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REVIEW OPEN ACCESS

Ceramides metabolism and impaired epidermal barrier in cutaneous diseases and skin aging: focus on the role of the enzyme PNPLA1 in the synthesis of ω-O-acylceramides and its pathophysiological involvement in some forms of congenital ichthyoses☆

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Abstract – The outermost layer of the skin, the *stratum corneum*, is essential for the protective barrier functions of the skin. It results from the stacking of corneocytes, the dead flattened cells resulting from epidermal terminal differentiation of underlying living keratinocytes. The cornified lipid envelope, encapsulating corneocytes, and the extracellular mortar-like multilayered lipid matrix, called *lamellae*, are two crucial elements of the epidermal barrier. *Stratum corneum* extracellular lipids are mainly composed of ceramides, cholesterol and free fatty acids. Ceramides, and more specifically the epidermis specific ω-O-acylceramides, are essential for lipid-matrix organization into *lamellae* and formation of the corneocyte lipid envelope. Pathophysiological studies of inherited lipid metabolism disorders recently contributed to a better understanding of *stratum corneum* lipid metabolism. In the lab, our data from patients with Autosomal Recessive Congenital Ichthyosis and a murine knock-out model showed that the enzyme PNPLA1 is essential for the last step of synthesis of omega-O-acylceramides. Skin aging is a complex biological process caused by genetic and extrinsic factors *e.g.* sun exposure, smoke, and pollution. Aging skin is marked by a senescence-related decline in lipid and water content, which ultimately impairs epidermal barrier function. Thus, aged epidermis is prone to develop altered drug permeability, increased susceptibility to irritants contact dermatitis and severe xerosis. Ceramide deficiency may account, at least in part, for the dysfunction of the *stratum corneum* associated with ageing. Hence, treatments able to increase skin-ceramide levels could improve the epidermal barrier function in aged skin. Many animal testing and clinical trials are taken in that regard.

Keywords: epidermal barrier / ichthyosis / omega-O-acylceramide / PNPLA1 enzyme / skin aging

Résumé – Métabolisme des céramides et altération de la barrière épidermique en pathologie dermatologique et au cours du vieillissement cutané : focus sur le rôle de l'enzyme PNPLA1 dans la synthèse des ω-O-acylcéramides et son implication physiopathologique dans certaines formes d'ichtyoses congénitales. La couche la plus superficielle de la peau ou *stratum corneum* est essentielle à la fonction de barrière protectrice de la peau. Elle est formée d'un empilement de cornéocytes, cellules mortes aplaties résultant de la différenciation terminale des kératinocytes épidermiques. L'enveloppe cornée lipidique, qui entoure les cornéocytes, et la matrice lipidique extracellulaire multi-lamellaire, appelée *lamellae*, sont deux éléments cruciaux de la barrière épidermique. Les lipides extracellulaires du *stratum corneum* sont essentiellement composés de céramides, cholestérol et acides gras libres. Les céramides, et plus particulièrement les ω-O-acylcéramides, sont nécessaires à l'organisation des *lamellae* et la formation

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de l'enveloppe cornée lipidique. L'étude physiopathologique de maladies héréditaires du métabolisme des lipides a récemment contribué à mieux comprendre le métabolisme des lipides du *stratum corneum*. Nos données issues de patients atteints d'ichtyose congénitale autosomique récessive et d'un modèle de souris knock-out ont montré que l'enzyme PNPLA1 est essentielle à l'ultime étape de synthèse des ω-O-acylcéramides. Le vieillissement cutané est un processus biologique complexe causé par des facteurs génétiques et extrinsèques (exposition solaire, tabac, pollution...). Au cours du vieillissement, le contenu de la peau en eau et en lipides, incluant les céramides, diminue progressivement ce qui, à terme, affecte la barrière épidermique. Ainsi, la peau âgée a tendance à développer des défauts de perméabilité aux drogues, une sensibilité accrue aux irritants, dermatites de contact et xérose sévère. De nombreux tests sur animaux et essais cliniques sont conduits dans le but d'augmenter le taux de céramides épidermiques et donc d'améliorer la barrière cutanée de la peau âgée.

