involved in the translocation process is not clear. Alternatively, these transporters may be indirectly involved in FA uptake by binding and creating high local concentrations of free FAs in close proximity to the membrane, as for CD36/FAT, or by vectorial acylation, as for FATPs.

After translocation to cytosol almost all FAs need to be activated by esterification with coenzyme A (CoA) before they can be utilized, a process which is catalyzed by a family of enzymes called acyl-CoA synthetases. FATPs possess acyl-CoA synthetase activity, generally preferring 16–18 carbon FAs, but can also activate FAs as long as 26 carbons. Activation of FAs into fatty acyl-CoA-forms diminish the intracellular pool of free FAs, thus creating a gradient across the membrane and enhancing influx of extracellular FAs, a process called vectorial acylation. The majority of the substrate (FA) and the end product (acyl-CoA) of the enzymatic reaction do not exist free in the cytosol under physiological conditions, but are rather bound to FABPs or ACBPs. Interestingly, all proteins in this pathway have been reported to mediate FA uptake, indicating the existence of tightly regulated mechanisms controlling concentration of the free FAs and the free acyl-CoA forms in the cytosol. Deficiency in ACSLs or in FABPs will lead to accumulation of LC FAs in the cytosol and indirectly decrease FA uptake. Deficiency in ACBPs will create local high concentration of free acyl-CoA, which may inhibit further esterification and indirectly affect FA transport. In addition, both free LC FAs and free acyl-CoA are potent signaling molecules which may regulate expression of genes involved in lipid metabolism through activation of nuclear hormone receptors. Moreover, if present in high concentrations, free FAs will lead to lipotoxicity and ultimately to cell death.

Surprisingly, taking into account the high rate of lipid synthesis in keratinocytes, only a few studies have been published reporting differential expression of putative FA transporters in epidermis and skin appendages. These studies have shown that FATP1, -3, -4 and -6, along with CD36/FAT, are expressed in adult murine epidermis. Interestingly, FATP1 and -3 were expressed predominantly by keratinocytes, whereas FATP4 was strongly expressed by sebaceous glands and FATP6 by hair follicle epithelium. As a result of permeability barrier disruption in mice, increase in expression of FATP1 and -6 have been observed, as well as robust increase in CD36 protein and mRNA. FATPs expression in humans was comparable to that in mice. Experiments with primary human keratinocytes show that the major FATP expressed in culture was FATP4, and induction of differentiation induced by high calcium conditions (1.2 mM Ca2+) results in approximately 50% reduction in the level of FATP4 protein. In marked contrast to human and mouse epidermis, neither FATP1, -3 nor -6 were expressed in either undifferentiated or differentiated human keratinocytes. In another study, in addition to FATPs and CD36/FAT, plasma-membrane FABPs and fatty acyl-CoA synthetase have been reported at different levels in undifferentiated and differentiated human keratinocyte cultures, as well as in mouse epidermis.

The crucial role for proteins in the efficient uptake of LCFAs was highlighted by a number of mouse models with impaired or enhanced FA transport. In the next section we will discuss cutaneous manifestations in these models as well as clinical skin abnormalities that occurs secondary to mutations in FA transporters.

### Role of Fatty Acid Transporters in Skin: Mouse Models and Possible Clinical Involvement

Among the number of mouse models with defective FA metabolism, we will in this review discuss only “classical” transporters such as CD36/FAT, FABPs and FATPs.

