Chapter 24
Atopic Dermatitis (AD) and Lipids

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Core Messages

- The stratum corneum (SC) of atopic dermatitis (AD) skin contains unusual lipids, particularly with respect to ceramides (CER).
- Abnormalities in the lipid bilayer at intercellular spaces of the SC cause an impaired barrier function as seen in higher values of transepidermal water loss.
- The mechanism underlying these lipid abnormalities could be altered enzymatic activities relevant to the *de novo* synthesis of CER in the epidermis of AD skin.
- Whether the abnormalities are primary or secondary to AD has not been fully elucidated although the outside–inside view has become more probable.

Introduction

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease characterized by pruritic and eczematous skin lesions. AD is thought to be caused by multiple pathogenic factors, such as genetic susceptibility, environmental triggers, cutaneous barrier dysfunction, bacterial infection, and/or immune dysregulation. About 20 % of Caucasian children as well as 2–10 % of adults are affected by AD (Alanne et al. 2011; Slattery et al. 2011). One of the biggest discoveries in recent studies of AD was an apparent loss-of-function that genetic variants in the gene encoding filaggrin demonstrated; those are a strong predisposing factor for the development of AD with very-high significance (Palmer et al. 2006). Up to 60 % of European AD patients have loss-of-function mutations in the filaggrin gene (Elias and Wakefield 2011).

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The relationship between AD and skin lipids was first reported by Melnik et al. 1988; Imokawa et al. 1991 then published an epoch-making article reporting lipid abnormalities with special reference to ceramides (CER) in the stratum corneum (SC) of AD skin. Since then, great attention has been paid to CER in the SC of patients with AD as well as their abnormal immune system. Until the beginning of the twenty-first century, thin-layer chromatography (TLC) was the only tool available to analyze CER in the SC, but the appearance of a new powerful technique, liquid chromatography-mass spectrometry (LC-MS), shifted the paradigm of dermatological studies regarding CER (Vietzke et al. 2001; Farwanah et al. 2005a). Progress worthy of special mention was that LC-MS analysis of CER in the SC of human skin revealed as many as 350 species that were structurally characterized (Masukawa et al. 2008), and that each of those diverse species could be quantified precisely and comprehensively using a newly developed LC-MS method (Masukawa et al. 2009). This method allowed the detailed features of the CER composition of the SC to be delineated.

The aim of this chapter is to clarify what has been known and unknown about the relationship between AD and skin lipids by answering the following four questions: (1) Are SC lipids in AD skin different from the lipids found in normal skin? (2) Do the lipid abnormalities affect the structures and/or properties of AD skin? (3) Is the mechanism underlying the lipid abnormalities known? (4) Are the lipid abnormalities primary or secondary to the development of AD? This chapter focuses on the relationships with skin lipids and not on relationships with skin proteins, such as filaggrin and cornified envelopes, except for those relevant to skin lipids. Readers who are interested in relationships between AD and skin proteins should consult other reviews (Proksch et al. 2008; Kypriotou et al. 2012; Nishifuji and Yoon 2013).

Are SC Lipids in AD Skin Different from the Lipids Found in Normal Skin?

Skin barrier function strongly relies on the SC (outermost layers of the skin), which consists of stacked layers of corneocytes (enriched proteins) “bricks” embedded in an intercellular lipid mixture “mortar” (Michaels et al. 1975). CER, cholesterol and free fatty acids (FFA) are the three abundant lipid classes in the free intercellular lipids of the SC of human skin and CER accounts for 40–50% of the total lipid mass (Wertz 1992). There are 12 CER subclasses (Robson et al. 1994; Ponec et al. 2003; Masukawa et al. 2008; Van Smeden et al. 2011), which can be expressed based on previous terminology (Motta et al. 1993; Robson et al. 1994) as depicted in Fig. 24.1; CER[ADS] consisting of α-hydroxy fatty acids and dihydrosphingosines (corresponding to CER 5 in the TLC analysis); CER[AH] consisting of α-hydroxy fatty acids and 6-hydroxysphingosines (CER 6II); CER[AP] consisting of α-hydroxy fatty acids and phytosphingosines (CER 6I); CER[AS] consisting of α-hydroxy fatty acids and sphingosines (CER 5); CER[EODS] consisting of ester-linked ω-hydroxy fatty acids and dihydrosphingosines (CER 1); CER[EOH] consisting of ester-linked ω-hydroxy fatty acids and 6-hydroxysphingosines (CER 4);