Mots clés : barrière épidermique / enzyme PNPLA1 / ichtyose / omega-O-acylcéramide / vieillissement cutané

1 Introduction

The epidermis is a stratified epithelium consisting mainly of keratinocytes (Fig. 1). These cells differentiate throughout their migration from the basal layer towards the external surface of the skin. At the latest stage, they undergo cornification, a programmed cell death leading to the transformation of granular keratinocytes, the last living cells in the program of keratinocyte terminal differentiation, into corneocyte. Stacking of the corneocytes forms the *stratum corneum*, the outermost layer of the epidermis. The *stratum corneum* is essential for the main function of the epidermis, the barrier function, vital for the organism. It mainly fulfils the permeability barrier function by dampening trans-epidermal loss of water and electrolytes and by preventing entry of toxic or pathogenic agents. An impaired epidermal barrier is associated with numerous skin diseases like atopic dermatitis, psoriasis or rare genodermatoses such as ichthyoses (Castiel-Higounenc *et al.*, 2004; van Smeden *et al.*, 2014). Elderly skin is typically thin and fragile, with increasing susceptibility to bruising and impaired wound healing (Gilchrest, 1996). The *stratum corneum* and more particularly the *stratum corneum* lipid structures are expected to undergo significant changes during the course of ageing. However, this has been little studied in comparison to other skin compartment like the dermis.

2 Epidermal barrier and *stratum corneum* lipids: importance of ω-O-acylceramides

Lipids play an important role in the epidermal permeability barrier function. Human *stratum corneum* lipids consist of 50% ceramides, 25% cholesterol, 10–15% free fatty acids (predominantly long-chain and saturated) and 5% of other various lipids. Sebaceous glands produce triglycerides, squalene, waxes, cholesterol and free fatty acids that form a hydro-lipid film at the surface of the skin while most components of the lipid-rich extracellular matrix of the *stratum corneum* are produced by the underlying living keratinocytes from the granular layer. Precursors of these *stratum corneum* lipids, such as glucosyl(acyl)ceramides, phospholipids and sphingomyelin, are stored in the tubulo-vesicular secretory organelles called lamellar bodies. At the *stratum granulosum*/*stratum corneum* interface, these precursors are released and processed into mature products that assemble into continuous hydrophobic lamellar lipid structures surrounding the corneocytes, the *lamellae*, or are crosslinked to the cornified envelope of corneocytes to form the cornified lipid envelope (Menon *et al.*, 2012).

Ceramides are sphingolipids resulting from the combination of a fatty acid and a sphingoid base via an amine link. They are the major lipid species in the *stratum corneum*, where they present a high degree of complexity linked to an important molecular heterogeneity. The sphingoid moiety can be sphingosine (S), dihydrosphingosine (D), phytosphingosine (P) or 6-hydroxy-sphingosine (H). The diversity of epidermal ceramides is additionally enhanced by the huge fatty acid moiety linked in amine position which can be non-hydroxy (N), α-hydroxy (A) or ester linked ω-hydroxy (EO) (Tab. 1). There is a high level of ω-hydroxyceramide with ultra-long carbon chain (C28-C38). Two major distinctive classes of ceramides derive from esterification of the ω-hydroxyl group: ω-fatty acid (predominantly linoleic acid) esterified ultra-long chain-ceramides, and protein bound ceramides due to esterification with glutamate side chains of cornified envelope proteins (Jennemann *et al.*, 2012; Rabionet *et al.*, 2014). These two ceramide species are specific to epidermis and are essential for lipid-matrix organization into *lamellae* and formation of the corneocyte lipid envelope (Menon *et al.*, 2012).

The specific importance of epidermal ceramides has been assigned definitively to their role in maintaining epidermal barrier homeostasis. Numerous enzymes and molecular actors are necessary for the synthesis, transport, secretion and extracellular maturation of all major epidermal ceramides. Most of them have been recently uncovered. In short, esterified and protein-bound ceramides (*i.e.* ceramides forming the cornified lipid envelope) require starts in late *stratum spinosum*/early *stratum granulosum* with the synthesis of ω-hydroxylated ultra-long fatty acids (C28-C38) at the endoplasmic reticulum, which requires the fatty acid elongase ELOVL4 (Li *et al.*, 2007) and the hydroxylase CYP4F22 (Ohno *et al.*, 2015). After activation, which involves SLC27A4/FATP4 (Herrmann *et al.*, 2005; Moulson *et al.*, 2007), a specialized ceramide synthase, CERS3, condenses the ultra-long ω-hydroxy-fatty acids with sphingoid bases to form ω-hydroxy-(dihydro)ceramides (Jennemann *et al.*, 2012). The transacylase PNPLA1, most probably enhanced by the co-factor ABHD5, allows ω-esterification of ceramides with linoleic acid released from triglycerides (Ohno *et al.*, 2017, 2018). The resulting ω-esterified ceramides then require