#### CD36/FAT

CD36 is an integral transmembrane glycoprotein with molecular mass of 88 kea in its fully glycosylated form, functioning as a scavenger receptor for oxidized low-density lipoproteins with multiple function in different cell types. CD36 was proposed to be a crucial transporter for long-chain fatty acids mediated through interaction with lipid rafts and caveolin. It is expressed in tissues with high FA metabolism, including adipose tissue, heart and skeletal muscles. CD36 has been also reported to be weakly expressed in normal epidermis, with increased expression after barrier disruption. However, CD36 knockout mice do not have any apparent skin phenotype, while defective uptake and utilization of LCFAs have been reported in muscles and adipose tissues. Recent finding indicate that CD36 mediates uptake of LC FAs, VLC FAs and cholesterol in the intestine. Interestingly, CD36 has four palmitoylation sites on both N- and C-termini, and it was shown that palmitoylation plays a crucial role in targeting CD36 to lipid rafts, where it mediates its function. In humans, CD36 deficiency has a prevalence of 0.3–11%, with higher incidences in Asian and African populations, while again skin phenotype was not obvious. Apparently, CD36-mediated uptake of FAs in keratinocytes can be easily compensated by other FA transporters.

#### FABP5

FABPs can be divided into two main groups: those associated with plasma membrane (FABP-pm) and with intracellular, cytoplasmic proteins (FABPc). So far, nine tissue-specific cytoplasmic FABPs with molecular mass 14–15 kea have been identified, including cytosolic epidermal FABP (FABP5, also termed E-FABP or PA-FABP). In contrast, FABP-pm has a higher molecular weight (43 kea) and seems to be identical to mitochondrial aspartame aminotransferase. Overexpression of FABP-pm in mammalian tissues can increase uptake of FAs, and in human keratinocytes induction of differentiation by high
calcium reduced FABP-pm mRNA by 50%. However, whether this finding is relevant to the regulation of FA uptake into keratinocytes was not studied. Another study has shown that antibodies directed against FABP-pm did not inhibit FA uptake in human keratinocytes, but did inhibit its uptake in hepatocyte cell line HepG2, indicating that FABP-pm is unlikely to be responsible for the bulk of transport activity in keratinocytes.

More is known about FABP5 which is predominantly expressed in keratinocytes, where it has been proposed to act as a cellular lipid chaperone in keratinocytes homeostasis. FABP5 has been detected also in tongue and thymus epithelia, as well as in mammary and adipose tissues, while it seems that FABP5 is the only FABP which is detected in epidermis. Compared with other tissues active in lipid metabolism, human keratinocytes contain a surprisingly low amount of FABP5, and its specific role in normal skin is not yet fully established. The amount of FABP5 increases with keratinocyte differentiation and immuno-histochemical studies have demonstrated that FABP5 expression is strongest in stratum granulosum, where activity of rate limiting enzymes required for barrier formation is the highest. Overexpression of FABP5 is associated with highly proliferative skin conditions, such as psoriasis, atopic dermatitis and basal and squamous cell carcinomas. It can be speculated that FABP5 expression is increased in response to increased lipid traffic, which may be related to abnormal proliferation and differentiation of keratinocytes in such conditions.

The skin of FABP5 knockout mice appears normal at the gross and histological level, but the water-barrier function of the epidermis is altered in these mice. It was shown that FABP5 deletion results in impaired keratinocyte migration, suggesting a connection between FABP5 and cell motility. The lack of obvious skin phenotype raises the possibility that other FABP members or even other FA transporters can compensate for FABP5 deficiency during development.

Recent study by Ogawa and colleagues helps to clarify the mechanism of FABP5 action in epidermis. It was shown that FABP5 deletion affects keratinocyte differentiation, but not proliferation. Total FA content in FABP5-deficient epidermis was decreased including decreased saturated, monounsaturated and polyunsaturated FAs. Detailed examination revealed that linoleic acid (C18:2, n6) was significantly decreased, while arachidonic acid (C20:4, n6) was unchanged. Furthermore, the authors showed that a linoleic acid derivative, 13-hydroxyoctadecatrienoic acid [13(S)-HODE], mediates keratinocyte differentiation by activating the NFkB pathway. Consequently, FABP5 deletion will lead to decreased cellular linoleic acid and 13(S)-HODE contents, resulting in downregulation of NFkB activity and decreased differentiation. Given the strong expression of FABP5 in hyperproliferative skin conditions, it can be suggested that FABP5 may have a role in the pathogenesis of such diseases through impaired metabolism of FAs and their derivatives. Except for hyperproliferation in keratinocytes, no other clinical conditions caused by either deficiency of overexpression of FABP5 have been reported so far in humans.