Fatty acid / Sphingoid	Non-hydroxy fatty acid [N]	α-hydroxy fatty acid [A]	Esterified ω-hydroxy fatty acid [EO]
Dihydrosphingosine [DS]	CER[NDS]	CER[ADS]	CER[EODS]
Sphingosine [S]	CER[NS]	CER[AS]	CER[EOS]
Phytosphingosine [P]	CER[NP]	CER[AP]	CER[EOP]
6-hydroxy sphingosine [H]	CER[NH]	CER[AH]	CER[EOH]

Fig. 24.1 Structures and nomenclature of ceramides (CER) in human stratum corneum (SC). (Note: this research was originally published in J. Lipid Res. (Masukawa et al. 2008). © the American Society for Biochemistry and Molecular Biology)

CER[EOP] consisting of ester-linked ω-hydroxy fatty acids and phytosphingosines (CER 2); CER[EOS] consisting of ester-linked ω-hydroxy fatty acids and sphingosines (CER 1); CER[NDS] consisting of nonhydroxy fatty acids and dihydrosphingosines (CER 2); CER[NH] consisting of nonhydroxy fatty acids and 6-hydroxysphingosines (CER 6I); CER[NP] consisting of nonhydroxy fatty acids and phytosphingosines (CER 3); and CER[NS] consisting of nonhydroxy fatty acids and sphingosines (CER 2). In addition to the 12 free CER subclasses, two subclasses of protein-bound CER are found in the SC, CER[OH] consisting of ω-hydroxy fatty acids and 6-hydroxysphingosines and CER[OS] consisting of ω-hydroxy fatty acids and sphingosines (Robson et al. 1994).

Table 24.1 shows a summary of intercellular lipids reported in the SC of AD lesional, AD nonlesional and controlled healthy nonlesional skin. Although numerous studies have emphasized diverse results due to the different subjects tested and the different methods used, there are common features for AD lesional skin as follows: (1) the level and/or wt.% of total CER is lower; (2) the CER composition is altered; and (3) the chain length of CER species is shortened. The first feature was confirmed by analyses done by Imokawa et al. 1991; Matsumoto et al. 1999; and Ishikawa et al. 2010. The second feature, i.e., that the balance of CER[EOS], other EO-containing CER subclasses and CER[NP] is commonly altered, was reported by Imokawa et al. (1991); Di Nordo et al. (1998); Matsumoto et al. (1999); Ishikawa

Table 24.1 Skin lipids for AD lesional (AL), AD nonlesional (ANL), and controlled healthy nonlesional (HNL)

Authors	Materials	Methods	Results
Melnik et al. 1988	SC from 10 ANL and 10 HNL	TLC	Lower wt. % of total CER in ANL
Imokawa et al. 1991	Cyanoacrylate-stripped SC from 35 AL, 35 ANL, and 65 HNL	TCL	Lower level of total CER in AL and ANL
			Lower wt. % of CER 1 (CER[EOS]) in AL and ANL
Yamamoto et al. 1991	Extracted SC lipids from 6 ANL and 6 HNL	TLC	Lower wt. % of CER 1 (CER[EOS]) in ANL
Di Nardo et al. 1998	Cyanoacrylate-stripped SC from 28 AL, 19 ANL, and 20 HNL	TLC	Lower levels of CER 1 (CER[EOS]) and CER 3 (CER[NP]) in AL
			Higher wt. % of CH in AL and ANL
			Intermediated levels in ANL between AL and HNL
Matsumoto et al. 1999	Extracted SC lipids from 14 AL,30 ANL, and 25 HNL	TLC	Lower levels of total CER and CER 1 (CER[EOS]) in AL
			Not different between ANL and HNL
Bleck et al. 1999	Cyanoacrylate-stripped SC from 10 ANL and 10 HNL	TLC	Lower wt. % of CER[EOS] and CER[NP], higher wt% of CER[EOP], and shorter chain in CER[AS] in ANL
		MALDI-MS	
Macheleidt et al. 2002	Biopsied epidermis from 10 AL, 8 ANL, and 5 HNL	TLC	Lower wt. % of ω-hydroxy CER in AL and ANL
		GC	Lower wt. % of very-long-chain FFA in AL and ANL
Arikawa et al. 2002	Tape-stripped SC from 73 AL, 83 ANL, and 69 HNL	TLC	Lower level of sphingosine in AL and ANL
Okamoto et al. 2003	Tape-stripped SC from 44 AL, 47 ANL, and 40 HNL	TLC	Lower level of sphingosylphosphorylcholine in AL and ANL
Ishibashi et al. 2003	Tape-stripped SC from 92 AL, 105 ANL, and 81 HNL	TLC	Lower level of glucosphingosine in AL and ANL
Farwanah et al. 2005b	Extracted SC lipids from 7 ANL and 7 HNL	TLC	Not different in CER between ANL and HNL
		LC-MS	
Ishikawa et al. 2010	Tape-stipped SC from 7 AL, 7 ANL, and 7 HNL	LC-MS	Lower levels of total CER, CER[NH], CER[NP], CER[EOS], CER[EOH], and CER[EOP], higher level of CER[AS], lower levels of longer chain in CER[NS], CER[NDS], CER[NH], CER[AS], and CER[AH], and higher levels of shorter chain in CER[NS] (especially with C34), CER[NDS] and CER[AS] in AL Intermediated level in ANL between AL and HNL

