Requirement of Fatty Acid Transport Protein 4 for Development, Maturation, and Function of Sebaceous Glands in a Mouse Model of Ichthyosis Prematurity Syndrome

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Background: Fatty acid transport protein 4 (FATP4) facilitates fatty acid uptake by converting fatty acids into active forms. FATP4 is expressed in several tissues, including skin. Mutations in human SLC27A4, which encodes FATP4, cause ichthyosis prematurity syndrome, characterized by a thick desquamating epidermis and premature birth. Mice lacking FATP4, which genetically model the human disease, are born with thick skin and a defective skin barrier; they die neonatally due to dehydration and restricted movements. Both the skin phenotype and the lethality are rescued by transgene expression of FATP4 in suprabasal keratinocytes. Sebaceous glands in Fatp4 null skin grafted onto nude mice were found to be dystrophic and enwrapped by thick layers of epithelial cells. Consistent with these results, transgene-rescued Fatp4 null mice showed a subnormal level of FATP4 expression in sebocytes and exhibited abnormal development of both sebaceous glands and meibomian glands, specialized sebaceous glands of the eyelids. Sebum from these mice contained a reduced level of type II diester wax, a major mouse sebum lipid species, and showed perturbations in mass spectrometric profiles of diester; TAG, triacylglycerol; ESI, electrospray ionization.

Results: Reduced FATP4 in mice leads to dystrophic sebaceous glands and abnormal production and function of sebaceous gland lipid secretions.

Conclusions: FATP4 plays crucial roles in the formation and function of lipid-secreting glands.

Significance: Understanding how FATP4 regulates lipid metabolism is important in elucidating the pathogenesis and potential therapies for ichthyosis prematurity syndrome.

The sebaceous glands of pilosebaceous units are epidermally derived appendages situated in the dermis. They are composed of two major types of cells: lipid-producing acinar cells (sebocytes) and ductal epithelial cells. During fetal development, sebaceous glands originate from the same epidermal buds as hair follicle epithelium. Sebaceous glands contain an outer row of progenitor cells that give rise to centripetally differentiating sebocytes. Sebocytes produce sebum, a lipid-rich fluid, that is released via holocrine secretion as lipid-filled sebocytes disintegrate (1, 2). Mature sebaceous glands remain attached to the permanent portion of hair follicles by a duct through which sebum flows into the follicular canal and onto the hair and skin surfaces. Sebum lubricates hair shafts (3) and plays important roles in skin homeostasis (reviewed in Ref. 4), e.g. by reinforcing the skin barrier, supplying antioxidants to the skin surface, and exhibiting antimicrobial activities. In furry mammals, sebum is crucial for water repulsion and thermoregulation (1).

In contrast to hair follicles, much less is known about the molecular mechanisms involved in sebaceous gland morphogenesis. Studies of mutant and chimeric mice have shown that tumor necrosis factor receptor-associated factor 6 (5), CCAAT/enhancer-binding protein (C/EBP)α and β (6), and peroxisome proliferator-activated receptor γ (7) play roles in sebaceous gland development. In addition, stearoyl-CoA desaturase 1 (SCD1) (3, 8), acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) (9), elongation of very long-chain fatty acids 3 (10), and acyl-CoA binding protein (11) are important for seboocyte differentiation and sebum production.

Meibomian glands are modified sebaceous glands that are embedded in the tarsal plate of the eyelids. They secrete meibum, a lipid-rich fluid that forms a superficial oily layer on...
the tear film to prevent the film from evaporating. Meibomian glands originate from the fused eyelid margin at the late fetal stage (12). Studies of mutant and transgenic mice have shown that tumor necrosis factor receptor-associated factor 6 (5), C/EBP\(\alpha\) and -\(\beta\) (6), ectodysplasin-A receptor signaling (13), bone morphogenetic protein signaling (14, 15), homeodomain transcription factor BARX2 (16), and kruppel-like factor 5 (17) are important for meibomian gland development. In contrast, SC\(D\)1 is important for meibum production (3, 8).

We previously identified an autosomal recessive mouse mutation called \textit{wrinkle free} (18). This mutation was caused by a spontaneous retrotransposon insertion into \(S\text{lc}\text{27a}4\), the gene encoding fatty acid transport protein 4 (FATP4). Mice lacking FATP4 are born with tight, thick, “wrinkle free” skin, a defective skin barrier, and sparse hair follicles; they die neonatally due to dehydration and restricted movements. FATP4 is one of a family of six integral membrane proteins that facilitate long- and very long-chain fatty acid uptake. FATP4 exhibits acyl-CoA synthetase activity and has been proposed to facilitate uptake of fatty acids indirectly by mediating their esterification to CoA (19, 20). FATP4 is expressed in several tissues including the epidermis, suggesting roles in multiple organs (18, 20). Suprabasal keratinocyte expression of a FATP4 transgene driven by the human involucrin gene promoter (Tg[IVL-Fatp4]) in \(\text{Fatp4}\) (21). Here we investigated abnormalities in sebaceous gland formation in \(\text{Fatp4}^{-/-}\) mice using grafting experiments. We also characterized the effects of FATP4 insufficiency on formation of sebaceous and meibomian glands, lipid production from sebaceous glands, and functions of sebum using \(\text{Fatp4}^{-/-}\); Tg[IVL-Fatp4] mice. Our results reveal important roles for FATP4 in the development, maturation, and function of sebaceous glands and have potentially important implications for ichthyosis prematurity syndrome patients who lack FATP4.

**EXPERIMENTAL PROCEDURES**

**Mice and Skin Grafting—Fatp4 mutant and Tg[IVL-Fatp4] transgenic mice have been previously described (18, 21). Embryos were dissected from pregnant females, with the morning when the copulation plug was observed considered embryonic day (E) 0.5. Skin grafting onto adult nude mice was performed as described (18) using dorsolateral skin from E18.5 donors. All experiments were approved by the Washington University Animal Studies Committee.

**Oil Red O Staining—**Frozen skin sections were fixed in 37% formaldehyde for 1 min, washed in PBS and tap water, and stained for 7 min in filtered Oil Red O solution that was freshly prepared by mixing the stock solution (0.8% Oil Red O in 98% isopropyl alcohol) and distilled water at a ratio of 1.5 to 1, followed by washing in tap water. Sections were then counterstained with hematoxylin and mounted in 90% glycerol.