FATP4. Recently, mutations in the FATP4 gene (also known as SLC27A4) were identified as causative for Ichthyosy Prematurity Syndrome (IPS). IPS is a rare disorder of cornification belonging to the heterogeneous group of autosomal-recessive congenital ichthyosy (ARCI). Ultrastructural investigation of the first IPS patients identified in Norway supported the idea that this was a distinct type of congenital ichthyosis, termed ichthyosy congenita type IV. IPS has been described almost exclusively in a region in the middle of Norway and Sweden where the estimated heterozygote carrier frequency is 1 in 50. Outside of this region, only a few cases have been reported in other European countries, including Germany, Finland, Italy, Denmark and France, as well as in families from North Africa and Middle East. IPS is characterized by the clinical triad of premature birth, scaly erythroderma and neonatal asphyxia in combination with pathognomonic ultrastructural findings, typically showing lipid membrane packages in the granular and horny cells. The severe skin phenotype at birth significantly improves during the first weeks of life, and recovers into a lifelong non-scaly ichthyosis with dermal atopic dermatitis-like inflammation and severe ichishing (D. Khynkin et al. unpublished). Interestingly, most of the Norwegian patients have extremely high numbers of cerosinophils in peripheral blood and very high serum-Age levels; both are central to etiology of atopic disorders (D. Khynkin et al. unpublished).

FATP4 knockout mice also show a dramatic skin phenotype, together highlighting the importance of this transporter for skin homeostasis. FATP4 belongs to the family of membrane associated FATPs consisting of six members, designated FATP1-6. They are proposed to mediate the translocation of FAs from the extracellular milieu into cells. However, not all members were able to complement FATP5 knockout mice in yeast studies. In addition, all six proteins have acyl-CoA synthetase activity and are able to activate LCFA and VLCFAs to their CoA hoisters for subsequent metabolism. The mechanism by which FATPs mediate FA uptake is not well understood, and it remains to be determined whether they participate in the physical translocation process or facilitate transport by trapping, as CoA derivatives, fatty acids that enter cells by diffusion.

Several FATPs, including FATP4, are expressed in the epidermis, and animal models suggest that FATP4 plays an important role in skin homeostasis. FATP4 knockout mice die either in uterus or shortly after birth, showing disturbed epidermal barrier and hyperkeratosis resulting in very tight, thick skin (wrinkle free phenotype). Given the well-known importance of lipids to the epidermal barrier, it was reasonable to hypothesize that the absence of FATP4 led to abnormal lipid trafficking and failed production of the lipids required to establish a proper skin barrier. Indeed, FATP4 was shown to be expressed in the epidermis primarily in the granular and upper spinous layers where epidermal barrier lipid synthesis is robust. Furthermore, analysis of epidermal lipid composition by mass spectrometry in the absence of FATP4 showed an increase in the fraction of ceramides with fatty acid moieties containing 24 or fewer carbon atoms, at the expense of those containing 26 or more.

Ceramides are crucial for barrier formation so this change in creased composition likely contributes to the barrier abnormalities. Studies
of dermal fibroblast cell lines established from FATP4 knockout mice revealed that FATP4 is in fact a principal VLCFA acyl-CoA synthetase in skin fibroblasts. However, mutant fibroblasts also showed reduced uptake of LCFA and a reduced level of long chain polyunsaturated FAs. Taking into account that FATP4-deficient cells also contain abnormal neutral lipid droplets, this indicates that the metabolic abnormalities in these cells are likely not limited only to VLCFAs. Despite the high expression of FATP4 in other tissues, such as intestine and brain, it was possible to rescue the lethal phenotype of FATP4 knockout mice by keratinocyte-specific transgenic expression of FATP4, resulting in viable and fertile mice with only mild skin and hair abnormalities. This suggests that the skin phenotype of FATP4 mutants is indeed due to the absence of FATP4 from epidermis. No structural or functional defects in other tissues have been observed in the transgene-rescued mutants that lack FATP4 expression in non-epidermal tissues. Importantly, keratinocytes-specific expression of FATP4 mutated in the acyl-CoA synthetase domain did not rescue the skin phenotype, indicating that the ability of FATP4 to activate fatty acids is a crucial for its function.