Table 24.1 (continued)

Authors	Materials	Methods	Results
Jungersted el al. 2010	Cyanoacrylate-stripped SC from 12 ANL-FLGm, 19 ANL-FLGw, 6 HNL-FLGm, and 12 HNL-FLGw	TLC	Lower wt.% of CER[EOP] and higher wt.% of CER[AP] in ANL-FLGm than HNL-FLGm and HNL-FLGw
			Lower wt.% of CER[EOS] and CER[AP] in ANL-FLGw than HNL-FLGm and HNL-FLGw
Angelova-Fischer et al. 2011	Cyanoacrylate-stripped SC from 14 AL/ANL-FLGm, 23 AL/ANL-FLGw, and 20 HNL	TLC	Lower levels of CER[EOH] and fatty acids in AL-FLGm than AL-FLGw
			Higher level of CH in ANL-FLGm
Janssens et al. 2011	Tape-stripped SC from 6 ANL and 6 HNL	LC-MS	Lower wt.% of CER[NP] and (CER[EODS]+CER[EOS]+CER[EOP]+CER[EOH]) in ANL
Janssens et al. 2012	Tape-stripped SC from 14 ANL-FLGm, 14 ANL-FLGw, and 15 HNL	LC-MS	Higher wt.% of shorter C34-CER[NS], C34-CER[NH], C34-CER[AS], and C34-CER[AH] and lower wt.% of (CER[EODS]+CER[EOS]+CER[EOP]+CER[EOH]) in ANL with no differences between FLGm and FLGw

TLC thin layer chromatography, *MALDI-MS* matrix-associated laser desorption-mass spectrometry, *GC* gas chromatography, *LC-MS* liquid chromatography-mass spectrometry, *FLGm* filaggrin gene mutation, *FLGw* no filaggrin mutation

et al. (2010); and Angelova-Fischer et al. (2011). The third feature, most recently unveiled, comes from the significantly higher levels of CER[NS], CER[NDS], and CER[AS] with shorter chain lengths, as represented in C34-CER[NS] (Ishikawa et al. 2010). The validity of the third feature is corroborated by the fact that there were significantly lower levels of CER[NS], CER[NDS], CER[NH], CER[AS], and CER[AH] with longer chain lengths in the AD lesions (Ishikawa et al. 2010), the fact that a CER[AS] species with a shorter chain length was detected in AD nonlesional skin but not in healthy skin (Bleck et al. 1999), and the fact that significantly higher wt.% of C34-CER[NS], C-34CER[NH], C34-CER[AS], and C-34CER[AH] were found in AD nonlesional skin (Janssens et al. 2012). Macheleidt et al. (2002) found a lower wt.% of very-long-chain FFA in the SC of AD lesional skin although this is not for CER.

Compared with the lipid abnormalities in AD lesional skin, AD nonlesional skin looks somewhat indefinite in terms of the levels and composition of CER. As shown in Table 24.1, the intermediate features of AD nonlesional skin between AD lesional skin and healthy skin were described in some articles (Di Nardo et al. 1998; Ishikawa et al. 2010) and characteristics in the lipid abnormalities similar to AD lesions were shown in AD nonlesional skin by others (Bleck et al. 1999; Janssens et al. 2011, 2012). On the other hand, Matsumoto et al. (1999) and Farwanah et al. (2005b) reported no differences between AD nonlesional skin and healthy skin. Those inconsistencies in results obtained for nonlesional SC of AD skin would be due to the varieties of subjects tested (severity, progress, and degree of nonlesions), sampling sites/procedures and analytical methods used. Filaggrin gene mutations do not appear to directly influence the lipid abnormalities for the nonlesional SC of AD skin. No significant differences at the nonlesional sites were found between individuals carrying and not carrying the mutations (Jungersted et al. 2010). In another study undertaken by Janssens et al. (2012), the nonlesional SC of AD subjects carrying filaggrin mutations did not have any differences in lipids with those not carrying the mutations. However, the lower level of CER[EOH] in the lesional SC of AD patients carrying the mutations than those not carrying them was pointed out (Angelova-Fischer et al. 2011). To define characteristics of the lipids in AD nonlesional skin and the impact of filaggrin gene mutations on the lipids in the SC of AD skin, much larger-scaled studies are needed.