For whole mount staining of meibomian glands, eyelids were resected, fixed in 4% paraformaldehyde for 1 h, washed in PBS and tap water, and stained for 1 h in Oil Red O as described above. For mice older than 1 month of age, the connective tissue and muscle covering the tarsal plate were removed before staining. Most of the tarsal plate and conjunctiva were removed prior to image capture. For whole mount staining of sebaceous glands, tail epidermis was peeled from skin soaked in 5 mM EDTA in PBS at 37 °C for 4 h, fixed, and stained in Oil Red O as described for meibomian glands. The stained epidermis was then incubated in 3% hydrogen peroxide in TBST for 20 min, subjected to immunostaining with keratins 6 (1:500) and 10 (1:200) antibody as described below, and mounted in 90% glycerol.

**Immunohistochemistry**—Frozen sections were prepared by equilibrating paraformaldehyde-fixed tissues in 10% and then in 30% sucrose in PBS at 4 °C for 1 h and overnight, respectively, embedding in OCT compound (Sakura Finetek, Torrance, CA), sectioning at 10 μm in a cryostat, and air drying. After rehydrating sections in PBS and blocking endogenous peroxidase activity in 3% hydrogen peroxide in methanol for 10 min, antibody staining was performed using the peroxidase Vectastain ABC kit (Vector Laboratories, Burlingame, CA) with 3,3’-diaminobenzidine as a chromogen (Pierce), as described in the manufacturer’s instructions. For detection of FATP4, sections were denatured in 6 M urea, 0.1 M glycine (pH 3.5) at 4 °C for 1 h prior to blocking endogenous peroxidase.

Dilutions of primary antibodies used were as follows: 1:500 for FATP4 (22); 1:1,000 for keratins 6 and 10 (Covance, Princeton, NJ), and 1:50 for fatty acid synthase (Cell Signaling Technology, Beverly, MA). Sections were counterstained with hematoxylin. For double immunofluorescence staining with antibodies to SC\(D\)1 (1:50; Cell Signaling Technology) and nido- gen (1:100; Millipore, Billerica, MA), signals were detected using Alexa 488-conjugated anti-rabbit and Alexa 594-conjugated anti-rat, respectively (Invitrogen), with Hoechst 33258 as a nuclear counterstain.

**Immunohistochemistry with DGAT1 antibody** (Santa Cruz Biotechnology, Santa Cruz, CA) was performed on paraffin sections at a 1:50 dilution of the primary antibody with antigen retrieval by Trilogy as described previously (23).

**In Situ Hybridization**—Preparation of paraffin sections and \textit{in situ} hybridization using digoxigenin/UTP-labeled Fatp4 riboprobes were performed as described (21), with the hybridization temperature at 60 °C.

**Extraction and Quantification of Hair Surface Lipids—**Solvents and other chemicals were purchased from Sigma. Hair surface lipids were extracted as described previously (9), but with minor modifications. Mice were anesthetized with isoflurane, and fur was clipped from the dorsal side using a pet trimmer. Hair (100 mg) was extracted twice with 8 ml of acetone by gentle shaking for 15 min. The lipid extracts were filtered using Whatman grade 1 filter paper (Whatman), pooled, and dried with a nitrogen evaporator (Organomation Associates, Inc., Berlin, MA). The lipids were redissolved in 200 μl of chloroform and stored under nitrogen at −20 °C. Quantification of lipids was performed by the colorimetric sulfo-phosphovanillin method in a microplate format as described (24), except that reactions were performed in strip tubes and transferred to 96-well plates for colorimetric measurement. A nonpolar lipid mixture (mix B from Matreya LLC, Pleasant Gap, PA) was used to make the standard curve.
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**Lipid Analysis and Quantification by Thin Layer Chromatography (TLC)—**Lipid extracts (50 to 100 μg) in chloroform, along with a nonpolar lipid standard mixture (50 μg of mix B from Matreya LLC), were applied to Silica Gel 60 plates (Flexible plates for TLC; Whatman) at 1 cm from the bottom. Plates were resolved in a solvent mixture of hexane/diethyl ether/acetone (88:12:1, v/v) in a pre-equilibrated tank until the solvent front reached the top of the plate. Plates were dried, and the lipid spots were visualized by iodine vapor or by spraying with 3% copper acetate in 8% phosphoric acid followed by chilling at 180 °C for 15 min in an oven (25). For quantification of lipid species, lipid spots on charred TLC plates were scanned and quantified in ImageJ with co-chromatographed standards in the range of 2.5 to 40 μg. Cholesteryl oleate, triolein, oleic acid, cholesterol (Matreya LLC), and 1,2-dodecanediol dioleate were used as the standard for cholesteryl ester (CE), triacylglycerol (TAG), free fatty acids, sterols, and type II diester wax (DW)/glyceryl ether diester (GED), respectively. 1,2-Dodecanediol dioleate was synthesized by reacting 1,2-dodecanediol with oleoyl chloride. 100 mg of oleoyl chloride was added dropwise to 20 mg of 1,2-dodecanediol dissolved in 1 ml of chloroform, all at room temperature. The mixture was vortexed for 10 min, and then mixed with 1 ml of water followed by vortexing. The organic phase was collected and examined by TLC. The final product was purified with CHROMABOND aminopropyl columns (see below), evaporated to dryness under nitrogen, and the dried sample was weighed several times until the final weight was constant. The structure of the compound was identified by electrospray ionization (ESI)-mass spectrometry (MS) (see below), which yielded a major [M + Na]+ ion at m/z 753 and a product-ion spectrum that contained fragment ions typical to 1,2-dodecanediol dioleate.

**Lipid Analysis by MS—**Extracted lipids were fractionated using CHROMABOND aminopropyl, solid phase extraction glass columns (Macherey-Nagel, Duren, Germany) as follows. Lipid extracts (500 μg in less than 100 μl of chloroform) were mixed with 1 ml of hexane and loaded to the columns preconditioned with 6 ml of hexane. The columns were washed with 3 ml of hexane, and the following hexane/diethyl ether (Hex/Et2O) mixtures (v/v) were used sequentially to elute the various lipid classes: CE, 3 ml of Hex/Et2O (99/1); DW, 2 × 3 ml of Hex/Et2O (98/2); GED, 3 ml of Hex/Et2O (96/4); TAG, 2 × 3 ml of Hex/Et2O (90/10); sterols, 2 × 3 ml of Hex/Et2O (60/40); free fatty acids, 2 × 3 ml of Hex/Et2O/acetate (60/40/2); and polar lipids, 3 ml of chloroform/methanol (2/1). Lipid eluates were dried under nitrogen, resuspended in chloroform, and examined for their recovery by TLC as described earlier.