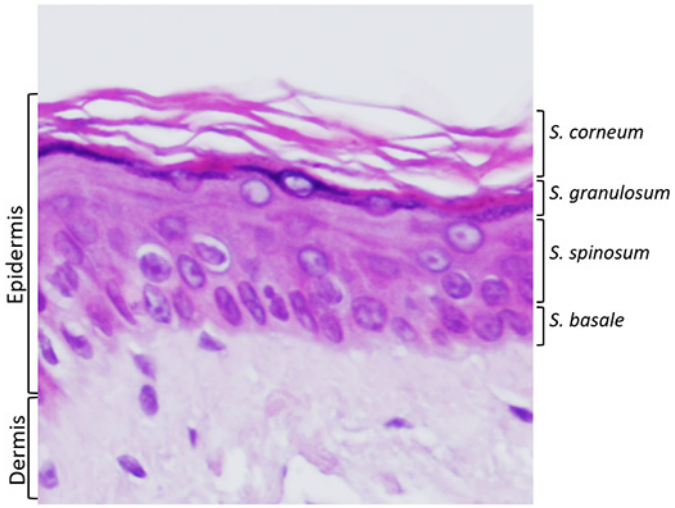

Fig. 1. Histology of normal human epidermis (hemalun-eosin staining).

transport to the Golgi where glucosylation involves the ubiquitously expressed glycosylceramide synthase UGGT to form ω-esterified glucosylceramides, a polar preform of the barrier ceramide (Jennemann *et al.*, 2007). Omega-esterified (glucosyl) ceramides then are packed together with polar precursors of other barrier lipids into lamellar bodies, which requires the ABC transporter ABCA12 (Smyth *et al.*, 2008). Fusion of lamellar bodies with the plasma membrane by the SNARE-complex may involve the isoform SNAP29 (Schiller *et al.*, 2016). Most of the secreted barrier lipids will form the extracellular lipid *lamellae*, but some are trans-esterified to proteins of the cornified envelope to form the cornified lipid envelope (Breiden and Sandhoff, 2014). This trans-esterification involves initial oxidation of the ω-bound linoleic acid by 12R-LOX and eLOX3, and finally transglutaminase TGase1 transfers ω-hydroxy-glucosylceramides onto proteins of the cornified envelope (Krieg *et al.*, 2013). Protein-bound glucosylceramides are processed by glucosylceramidase, an acidic ceramidase, with the help of activator proteins to form protein-bound ω-hydroxy-ceramides and later protein bound ω-hydroxy-fatty acids (Breiden and Sandhoff, 2014; Rabionet *et al.*, 2014) (Fig. 2).

3 Omega-O-acylceramides and pathophysiological study of ichthyoses: example of the elucidation of PNPLA1 biological role

Numerous inherited skin diseases directly result from abnormalities in sphingolipids. Identification and characterization of the corresponding genes, as well as the study of the corresponding KO mouse models, largely contributed to a better knowledge of fundamental aspects of ceramide metabolism (Elias *et al.*, 2008; Borodzicz *et al.*, 2016). This was particularly the case with the pathophysiological study of Autosomal Recessive Congenital Ichthyoses (ARCI), a subgroup of rare monogenic skin diseases due to mutation of genes involved in cornification.

At birth many ARCI patients are "collodion babies", a descriptive term for infant born encased in a tight shiny skin that resembles plastic wrap. Later, the skin phenotype consists in generalized scaling and variable erythroderma, with a wide spectrum of clinical presentations from lamellar ichthyosis to congenital ichthyosiform erythroderma. To date, mutations associated with ARCI have been described in 10 genes: *ABCA12*, *ALOX12B*, *ALOXE3*, *CERS3*, *CYP4F22*, *NIPAL4*, *PNPLA1*, *SDR9C7*, *SULT2B1* and *TGM1*. Some of them have been addressed in many works which demonstrated their involvement in *stratum corneum* lipid metabolism. Recently, our group as well as others showed that PNPLA1 was a key player in the formation of ω-esterified ceramides (Grond *et al.*, 2017; Hirabayashi *et al.*, 2017; Ohno *et al.*, 2017; Pichery *et al.*, 2017).