Another strategy was chosen by Herrmann et al. who created mice with conditional FATP4 deficiency in the epidermis. This mouse line displays distinct changes in the structure of the epidermis and a compromised barrier function, but the phenotype was not nearly as severe as that seen in mutant neonates. Interestingly, IPS patients carrying mutations in FATP4 gene show much more severe phenotype immediately after birth and in neonatal period, and show a mild form of ichthyosis in later life (see below). The explanation for this apparent difference could be that in adult skin FATP4 is compensated by other FATPs. As was discussed above, adult skin expresses several FATPs, including FATP1, -3, -4 and -6, and FATP1 and 3 are predominantly expressed by keratinocytes. However, studies of embryonic expression at day 18.5 revealed that FATP1 is not expressed in epidermis, whereas expression of FATP4 was relatively increased compared with expression in adult epidermis. It can be speculated therefore, that FATP4 is more critical for the generation of the epidermal barrier and less important for its maintenance, because after birth the other FATPs may partially compensate for the deficiency of FATP4.

To gain insights into why the absence of FATP4 in utero causes the severe skin defects in mice, gene expression profiling was used to search for significant changes at embryonic day 15.5. This is the gestational age at which the wrinkle free phenotype first becomes detectable in some but not all Fatp4 mutants; thus, the hope was that any changes observed would be directly related to the absence of FATP4 rather than secondary to the abnormal skin phenotype. The results suggested that there was premature expression of genes relevant to establishing the barrier, and this was validated by the presence of a precocious but incomplete skin barrier at E16.5 that never progressed into a complete barrier. Four members of the epidermal growth factor (EGF) family of ligands were also upregulated. This was associated with increased activation of the EGF receptor (EGFR) and of at least one of its downstream effectors, signal transducer and activator of transcription-3 (STAT3). Pharmacological studies in vivo with curcumin and AG1478, inhibitors of EGFR and/or STAT3 signaling, showed that activation of EGFR and STAT3 caused suprabasal keratinocyte hyperproliferation and thickening, as well as precocious barrier formation. However, the lack of skin wrinkles was not ameliorated, indicating that other signaling pathways must contribute to that aspect of the phenotype.

The Fatp4 gene (also known as Slc27a4) consists of 1 non-coding and 12 coding exons, encoding a protein of 643 residues with a predicted size of 71 kDa. Fatp4 is highly conserved among widely divergent species and contains an N-terminal transmembrane (TM) region, an ER localization signal (ERx), as well as two functioning domains: ATP/AMP motif, responsible for ATP binding and FATP/VLACS motif, possessing very long chain acyl-CoA synthetase activity and contributing to fatty acid transport. Molecular genetic analysis of DNA from members of 18 Scandinavian IPS patients revealed that they all were either homozygous or compound heterozygous for a nonsense mutation in exon 3 of the FATP4 gene, whereas a North African patient and a patient from the Middle East were homozygous for a splice site mutation in exon 5 and a missense mutation in exon 12, respectively. Two new mutation in the Fatp4 gene leading to IPS were identified recently: missense mutation c.1120C>T in a highly conserved region of exon 8, and a homozygous A>G mutation in exon 2 (c.1a>g) which leads to conversion of the initiation codon ATG to GTG (p.M1V) (Inhoff et al. in press).