Fractionated DW, GED, and TAG were analyzed as ammonium adduct ions ([M + NH4]+) desorbed by ESI using a Thermo Finnigan (San Jose, CA) Vantage triple stage quadrupole or a LTQ-Velo linear ion-trap instrument. CE species were detected as ammonium adduct ions by precursor ion scanning of m/z 369, following collision-induced dissociation in a triple-stage tandem quadrupole mass spectrometer. Free fatty acid species were detected as [M – H]− ions in the negative ion mode; whereas sterol fractions were not analyzed by MS in this study.

**Water Repulsion Assays—**Mice at 9 to 10 weeks of age were subjected to a water repulsion assay as described (9, 10). Weights and rectal temperatures were determined, and then the mice were dropped into 37 °C water. After swimming for 4 min, they were removed and allowed to walk on dry paper towels at ambient temperature for 5 s to remove excess water. Their weights and rectal temperatures were measured at 1, 5, and every 5 min thereafter up to 40 min. Between measurements, mice were caged individually. The retained water weight at each time point was calculated by subtracting the weight before the swim from the weight at each time point afterward.

**Statistical Analysis—**Two-tailed, unpaired Student’s t tests were used to determine statistical significance in the assays. Differences were considered significant when the p value was <0.05.

**RESULTS**

**FATP4 Is Normally Expressed in Skin and Its Appendages—**By in situ hybridization, Fatp4 was expressed in the epidermis from E15.5 onwards (Fig. 1A). At later stages of development, Fatp4 RNA was detected in suprabasal keratinocytes, in hair follicle progenitors (Fig. 1C), and in sebaceous gland progenitors (Fig. 1E). At birth, Fatp4 RNA was reduced in the epidermis and hair follicle progenitors (Fig. 1E) but was later detected in differentiated hair follicles (data not shown). In contrast, Fatp4 mutants showed nuclear localization of Fatp4 RNA in the skin during fetal development and at birth (Fig. 1, D and F), perhaps due to mislocalization of the mutant transcripts caused by the inclusion of the retrotransposon in the transcript. By immunohistochemistry, FATP4 protein was detected primarily in the granular layer in fetal epidermis (Fig. 1G) and after birth (not shown). Consistent with the RNA patterns, FATP4 was detected in sebaceous gland progenitors (Fig. 1G) and differentiated hair follicles (see below). We have previously shown that Fatp4 mutant embryos manifest ichthyosis prematurity syndrome-related thickened epidermis (Fig. 1, B, D, F, and H) resulting from hyperproliferation of suprabasal cells (18, 23). In addition, those mutant embryos display hyperkeratosis, a defective barrier, and sparse hair follicles (18, 23). Together with these phenotypes, the normal expression pattern of Fatp4 suggests crucial, cell autonomous roles for FATP4 in the formation of skin and two appendages, sebaceous glands and hair follicles.

**Sebaceous Glands Lacking FATP4 or with Subnormal Levels of FATP4 Are Dystrophic and Enwrapped by Ectopic Epithelial Cells—**By Oil Red O staining for neutral lipids, early sebocytes showed accumulation of lipids from E18.5 and onwards (Fig. 2A). At later stages of development, Fatp4 RNA was detected in sebaceous gland progenitors (Fig. 2A) and after birth (not shown). Consistent with the RNA patterns, FATP4 was shown). Consistent with the RNA patterns, FATP4 was detected in sebaceous gland progenitors (Fig. 1G) and differentiated hair follicles (see below). We have previously shown that Fatp4 mutants showed nuclear localization of Fatp4 RNA in the skin during fetal development and at birth (Fig. 1, D and F), perhaps due to mislocalization of the mutant transcripts caused by the inclusion of the retrotransposon in the transcript. By immunohistochemistry, FATP4 protein was detected primarily in the granular layer in fetal epidermis (Fig. 1G) and after birth (not shown). Consistent with the RNA patterns, FATP4 was detected in sebaceous gland progenitors (Fig. 1G) and differentiated hair follicles (see below). We have previously shown that Fatp4 mutant embryos manifest ichthyosis prematurity syndrome-related thickened epidermis (Fig. 1, B, D, F, and H) resulting from hyperproliferation of suprabasal cells (18, 23). In addition, those mutant embryos display hyperkeratosis, a defective barrier, and sparse hair follicles (18, 23). Together with these phenotypes, the normal expression pattern of Fatp4 suggests crucial, cell autonomous roles for FATP4 in the formation of skin and two appendages, sebaceous glands and hair follicles.
layers of epithelial-like cells, the inner layers of which expressed K6, a marker of activated keratinocytes (Fig. 2, I and J). In control grafts, FATP4 was highly expressed in immature sebocytes, but its level diminished as sebocytes matured and were near rupturing (Fig. 2K). Weakly Oil Red O-positive cells were found in the granular layer of the mutant grafts but not in controls (Fig. 2, C–F), reminiscent of our previous finding in the epidermis of Fatp4 mutant newborns (18) and consistent with the notion that the defective skin barrier may result from impaired lipid transport or processing.