Patatin-like phospholipase domain containing 1 (PNPLA1) is one of the 9 members of the PNPLA family, characterized by a highly conserved "patatin" domain. These proteins have diverse lipolytic and acyltransferase activities and play a key role in the lipid metabolism (Wilson *et al.*, 2006; Kienesberger *et al.*, 2009). PNPLA1 was the less characterized member of this family. Interest in the biological function of this protein was enhanced by the recent identification of *PNPLA1* gene as an ARCI-associated gene, originally in a spontaneous mutant dog model then in patients affected by ARCI for which no mutation in other already known ARCI-causing gene was detected (Grall *et al.*, 2012). However, the enzymatic function of PNPLA1 remained unknown. In human, the protein is expressed in the epidermis, predominantly in the granular layer (Toulza *et al.*, 2007; Grall *et al.*, 2012). The only functional data concerning PNPLA1 arose from the work of Grall and coworkers. Their analyses of triglyceride hydrolase activity and [^{14}C]-linoleic acid incorporation of normal and PNPLA1-deficient cultured human keratinocytes indicated that PNPLA1 had a role in the metabolism of glycerophospholipid rather than neutral lipids (Grall *et al.*, 2012). Thus, deciphering the biological function of PNPLA1 could allow understanding its physiological role in the epidermis and its pathophysiological implication in ARCI.

In the lab, we searched for *PNPLA1* mutations in patients suffering from ARCI from our biological collection. Among 105 patients analyzed, 5 novel *PNPLA1* mutations were identified in 5 patients from 3 non-consanguineous Caucasian families. Four of these mutations corresponded to amino acid substitution in the highly conserved patatin domain and the fifth was a frameshift with a premature terminal codon that can be predicted to result either in mRNA decay or in the synthesis of a truncated protein. Thus, all these newly identified mutations were strongly suspected to affect the biological function of the protein. In order to better understand the function of PNPLA1 in the epidermis, we developed *Pnpla1* knockout (KO) mice. Pnpla1 deficiency in mice led to neonatal lethality. *Pnpla1* KO E18.5 embryos and newborns had a thick, taut and shiny skin with a shellacked appearance that led to reduced mobility and thus failure to suckle maternal milk. This "collodion-like" appearance was strongly evocative of the collodion baby observed in humans. Histological examination of the skin revealed an increased number of epidermal living layers (acanthosis) and a thick, compact *stratum corneum*, consistent with proliferation/differentiation disturbance. This phenotype was associated with impairment in the outside-in and inside-out permeability barrier function. *Pnpla1*-deficient mice lethality

Table 1. Structure and correspondent nomenclatures of human epidermal ceramides.

		Fatty acid moiety			
		Non-hydroxyl fatty acid (N)	Alpha-hydroxyl fatty acid (A)	Omega-hydroxyl fatty acid (O)	Esterified omega-hydroxyl fatty acid (EO)
Sphingoid base moiety	Dihydrosphyngosine (dS)	Cer[NdS]	Cer[AdS]	Cer[OdS]	Cer[EOdS]
	Sphingosine (S)	Cer[NS]	Cer[AS]	Cer[OS]	Cer[EOS]
	Phytosphingosine (P)	Cer[NP]	Cer[AP]	Cer[OP]	Cer[EOP]
	6-Hydroxysphingosine (H)	Cer[NH]	Cer[AH]	Cer[OH]	Cer[EOH]

Note: Epidermal ceramides (Cer) are classified into 19 classes depending on their sphingoid base and fatty acid moieties. Ceramide species are additionally defined by fatty acid chain length. Fatty acids [N] and [A] contain C16-C30 whereas fatty acids [O] and [EO] contain C28-36. Ceramides can be glycosylated (not represented) and Cer[OS] can be covalently bound to cornified envelope protein shell. Omega-hydroxy-ceramide include: Cer[Ods], Cer[OS], Cer[OP], Cer[OH]; omega-O-acylceramide include: Cer[EOds], Cer[EOS], Cer[EOP], Cer[EOH]. Ceramide name in square brackets correspond to the Motta nomenclature (Motta *et al.*, 1993).