Collectively, the answer for the question “are SC lipids in AD skin different from the lipids found in normal skin?” is likely “yes” for the SC of AD lesional skin, as indicated by the lower level of total CER, the altered CER composition and the CER species with shorter chain lengths. For AD nonlesional skin, the abnormalities may be present with slight but similar characteristics to AD lesional skin, but further studies are required in a way that the subjects tested are standardized in terms of severity, progress, and degree of nonlesional skin. The filaggrin gene mutations do not seem to directly affect the lipid abnormalities, at least for the nonlesional SC of AD skin but this also remains to be defined.

Do the Lipid Abnormalities Affect the Structures and/or Properties of Skin?

No studies have been reported that characterized structures of the lipid bilayer at intercellular spaces in the SC of AD lesional skin, and only structures in nonlesional SC have been investigated. The long-periodicity phase in the lipid bilayer in the nonlesional SC was found to be slightly but significantly reduced in the repeat distance or repeat quantity compared to healthy SC (Janssens et al. 2012). Regarding the lateral lipid packing, it was found that the nonlesional SC of AD patients has an increased percentage of hexagonal lattice, gel phase, compared to healthy skin which is characterized by a larger presence of orthorhombic packing, crystalline phase (Pilgram et al. 2001; Janssens et al. 2012, 2013). These differences could be interpreted as originating from the lipid abnormalities, such as a lower level of total CER, an altered CER composition, and/or CER species with shorter chain lengths.

The diminished level of total CER in the SC of AD skin had a negative correlation with transepidermal water loss (TEWL), which is an index of impaired barrier function (Ishikawa et al. 2010). Also, there was a significantly negative correlation of the TEWL value versus the level of each CER subclass other than CER[AS] and CER[NS]. The subclass of CER[AS] had a significantly positive correlation with TEWL (Ishikawa et al. 2010). Only the subclass CER[AS] seems to have a different nature in terms of the involvement with the barrier function in AD skin. An effect of chain lengths of CER species on the TEWL has also been revealed. Thus, the more abundant the CER species with shorter chain lengths are, the higher the TEWL values (Ishikawa et al. 2010; Joo et al. 2010; Janssens et al. 2012). Since the level of C34 CER species sounds strongly correlated with TEWL (Ishikawa et al. 2010; Janssens et al. 2012), it may be a characteristic marker for the diagnosis of AD. Janssens et al. (2013) showed that the change in tendency in the lateral packing is correlated with the TEWL value. That correlation could be interpreted by the physicochemical nature that the hexagonal lattice is a less-packed structure in the lateral direction, where water can be less disturbed through the lipid bilayer.

The structure of the lipid bilayer in the SC of AD skin is likely to be changed into a bilayer with the reduced repeat distance or repeat quantity in the long-periodicity phase and with an increase in the hexagonal lattice, which may be due to the lipid abnormalities. This change in structure would cause a higher TEWL value corresponding to the impaired barrier function of AD skin.

Is the Mechanism Underlying the Lipid Abnormalities Known?

An ultrastructural study of AD skin versus healthy skin indicated the immature formation of lipid lamellae at the border between the stratum granulosum and the SC of AD skin (Fartasch et al. 1992). Thus, in AD skin, lamellar body-discs remained