We previously showed that suprabasal keratinocyte expression of the Tg(ILV-Fatp4) transgene rescued the neonatal lethality and ameliorated the skin phenotype of Fatp4 mutant mice. However, Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice showed matted, ungroomed coat hair usually apparent at 1 month of age; this progressed into thinning hair and alopecia with varying severity at 2 months of age (21). It has been shown that sebaceous gland malfunction can be associated with blockage in hair shaft eruption (3). To investigate whether the hair defects of Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice correlated with abnormal sebaceous glands, dorsal skin of these mice and control littermates was analyzed by Oil Red O staining and immunohistochemistry. At postnatal day (P)13.5, sebaceous glands in both controls and Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice released sebum into the hair canal (Fig. 3, C and D). However, sebaceous glands in Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice showed less translucent cytoplasm and less dense lipid droplets in differentiating sebocytes, and eosinophilic cytoplasm in disintegrating sebocytes (Fig. 3, A–D). Sebaceous glands in the Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice were also elongated, with the upper part of the glands enwrapped by ectopic K6-positive cells (Fig. 3, C–F), reminiscent of the abnormalities in the Fatp4 null grafts. The elongated gland phenotype became less distinct as mice aged (data not shown). The ectopic K6-positive cells also expressed K10 (Fig. 3H), normally a marker of sebaceous duct epithelium (26). In contrast, K10 was expressed in the luminal layer of the ductal epithelium in controls (Fig. 3G). The dystrophy and K6- and K10-positive ectopic cells in sebaceous glands of Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) dorsal skin were also detected in whole mount epidermis of adult tails (Fig. 3, Q–T).

In sebaceous glands of Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) pups, both the ectopic cells and sebocytes in the lower part of the glands were unexpectedly expressing the Tg(ILV-Fatp4) transgene; however, FATP4 levels were lower than those seen in controls (Fig. 3, I and J). In most of the skin samples examined, sebaceous glands of Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) skin also displayed higher levels of SCD1, a key enzyme for synthesizing monounsaturated fatty acids (27) (Fig. 3, O and P). In contrast, the glands of Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) skin appeared to express a normal level of fatty acid synthase (Fig. 3, K and L), a multi-enzyme protein that catalyzes fatty acid synthesis (28), and DGAT1 (Fig. 3, M and N), a multifunctional transferase that catalyzes the synthesis of various lipids including diester wax (29).

In summary, either absence or subnormal levels of FATP4 in sebocytes results in defective sebocyte differentiation and ectopic formation of epithelial cells around the glands. Moreover, the ruffled fur phenotype in the Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice suggests that FATP4 is also required for proper sebaceous gland function.

**Meibomian Glands with Subnormal Levels of FATP4 Are Underdeveloped with Thickened Sebaceous Ducts**—Besides hair defects, Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice also showed incomplete eyelid opening at both pup and adult stages. To investigate whether these eyelid abnormalities were associated with defects in meibomian gland formation, eyelids of mice were dissected and examined. At P12.5, the meibomian glands within the tarsal plate of Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice were less “fluffy” than controls and also displayed much larger gland orifices (Fig. 4, A and B). Upon removal of the tarsal plate using collagenase and dispase, the meibomian ducts of Fatp4<sup>−/−</sup>;Tg(ILV-Fatp4) mice were especially distinct, whereas those in controls were rarely visible (Fig. 4, C and D). To investigate the
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Figure 2. Sebaceous glands in Fatp4<sup>-/-</sup> mutant skin are dystrophic and enwrapped by ectopic epithelial cells. Skin sections from the dorsum of E18.5 control and Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice were properly arranged in at least 12 clusters vertically along the margin of each upper and lower eyelid (Figs. 4, E and F). However, beginning at P10.5, meibocytes in Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice showed less dense lipid droplets and appeared dystrophic (Figs. 4, G–J). By 3 months of age, only a few residual Oil Red O-positive cells were present (Fig. 4, K and L).

By Oil Red O staining of frozen eyelid sections, meibomian glands of control mice were well developed by P10.5, with the meibocytes filled with neutral lipids (Fig. 5A). In contrast, meibomian glands of the Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice appeared dystrophic and contained many Oil Red O-negative epithelial cells at P10.5 (Fig. 5B), a phenotype that worsened as the mice aged (Fig. 5L). The meibomian glands of Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice also manifested eosinophilic and less translucent cytoplasm by H&E staining (data not shown). By immunohistochemistry, K6 and FATP4 were detected in control glands in the surface layer of the luminal epithelium lining the main excretory ducts (Fig. 5, C, E, and G) and the intralobular ducts (Fig. 5, M, O, and Q). In juvenile and adult glands of Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice, the residual meibocytes were enwrapped in thick layers of K6 (Fig. 5, D, F, N, and P), K10 (data not shown), and transgenic FATP4-positive epithelial cells (Fig. 5, H and R), reminiscent of the ectopic cells observed in sebaceous glands (Fig. 3). Similar to sebocytes, the residual meibocytes in pups also expressed transgenic FATP4 at levels lower than those seen in controls (Fig. 5, G and H). The presence of the enwrapping epithelial cells was consistent with the enlarged meibomian gland orifice and distinct meibomian ducts (Fig. 4, A–D). Note that the level of FATP4 in control meibocytes diminished when they matured centripetally (Fig. 5Q), reminiscent of sebocytes (Figs. 2K and 3I). Whereas Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice showed a higher level of SCD1 in sebocytes, they did not show changes in SCD1 expression in residual meibocytes (Fig. 5, I, J, S, and T); expression of fatty acid synthase and DGAT1 in meibocytes also appeared normal (data not shown). These data suggest that subnormal levels of FATP4 in meibocytes result in defective meibocyte differentiation and ectopic formation of epithelial cells around the glands.

Alterations of Lipid Species in Sebum of Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) Mice—Although the sebaceous glands of Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice appeared dystrophic, their sebum was secreted into the hair canal similar to controls (Fig. 3, C and D). To test whether the ruffled hair phenotype of Fatp4<sup>-/-</sup>;Tg(IVL-Fatp4) mice was associated with abnormal sebum production that could correlate with abnormal lubrication of hair shafts, sebum was isolated from the surface of hair clipped from....
Sebum lipids in animals have been characterized previously (reviewed in Ref. 30): CE/monoester wax, type II DW, TAG, free fatty acids, and sterols (in the increasing order of polarity) are the five major nonpolar sebum lipid classes found in mice (31). Consistent with the previous report, our results from TLC analysis showed the presence of the five major nonpolar lipid bands in both the control and Fatp4+/−;Tg(IVL-Fatp4) mice.