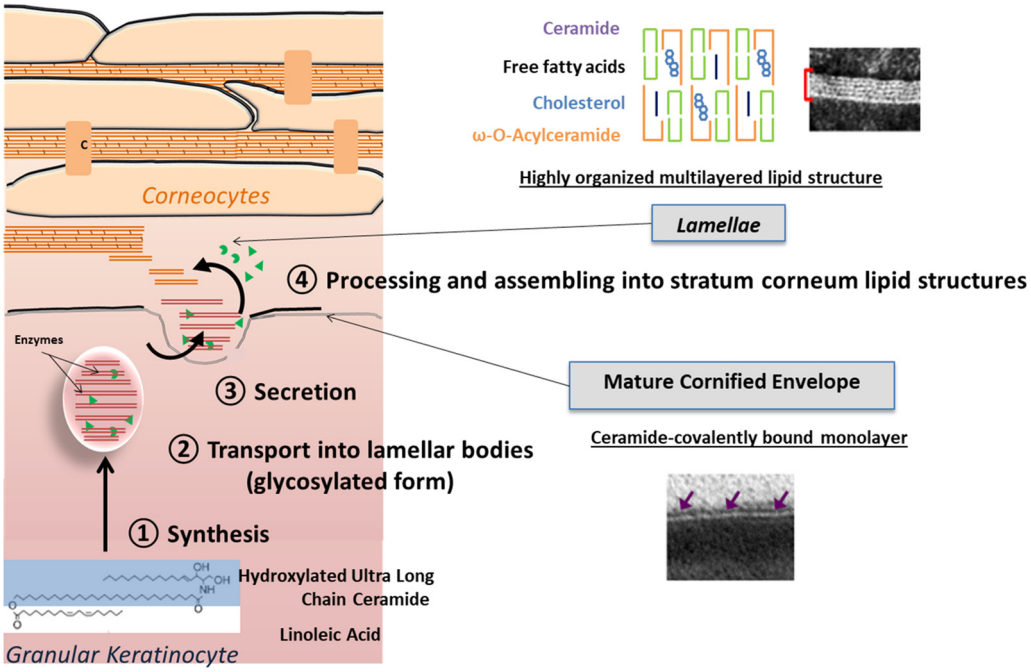

Fig. 2. Omega-O-acylceramide is an epidermis specific sphingolipid with a crucial role for epidermal barrier function.
Diagram showing the synthesis and transport of omega-O-acylceramide in the granular keratinocyte, their secretion at the interface *stratum granulosum*/*stratum corneum* and their extracellular processing and assembling into the *stratum corneum* lipid structures (*lamellae* and cornified lipid envelope) (see text for details).

was most probably due to severe dehydration caused by both an inability to feed and epidermal barrier impairment. Similar lethal phenotypes have also been reported in mice invalidated for other genes causing ARCI (Matsuki *et al.*, 1998; Epp *et al.*, 2007; Jennemann *et al.*, 2012; Krieg *et al.*, 2013).

Pnpla1-deficient murine skin showed an important alteration in epidermal lipid composition and organization. Ultrastructural analyses of the KO skin by transmission electron microscopy showed an impaired organization of the extracellular lipid matrix in the *stratum corneum*. Indeed, the typical arrangement in *lamellae* was observed only in wild-type mice. Moreover, analyses of cornified fluorescent-labelled envelopes showed that when Pnpla1 was absent, cornified envelopes are mainly composed of crosslinked proteins with an obvious defect in lipid coverage (*i.e.* quasi-absence of cornified lipid envelope). Finally, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) revealed that Pnpla1-deficient mice had a modified sphingolipid profile. Quantification of different species of ceramides in the mutant epidermis highlighted a drastic reduction in ω-esterified ceramides with a concomitant accumulation of their metabolic precursors, ω-hydroxyceramides. In accordance with the reduced level of esterified ceramides, we also observed a drastic reduction of their derivatives, the ceramides crosslinked to the cornified envelope,

relevant with the impaired lipid coverage observed by fluorescence microscopy. These data obtained in mice were confirmed on patients suffering from ARCI. We evidenced in *PNPLA1*-mutated patients an alteration of the cornified lipid envelope. Moreover, *PNPLA1* mutations resulted in an important decrease in *stratum corneum* esterified ceramides accompanied by an accumulation of its precursors.