All together, nine mutations in Fatp4, affecting several domains of Fatp4 protein, have been found so far in IPS patients worldwide. The most frequent mutation is located in exon 3. If the mRNA for this allele is translated it will produce truncated protein without any FA transport or acyl-CoA synthetase activity. Of interest, mutation in mouse Fatp4 leads to generation of a protein containing the first 133 amino acids of FATP4 fused to 50 amino acids encoded by retrotransposon antisense RNA which will also lack acyl-CoA synthetase activity. However, the mice exhibit a much more severe, lethal phenotype, which may be due to a higher surface to volume ratio as compared with humans, leading to excess loss of water through the skin.

The mechanism by which Fatp4 deficiency leads to a defective barrier and skin inflammation is not known and is only beginning to be elucidated. Some of the possible mechanisms are shown in Figure 1. It would be tempting to speculate that impaired acyl-CoA synthetase activity is the major trait associated with IPS pathology. Fatp4 carrying a mutated acyl-CoA domain is functionally inactive in vivo and not able to rescue the skin phenotype in Fatp4 deficient mice, indicating a critical role for FA esterification by Fatp4 in skin barrier formation.
FATP4 deficient mice, as well as both human and mouse FATP4 deficient fibroblasts, show a decreased activation and incorporation of very VLCFAs into complex structural lipids.

Analysis of lipid composition from FATP4 deficient epidermis revealed deficiency of VLCFAs (C≥26) in epidermal ceramides, which might affect barrier properties of the skin. But barrier function may not be the only aspect of the epidermis affected by the lack of FATP4 activity. The inability of FATP4 to activate VLCFA and LCFA could lead to intracellular accumulation of free FAs in the cell, leading to ER-stress and activation of proinflammatory pathways.

Mouse FATP4 shows considerable activity in the esterification of arachidonic acid in uptake- and activation-studies in yeast. Moreover, the level of arachidonic acid was almost 50% lower in FATP4 deficient fibroblasts compared to control cells, while the level of linoleic acid was unchanged. Arachidonic acid is a precursor of potent pro- and anti-inflammatory eicosanoids, and it appears that the 12(R)-lipoxigenase pathway of arachidonic acid utilization plays a critical role in epidermal barrier formation.

Therefore, it cannot be excluded that altered arachidonic acid metabolism are directly involved in the skin pathology observed in humans and mice with FATP4 deficiency.

Given the highly purity nature of IPS, another possible factor affecting the phenotype could be related to regulation of skin pH. The changes in stratum corneum pH may be due to accumulation of free FAs, generated both in epidermis and sebaceous glands. Local changes in pH can activate proteases and their receptors in inappropriate temporal and spatial patterns, leading to inflammation and itching. One more family of proteins which are expressed in epidermis and are pH-sensitive is the transient receptor potential (TRP) channel family. Members of this family has been shown to be involved in the pathogenesis of itch and in skin barrier formation, where it regulates EGFR signaling.

Finally, proinflammatory signals may be generated not only in epidermis but also in skin appendages. FATP4 is highly expressed in sebaceous glands, and absence of its acyl-CoA synthetase activity may lead to accumulation of free fatty acids in sebocytes, as well as in keratinocytes. Sebum free FAs may enhance production and secretion of antimicrobial peptides, which in turn may stimulate production of proinflammatory cytokines and chemokines by keratinocytes.

**Concluding Remarks**

Although the exact mechanism by which FAs are transported into keratinocytes remains unclear, studies of different FA transporters demonstrate their unique and important roles in the epidermis for maintaining skin barrier and keratinocyte homeostasis. Animal models of FATP4 deficiency, as well as human phenotype in IPS, have demonstrated a vital role for FATP4 in mammalian skin development and formation of epidermal barrier. Pathomechanism of FATP4 deficiency in skin is still poorly understood and clarifying of specific pathways affected by such deficiency may yield new insights for the role of fatty acid metabolism in epidermal biology.

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