FIGURE 3. Sebaceous glands of Fatp4+/−;Tg(IVL-Fatp4) mice are dystrophic and enwrapped by epithelial cells. A–P, dorsal skin sections from Fatp4+/−; Tg(IVL-Fatp4) mice and control littersmates at P13.5 (A–L), P6.5 (M and N), and P10.5 (O and P) were stained (A–D) or immunostained (E–P) as indicated. Sebocytes in Fatp4+/−; Tg(IVL-Fatp4) mice showed less translucent cytoplasm (compare arrowheads in A and B) and less dense lipid droplets (compare arrowheads in C and D). In both controls and Fatp4+/−; Tg(IVL-Fatp4) mice, sebaceous glands were connected to the hair canal (arrows in A and B) and excreted sebum into the hair canal (arrows in C and D). Note the eosinophilic material in rupturing sebocytes of Fatp4+/−; Tg(IVL-Fatp4) mice (asterisks in B). In controls, K6 was detected in both the infra-infundibulum directly above the sebaceous glands (arrows in E) and the outer root sheath of hair follicles (asterisk in E), whereas K10 was detected in sebaceous duct epithelium (white arrows in G; inset shows 1 month), in the infundibulum and the isthmus (black arrows in G), and in suprabasal epidermis (asterisk in G). Sebaceous glands in the Fatp4+/−; Tg(IVL-Fatp4) mice were elongated (F, H, and J), with sebocytes in the deeper segment expressing transgenic FATP4 (black arrowheads in J). Sebocytes in the upper segment were enwrapped by K6, K10, and transgenic FATP4-positive cells (white arrowheads in F, H, and J). Note that the FATP4 level in control mature sebocytes declined (arrow in I). Fatp4+/−; Tg(IVL-Fatp4) skin showed normal expression of fatty acid synthase (K and L) and DGAT1 (M and N) and normal basement membranes by nidogen counterstain (red). Dashed lines demarcate the sebaceous glands. Bar is 50 μm. Q–T, whole mount tail epidermis from Fatp4+/−; Tg(IVL-Fatp4) mice and age-matched controls at 1 year of age was stained with Oil Red O (insets in S and T) or double-stained with Oil Red O and K6 (Q and R) or K10 (S and T) antibodies. The Fatp4+/−; Tg(IVL-Fatp4) mice showed less dense lipid staining by Oil Red O (black arrowhead and inset in T). K10 signals were detected in sebaceous duct epithelium in controls (arrows in S) and the K6 and K10 signals in the ectopic epithelium enwrapping the sebaceous glands in Fatp4+/−; Tg(IVL-Fatp4) mice (white arrowheads in R and T). Bar is 100 μm.

the dorsum of adult mice and characterized in detail. Male Fatp4+/−; Tg(IVL-Fatp4) mice at 2 to 2.7 months of age showed a substantially lower overall yield of sebum compared with male controls (728 ± 84 μg in controls, n = 4 versus 377 ± 169 μg in mutants, n = 4; p < 0.01). This abnormality was accompanied by a substantially lower weight of hair (102 ± 21 mg in controls, n = 4 versus 63 ± 10 mg in mutants, n = 4; p < 0.05).
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From 2 months of age, both male (Fig. 6) and female (data not shown) Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice showed a substantial reduction in the proportion of type II DW, a diester of alkane 1,2-diol with fatty acids that is the most abundant lipid species in normal sebum, as compared with gender-matched controls. By quantifying the lipid species isolated from male mice ranging from 2 to 6 months of age, Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice showed a statistically significant decrease in the proportion of DW (43.22 ± 9.47% in controls versus 19.30 ± 5.70% in mutants; p < 0.01) (Table 1). This substantial decrease in DW was accompanied by a moderate increase in the proportion of sterols (13.87 ± 3.00% in controls versus 23.55 ± 2.54% in mutants; p < 0.01) (Table 1). In contrast, the proportions of the other four nonpolar lipid species were unchanged in Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice.

By ESI-MS analysis, sebum of both control and Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice contained similar ion species of DW. However, sebum of Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice showed a substantial increase in the abundance of DW species with two unsaturated bonds (30.03 ± 2.41% in controls versus 40.26 ± 4.28% in mutants). This change was mainly seen as increases of C54:2, C56:2, and C58:2 DW species containing an even total number of carbons along the fatty acyl and alkyl chains in the low m/z region (Fig. 7A). Sebum from Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice also displayed decreases in the abundance of DW species with one unsaturated bond (49.44 ± 2.73% in controls versus 43.34 ± 2.56% in mutants) and of fully saturated DW species (20.49 ± 2.09% in controls versus 16.41 ± 2.33% in mutants). Those changes were manifested by decreases in DW species in the high m/z region, such as C59:0, C61:0 (Fig. 7C), C59:1, and C61:1 (Fig. 7B).

CE is another abundant lipid species present in normal mouse sebum. As shown in Table 2, the Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice showed a statistically significant increase in CE species esterified with C22:1 (6.62 ± 2.97% in controls versus 14.13 ± 6.52% in mutants; p < 0.05) and C22:0 (3.17 ± 1.42% in controls versus 7.07 ± 0.32% in mutants; p < 0.001) fatty acyl substituents. In contrast, no significant changes in the MS profiles of TAG, GED, or fatty acids were observed (data not shown). These results suggest that an insufficient level of FATP4 in sebaceous glands affects both the amount of sebum recovered from the fur and the fatty acyl composition of sebum type II DW and CE, possibly resulting in altered sebum fluidity and function.

**Defects in Water Repulsion and Thermoregulation in Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4)**—To examine whether the alterations in sebum amount and composition would affect the protective function of fur, Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) and control mice at 2.5 months of age (before signs of hair thinning in the former became distinct) were subjected to a water repulsion assay. In this assay, mice swam in 37 °C water for 4 min and were tested for their abilities to repel water and maintain normal body temperature. Five min after swimming, Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice looked much wetter than controls, and whereas control mice looked nearly dry 15 min after swimming, Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice were still wet (Fig. 8A). This delayed drying correlated with significant increases in water weight retention up to 25 min after swimming (Fig. 8B).

plus an additional band migrating between type II DW and TAG (Fig. 6). We identified this band by ESI-MS as GED, which was previously reported to be a slower migrating type II DW (32).