Altogether, these results clearly showed that PNPLA1/Pnpla1 deficiency provoked a blockade in ω-esterified ceramide synthesis. Pnpla1-deficient mice were developed and analysed by two other groups that reported very similar and concordant results (Grond *et al.*, 2017; Hirabayashi *et al.*, 2017). Furthermore, the work of Kihara's group allowed demonstrating the enzymatic activity of PNPLA1. Indeed, the experiments they performed *in vitro* or using transfected cell models demonstrated that PNPLA1 was able to transfer linoleic acid from triglyceride to ω-hydroxyceramides (Ohno *et al.*, 2017). In the end, these studies demonstrated that PNPLA1 is a transacylase essential for the synthesis of ω-esterified ceramides.

4 Ceramides and skin ageing

Skin changes constitute the first obvious evidence of aging, a complex and multifactorial biological process affecting the whole body. Skin aging is influenced by several factors including genetics, environmental exposure (UV radiation, smoke, pollution...), hormonal changes and metabolic process. These intrinsic and environmental factors gradually make the skin thin, translucent, and more susceptible to trauma and bruise. Aged skin also presents marbled pigmentation, aged spots, loss of elasticity and wrinkles (Gilchrest, 1996).

Histologically, one of the most prominent changes with intrinsic aging is flattening of the dermal-epidermal junction, making it less resistant to shearing forces, as well as decreased thickness of the dermis and the epidermis. Concerning resident cells of the skin other than keratinocytes, a reduced number of melanocytes and Langerhans cells (the epidermal "sentinel" immune cells) have also been described. Comparatively to the dermis and the living layers of the epidermis, little histological changes appear in the *stratum corneum* of aged skin in comparison to young skin. Notably, it has a virtually normal thickness and basket-weave appearance. Age-dependent functional changes in the *stratum corneum* have been assessed by measuring different parameters like transcutaneous water loss (transepidermal water loss, TEWL), *stratum corneum* hydration or skin-surface pH. Some variations in one or more of these parameters were detected in aged skin in comparison to young skin of healthy male and female groups of volunteers, but globally the functionality of the *stratum corneum* appeared little or non-affected (Leveque *et al.*, 1984; Ghadially *et al.*, 1995; Tagami, 2008; Luebberding *et al.*, 2013, 2014; Boireau-Adamezyk *et al.*, 2014). However, when the barrier was experimentally perturbed, for instance with either acetone or tape stripping, it recovered more slowly in aged than in young human subjects (Ghadially *et al.*, 1995). Moreover, it is well documented that aged skin is prone to develop altered drug permeability, increased susceptibility to irritants, contact dermatitis and severe xerosis. Thus the *stratum corneum* barrier function appears more "fragile" in elderly. Lipids and more particularly ceramide deficiency may account, at least in part, for this dysfunction of the *stratum corneum* associated with ageing.

A detailed analysis of the effect of aging on the lipid structures in the *stratum corneum* was performed by the group of P. Elias (Ghadially *et al.*, 1995). They examined by transmission electron microscopy the lipid *lamellae* and the cornified lipid envelope of young *vs.* aged skin. In both aged human and mouse epidermis they observed a normal number of lamellar bodies in the granular keratinocytes cytosol, with normal internal structure. However, a paucity of secreted lamellar body contents was present at the *stratum granulosum*/*stratum corneum* interface. Moreover, the *stratum corneum* interstices contained focal domains that were either depleted or virtually devoid of *lamellae*. This study was completed by the analysis of lipid content which showed that the quantity of lipids in aged murine *stratum corneum* was globally reduced (~30%), without exhibiting any specific abnormality in species distribution or fatty acid composition (Ghadially *et al.*, 1995). Significantly decreased levels of all major lipid species, in particular ceramides, with increasing age, were also reported in human (Rogers *et al.*, 1996).