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**FIGURE 4. Defects in meibomian glands of Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice.** Eyelids (A and B) or meibomian glands within the tarsal plate (C–L) of Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice and control littermates were untreated (A and B), digested in collagenase and dispase (C and D), or subjected to Oil Red O staining (E–L). At P12.5, the meibomian glands of the Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice were much less fluffy (compare arrowheads in A and B) and displayed larger gland orifices (arrows in B) than controls. These abnormalities (arrowheads and arrows in D) were also observed upon removal of the tarsal plate using collagenase and dispase. Glands in C and D are those from the upper eyelids in A and B, respectively. Meibocytes were properly arranged in vertical clusters at P6.5 (arrowheads in E and F). From P10.5 and onwards, compared with controls (G, I, and K), meibocytes of Fatp4<sup>i−/−</sup>;Tg(IVL-Fatp4) mice showed less dense lipid droplets (H), became dystrophic (J), and eventually atrophied by 3 months of age (arrowheads in L). Arrows in E and F mark eyelash follicles.
Before swimming, both control and Fatp4−/−;Tg(IVL-Fatp4) mice had a body temperature of ~35 °C. The body temperature of control mice dropped to 33.7 ± 1.0 °C at 5 min and returned to the pre-swim temperature at 15 min after swimming (Fig. 8C). In stark contrast, the body temperature of Fatp4−/−;Tg(IVL-Fatp4) mice dropped to a low level of 28.6 ± 1.1 °C at 15 min and remained at low levels until 30 min after swimming (Fig. 8C), when the mice were nearly dry (Fig. 8B). The Fatp4−/−;Tg(IVL-Fatp4) mice were less active and exhibited little grooming behavior after water immersion as compared...
FATP4 and Sebaceous Glands

Sebum lipids were isolated from the hair surface of 4 male control mice and 4 male Fatp4−/−;Tg(IVL-Fatp4) mice. Sebum lipids were isolated from the hair surface of male Fatp4−/−;Tg(IVL-Fatp4) mice and male control littermates with the ages indicated. Lipids were resolved by TLC, and quantified as described under “Experimental Procedures.” Data are indicated as percentage of each lipid species with S.D. and statistical significance indicated.

| Species   | Mean ± S.D. in controls | Mean ± S.D. in mutants |
|-----------|-------------------------|------------------------|
| CE*       | 20.43 ± 2.80            | 27.23 ± 6.62           |
| DW        | 43.22 ± 9.47            | 19.30 ± 5.70           |
| GED       | 5.49 ± 2.11             | 6.38 ± 2.57            |
| TAG       | 6.36 ± 3.20             | 9.96 ± 1.39            |
| FA        | 10.64 ± 5.19            | 13.57 ± 5.35           |
| S         | 13.87 ± 3.00            | 23.55 ± 2.54           |

* CE, cholesteryl ester; DW, type II diester wax; GED, glyceryl ether diester; TAG, triacylglycerol; FA, free fatty acids; S, sterols; PL, polar lipids.

FIGURE 6. Alterations in sebum from Fatp4−/−;Tg(IVL-Fatp4) mice. Sebum lipids were isolated from the hair surface of male Fatp4−/−;Tg(IVL-Fatp4) mice and male control littermates with the ages indicated. Lipids were resolved by TLC in a solvent mixture of hexane/ethyl acetate (68/12/1, v/v), and stained by iodine vapor. Components in the nonpolar lipid marker are indicated on the left. The arrow marks the origin of sample application. FA, free fatty acids; S, sterols; PL, polar lipids.

TABLE 1
Lipid species in sebum of control and Fatp4−/−;Tg(IVL-Fatp4) mice

Sebum lipids were isolated from the hair surface of 4 male control mice and 4 male Fatp4−/−;Tg(IVL-Fatp4) mice (mutants) ranging from 2 to 6 months old, analyzed by TLC, and quantified as described under “Experimental Procedures.” Data are indicated as percentage of each lipid species with S.D. and statistical significance indicated.

DISCUSSION
Roles for FATP4 in Formation of Sebaceous and Meibomian Glands—FATP4 is the only FATP family member that has been detected in mouse sebocytes (33). Although K14 promoter-driven, tamoxifen-induced deletion of Fatp4 in the skin leads to reduced FATP4 in sebaceous glands, its consequences on sebaceous gland formation have not been reported (34). Using skin grafting experiments, we found that FATP4 is not required for initial differentiation of sebocytes, but rather for the maintenance of sebocytes and sebaceous gland structure. In both Fatp4−/− skin grafts and skin of Fatp4−/−;Tg(IVL–Fatp4) mice, sebaceous glands showed abnormalities in histology and reduced lipid droplet density in maturing sebocytes. Sebaceous glands of Fatp4−/−;Tg(IVL–Fatp4) mice were also dystrophic, with mature sebocytes enwrapped by K6- and K10-positive epithelial cells. Although sebocytes in Fatp4−/−;Tg(IVL–Fatp4) mice expressed transgene-derived FATP4, the levels were lower than endogenous FATP4 levels in controls, and the sebaceous gland structural defects were similar to those in Fatp4−/− skin grafts. Together with the normally high level expression of FATP4 in sebocytes, the similar sebaceous gland abnormalities in Fatp4−/− skin grafts and Fatp4−/−;Tg(IVL–Fatp4) skin suggest that FATP4 functions in a cell autonomous fashion for sebocyte differentiation and that a low level of exogenous FATP4 in Fatp4 null sebocytes is not sufficient for normal function.

Similar to the abnormalities in sebaceous glands, Fatp4−/−; Tg(IVL–Fatp4) mice displayed dystrophic meibomian glands with residual meibocytes enwrapped by thick layers of K6- and K10-positive epithelial cells. However, they also exhibited distinct/enlarged meibomian ducts and orifices. Like the epidermis, normal meibomian duct epithelium is a keratinizing stratified squamous epithelium, but it lacks a granular layer (35). In our studies, both K6 and K10 were detected in control mice in the epithelial layer lining the main excretory and intralobular ducts of meibomian glands (Fig. 5). Thus, it would be interesting to find out whether the abnormalities in the formation of sebaceous glands in Fatp4−/−;Tg(IVL–Fatp4) mice were also associated with thickening of ducatal epithelium. Ductal structures of sebaceous glands have been identified in the mouse tail skin, with K6 expression activated in the surface layer of the luminal epithelium of the sebaceous duct (36). In our analysis of both dorsal skin and tail skin, we found short sebaceous ductal structures positive for K10, whereas K6 was not detected in the ductal epithelium but rather in the infra-infundibulum directly above the ductal opening (Fig. 3). The shortness of the sebaceous duct may contribute to its inability to be traced in a previous lineage analysis (37).

In Fatp4−/−;Tg(IVL–Fatp4) mice, both sebaceous and meibomian glands were dystrophic and enwrapped by ectopic K6- and K10-expressing epithelial cells. It is therefore possible that the ductal epithelium in Fatp4−/−;Tg(IVL–Fatp4) mice displayed dystrophic meibomian glands with residual meibocytes enwrapped by thick layers of K6- and K10-positive epithelial cells. However, they also exhibited distinct/enlarged meibomian ducts and orifices. Like the epidermis, normal meibomian duct epithelium is a keratinizing stratified squamous epithelium, but it lacks a granular layer (35). In our studies, both K6 and K10 were detected in control mice in the epithelial layer lining the main excretory and intralobular ducts of meibomian glands (Fig. 5). Thus, it would be interesting to find out whether the abnormalities in the formation of sebaceous glands in Fatp4−/−;Tg(IVL–Fatp4) mice were also associated with thickening of ducatal epithelium. Ductal structures of sebaceous glands have been identified in the mouse tail skin, with K6 expression activated in the surface layer of the luminal epithelium of the sebaceous duct (36). In our analysis of both dorsal skin and tail skin, we found short sebaceous ductal structures positive for K10, whereas K6 was not detected in the ductal epithelium but rather in the infra-infundibulum directly above the ductal opening (Fig. 3). The shortness of the sebaceous duct may contribute to its inability to be traced in a previous lineage analysis (37).

In Fatp4−/−;Tg(IVL–Fatp4) mice, both sebaceous and meibomian glands were dystrophic and enwrapped by ectopic K6- and K10-expressing epithelial cells. It is therefore possible that the ductal epithelium in Fatp4−/−;Tg(IVL–Fatp4) mice was hyperproliferative and hyperkeratinized due to dystrophy of acinar cells and a subsequent imbalance between acinar cells and ductal cells, or due to activation by abnormal lipid or fatty acyl components excreted along the ductal epithelium. It is also possible that the ductal epithelium in Fatp4−/−;Tg(IVL–Fatp4) mice became activated as a cell autonomous response to the reduction in FATP4. Alternatively, the ectopic epithelial cells might arise as a result of hyperkeratinization of differentiating acinar cells due to the reduction in FATP4. Defective acinar cells and hyperkeratinization of ductal epithelial cells are two major areas of pathogenesis of meibomian gland dysfunction in humans (38). Our Fatp4−/−;Tg(IVL–Fatp4) mice could be a useful mouse model for elucidating the pathogenesis and identifying potential therapies for meibomian gland dysfunction.
TABLE 2
Lipid profiles of CE in sebum of control and Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice

CE was isolated from the hair surface of 7 control mice and 5 Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice (mutants) and analyzed as ammonium adduct ions by ESI-MS as described under “Experimental Procedures.” Data are indicated as percentage of each lipid species in CE with S.D. and statistical significance indicated. Each species is marked by its m/z (unaccountable for mass defect) on the mass spectrum and its total number (9) of carbons:total number of unsaturated (unsat.) bonds in the fatty acyl moiety of CE.

| m/z (M + NH<sub>4</sub><sup>+</sup>) | Species | Mean ± S.D. in controls | Mean ± S.D. in mutants |
|-----------------------------|---------|-------------------------|------------------------|
| 640                        | C16:1   | 2.39 ± 1.80             | 1.66 ± 0.79            |
| 642                        | C16:0   | 3.24 ± 1.59             | 3.29 ± 2.43            |
| 666                        | C18:2   | 13.93 ± 7.42            | 16.22 ± 4.91           |
| 668                        | C18:1   | 36.37 ± 16.42           | 28.73 ± 10.71          |
| 670                        | C18:0   | 4.26 ± 1.36             | 2.85 ± 1.47            |
| 696                        | C20:1   | 6.41 ± 4.66             | 5.41 ± 1.32            |
| 698                        | C20:0   | 5.39 ± 3.49             | 5.67 ± 2.10            |
| 712                        | C21:0   | 5.78 ± 3.74             | 4.61 ± 2.16            |
| 724                        | C21:1   | 6.62 ± 2.97             | 4.13 ± 6.52<sup>a</sup>|
| 726                        | C22:0   | 3.17 ± 1.42             | 7.07 ± 0.32<sup>b</sup>|
| 752                        | C24:1   | 7.52 ± 4.43             | 7.35 ± 1.86            |
| 754                        | C24:0   | 4.92 ± 3.57             | 3.01 ± 1.35            |

<sup>a</sup> p < 0.05.
<sup>b</sup> p < 0.001.

Roles for FATP4 in Production and Function of Lipids in Sebaceous Glands—The ruffled hair phenotype of Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice was associated with a 48% reduction in the amount of lipids recovered from the fur per mouse dorsum. This abnormality might relate to the reduced weight of hair obtained from the Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice. Alternatively, the reduced amount of sebum recovered could stem from reduced sebum synthesis in each gland, as suggested by the Oil Red O staining in both skin sections and whole mount tail epidermis (Fig. 3), and/or impaired coating and retention of sebum on the fur due to structural defects in sebum lipids. It is unclear whether the reduced sebum recovery from the Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice is related to abnormalities in lipid secretion rate or the hair loss phenotype apparent as the mice age.

Our TLC results showed that Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice exhibited a substantial reduction in the amount of type II DW in sebum lipids, dropping to 19% of the total, versus 43% for controls (Table 1). In contrast, DW has been reported to be ~60% of the total in normal mouse sebum (31, 32). This discrepancy is primarily due to differences in sample collection methods (data not shown); we collected only hair surface lipids, whereas both hair and skin surface lipids were collected in the other reports.

The substantial reduction in the proportion of DW in sebum of Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice may make the sebum more fluid and volatile. In many ground-nesting shorebirds and ducks, the usual mixtures of monoester wax in preen gland lipids are replaced by mixtures of type II DW to reduce nest predation when the breeding season begins (39). This is attributed to the high molecular mass of DW, making it less volatile and more difficult to be detected by predators via olfaction.