Hence, treatments able to restore skin-ceramide levels and lipid organization in the *stratum corneum* could improve the epidermal barrier function in aged skin. For some years now many studies have been conducted to identify such treatments. Besides clinical trials performed on aged *vs.* young subjects (Seyfarth *et al.*, 2011; Danby *et al.*, 2016; Chang *et al.*, 2018), one can also find interesting information from studies done on patients with skin diseases characterized by a perturbation of *stratum corneum* lipids and a defective barrier, like atopic dermatitis, psoriasis or ichthyoses (Lowe *et al.*, 2012; Liu *et al.*, 2015; Sahle *et al.*, 2015), as well as studies using animal or *in vitro* models (Di Marzio *et al.*, 2008; Moner *et al.*, 2018; Popa *et al.*, 2018).

Different strategies were developed in order to improve skin-ceramide levels. One consisted in direct replenishment of the missing lipids or their analogues. Several studies reported positive effects of topically applied preparations containing ceramides on skin barrier function (Kucharekova *et al.*, 2002; Lowe *et al.*, 2012; Liu *et al.*, 2015; Sahle *et al.*, 2015). Importantly, it seems that the therapeutic ceramide may not be applied alone but in combination with other lipids in order to maintain the ratio between the three major lipid components of the *stratum corneum*. Indeed, partial lipid compositions may result in abnormal lamellar bodies' contents and hence may interfere with the formation of *lamellae* and cornified lipid envelope. The vehicle used to penetrate the *stratum corneum* is also important. Efforts are still done in the development of technologies with the aim of highly efficient delivery of the product. For instance, sphingomyelin-based liposomes and lamellar body mimetic system have recently been proposed (Itaya and Tokudome, 2016; Moner *et al.*, 2016). Efficiency of topically applied substitutive lipid mixture may also differ regarding the nature of the topically applied ceramide. Different sources of therapeutic lipids have been proposed, including synthetic or animal-based ceramides as well as plant-derived ceramides (Tessema *et al.*, 2017). Sometimes, therapeutic lipids were administered by oral dietary supplement. Although some clinical studies reported skin-moisturizing and skin barrier recovery effects of such dietary supplement, the fate of the ingested ceramide and the mechanisms underlying skin barrier improvement are still obscure (Tessema *et al.*, 2017).

Another approach is to facilitate the production of the lipids *in vivo*. For instance, the administration of agents such as nicotinamide, ascorbic acid and its derivatives, or ursolic acid, a plant-derived triterpenoid, was found to effectively increase the synthesis of epidermal ceramides (Tanno *et al.*, 2000; Yarosh *et al.*, 2000; Katsuyama *et al.*, 2017). Furthermore, the age-dependent increase of pH in the *stratum corneum* is known to have an impact on the activity of acidic-dependent lipid hydrolases involved in ceramide processing (*e.g.* β-glucocerebrosidase, sphingomyelinase). It has been shown that re-acidification of the *stratum corneum* with lacto bionic acid accelerated barrier recovery in old individual and led to increased formation of fully processed *lamellae* (Choi *et al.*, 2007). In another study, researchers showed that topical application of bacterial sphingomyelinase from *Streptococcus thermophilus* increased skin-ceramide levels, improved the lipid barrier and augmented a resistance against ageing-associated xerosis in aged subjects (Di Marzio *et al.*, 2008).

5 Conclusion

Stratum corneum lipids, and more particularly ω-O-acylceramides, play a crucial role in the epidermal barrier function. They are mainly produced by the differentiating living keratinocytes beneath the *stratum corneum*, undergo extracellular maturation in the lower *stratum corneum* to finally form extracellular lipid matrix *lamellae* and cornified lipid envelopes, the two essential lipid structures of the *stratum corneum*. Clarifying the role of the enzyme PNPLA1, which gene is mutated in some forms of congenital ichthyoses, is a further illustration emphasizing the crucial role of ω-O-acylceramides in the epidermal barrier function. Perturbation of the lipid organization in the *stratum corneum* is associated with skin conditions of impaired epidermal barrier, including numerous skin diseases (atopic dermatitis, psoriasis, ichthyoses), as well as aged skin in which the permeability barrier is more "fragile". Age-dependent ceramide deficiency contributes to skin fragility in elderly. Restoration of skin-ceramide levels is part of treatments under development for combating the effects of skin aging. Many *in vitro*, animal and clinical studies are conduced to this end with encouraging and positive results. Continuation of those efforts and further experiments are needed to improve such treatments and better understand the underlying molecular mechanisms of lipid barrier restoration.

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