The abnormality in DW level in sebum of Fatp4<sup>−/−</sup>;Tg(IVL-Fatp4) mice by TLC was also accompanied by statistically significant decreases in the abundance of DW species with one
unsaturated bond and of saturated DW species in the high m/z region (Fig. 7, B and C). Further MS analyses revealed that the DW species in control sebum with one unsaturated bond in the high m/z region consisted of C26 or longer fatty acyl chains, suggesting that the incorporation of very long fatty acyl chains into DW is altered in sebocytes of Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice. This is consistent with the previous notion that Fatp4 mutant mice showed a significant reduction in incorporation of very long-chain fatty acids into ceramides of the epidermis (21, 40). In contrast, sebum lipids of Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice displayed a statistically significant increase in the abundance of DW species with two unsaturated bonds in the low m/z region, such as C56:2 and C58:2 (Fig. 7 A). By MS analysis of control sebum, C20:0 (alkyl)/C16:1 (acyl)/C20:1 (acyl) and C20:0 (alkyl)/C18:1 (acyl)/C20:1 (acyl) were identified to be the major components of C56:2 and C58:2, respectively (data not shown). This suggests that in sebaceous glands of Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice, reduced FATP4 led to more frequent incorporation of long-chain or medium-chain fatty acids into DW. Our data also revealed higher SCD1 expression in sebocytes of Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice (Fig. 3). This may lead to an increase in the synthesis of monounsaturated fatty acids (by SCD1) and a subsequent increase in DW species with two unsaturated bonds. Although Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice displayed higher expression of SCD1 in sebocytes, the SCD1 expression in meibocytes appeared normal (Fig. 5). This suggests that dystrophy of the acinar cells in those two glands may involve different mechanisms resulting from defects in FATP4.

For waxes, the melting temperature is determined by the degree of unsaturation, the carbon chain length, and the site of the ester bond in the wax (41). For example, a higher degree of unsaturation and shorter carbon chain length in the wax result in lower melting temperatures and thus higher fluidity and less waterproofing. In sebum from Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice, the structural defects in DW, together with the reduction in the overall proportion of DW and the reduction in the amount of sebum recovered from the hair surface, suggest an increased sebum fluidity and less waterproofing. After swim tests, Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice indeed displayed defects in repelling water and in regulating body temperature (Fig. 8).

The abnormality in thermoregulation in Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice could also result from reduced grooming behavior after water immersion and/or the significant reduction in incorporation of very long-chain fatty acids into ceramides of the epidermis as reported previously (21, 40). In addition to structural defects in DW, Fatp4/H11002/H11002;Tg(IVL-Fatp4) mice showed a statistically significant increase in the proportion of cholesterol that was esterified with fatty acids of C22:1 and

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3 M-H. Lin, F-F. Hsu, J. H. Miner, unpublished data.
C22:0 (Table 2). It remains to be determined how the fatty acyl incorporation in CE is changed by the insufficient level of FATP4 and whether these changes contribute to the altered fluidity and function of sebum. Similarly, it is not known whether or how the increases in proportions of sterols in sebocytes of Fatp4−/−;Tg(IVL-Fatp4) mice alter the sebum. But taken together, our data indicate that FATP4 plays crucial roles in sebaceous glands in part by regulating the production of sebum and composition of DW.

How Does FATP4 Activity Influence Formation of DW in Sebum?—The proportions of different lipid species in sebum vary significantly among different animals (reviewed in (30)). DW is the most abundant lipid species in mouse sebum and is thought to be important for waterproofing the fur (1). In both Dgat1−− mice (9) and aselia mice carrying a deletion in the Scd1 gene (31), the sebaceous glands are atrophic and the sebum DW proportions are substantially reduced. DGAT1 is a multifunctional enzyme possessing a wax diester synthase activity that catalyzes the formation of DW from alkane 1,2-diol (29). Although DGAT1 expression was not affected in sebocytes of Fatp4−/−;Tg(IVL-Fatp4) mice (data not shown) in Fatp4−/−;Tg(IVL-Fatp4) mice by immunohistochemistry, it is possible that an insufficient level of FATP4 affects DGAT1 activity or substrate availability and subsequently the synthesis of DW. Alternatively, FATP4 insufficiency may result in reduced activation of very long-chain fatty acids, leading to an unbalanced supply of fatty acids and subsequently an abnormal DW composition. In contrast to DW species, CE (Table 2) and two other esters (GED and TAG)3 in normal sebum carried no or few very long-chain fatty acids (≥C26). These data suggest that DW would be the sebum lipid species most affected by a subnormal level of FATP4 and that other long-chain acyl-CoA synthetases are responsible for converting long-chain fatty acids into CoA derivatives for the synthesis of various ester lipids in mouse sebum. It remains to be determined whether the abnormalities in DW lead to sebaceous gland dystrophy, or perhaps impaired FATP4 activity impedes the growth of sebocytes due to altered signaling pathways as a result of an unbalanced free fatty acid pool.

In contrast to the abundance of DW in mouse sebum, human sebum does not contain DW, but contains squalene, monoester wax, and TAG as the major ester lipids (reviewed in Ref. 30). What roles then might FATP4 play in the production and composition of human sebum, the main function of which is skin homeostasis but not waterproofing of the hair (4)? FATP4 mutations have been identified in human patients with ichthyosis prematurity syndrome (42, 43). This makes it an especially important goal to understand the mechanism whereby the absence of FATP4 in mice causes the wrinkle free and sebaceous and meibomian gland phenotypes. It will be interesting to examine whether human FATP4 plays important roles in incorporating very long-chain fatty acids into various ester lipids in human sebum, and whether these patients show defects in sebaceous and meibomian glands similar to those observed in Fatp4−/−;Tg(IVL-Fatp4) mice.

In summary, our results suggest that FATP4 plays crucial roles in the development and maturation of sebaceous and meibomian glands and in the production and composition of sebum, likely by regulating the supply and balance of fatty acids necessary for synthesizing secreted lipids. In contrast, our previous results show that FATP4 regulates epidermal proliferation and differentiation during fetal development. Thus, FATP4 may have separable roles in the epidermis and appendages. With the Fatp4−/−;Tg(IVL-Fatp4) mice as an animal model for ichthyosis prematurity syndrome, its pathogenesis and potential therapies can be further investigated.

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