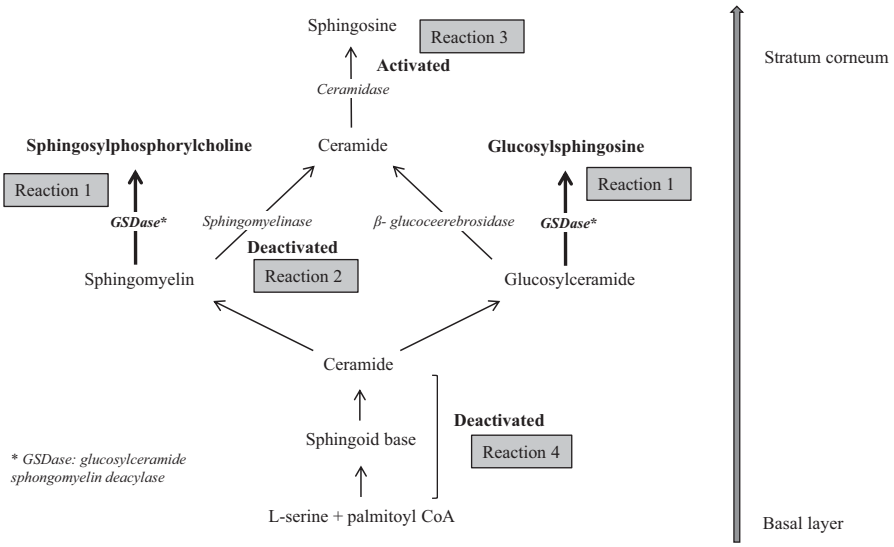

Fig. 24.2 Metabolism of ceramides (CER) in human skin

undelivered and were found even within the horny cells, in contrast to healthy skin where the body-discs completely disappeared. This suggested an abnormal keratinization coming from the unusual lipid metabolism in AD skin. The deficiency of CER in the SC of patients with AD can be explained by the extraordinary upregulation of glucosylceramide sphingomyelin deacylase (GSDase), which hydrolyzes glucosylceramide (GlcCER) or sphingomyelin (SM) at an acyl site to yield sphingosylphosphorylcholine (SPC) or glucosylsphingosine (GSP), respectively, instead of CER (Imokawa 2009), as illustrated in Reaction 1 of Fig. 24.2. The substantiality of the enzyme is supposed to be the β-subunit of acid ceramidase (CDase) based on a study using rat skin (Nogami-Itoh et al. 2010). At first, it was found that in the skin of patients with AD, the activities of three sphingolipid hydrolysis enzymes, β-glucocerebrosidase, sphingomyelinase (SMase), and CDase were not changed (Jin et al. 1994; Murata et al. 1996) whereas SM hydrolysis was increased with the occurrence of SPC as a reaction product and this hitherto undiscovered enzyme was tentatively termed SM deacylase (Murata et al. 1996; Hara et al. 2000). In a subsequent study, this enzyme was then termed GSDase because it hydrolyzes not only SM but also GlcCER in AD skin (Higuchi et al. 2000). The fact that the levels of SPC and GSP were both significantly higher in the epidermis of AD patients (Okamoto et al. 2003; Ishibashi et al. 2003), as listed in Table 24.1, corroborates the mechanism that the upregulation of GSDase generates the CER deficiency.

Other possible mechanisms underlying the diminished level of CER were proposed regarding reduced SMase activity (Reaction 2 of Fig. 24.2) and the involvement of bacterial CDase (Reaction 3 of Fig. 24.2). Acid SMase as well as neutral SMase, which produce CER from SM in the epidermis, were decreased both in the lesional and nonlesional skin from AD patients compared to control healthy skin

(Jensen et al 2014). The involvement of bacteria secreting CDase by which CER would be decomposed in the SC of AD skin was proposed (Ohnishi et al. 1999). Those mechanisms might be responsible in part for the diminished level of total CER. However, the altered CER composition cannot be explained only by the SMase activity or bacterial CDase because CER[NS] and CER[AS] are derived in part from the corresponding SM precursors while other subclasses such as EO-containing CER are derived only from GlcCER (Uchida et al. 2000; Hamanaka et al. 2002). Those mechanisms are not enough to explain the altered CER composition in the selective changes in the balances of CER[EOS], other EO-containing CER subclasses, and CER[NP].

Macheleidt et al. (2002) compared the *de novo* synthesis of GlcCER and CER in lesional AD skin with healthy skin using a metabolic labeling technique, which revealed remarkable decreases of newly biosynthesized ClcCER and CER in lesional AD skin. An experimental system using a reconstructed human epidermal keratinization model suggested that the Th2 type of inflammation evoked in AD skin may be one factor involved in the downregulated biosynthesis of CER, which results in the reduced levels of CER in the SC (Sawada et al. 2012). Therefore, the deficiency of CER in the SC of AD skin is likely to be caused not only by the abnormal pathway from GlcCER and SM to CER, such as the upregulation of GSDase (Reaction 1 of Fig. 24.2), but also reduced the *de novo* synthesis of CER skeletons themselves (Reaction 4 of Fig. 24.2). As for the chain length, elongases in the epidermis seem to be involved. Although the results were obtained in an experimental system using mice but not humans, some elongases that synthesize very-long-chain FFA were downregulated in a hapten-induced AD model (Park et al. 2012). It could be assumed that the downregulated elongases resulted in the decreased levels of CER species with longer -chain lengths (Bleck et al. 1999; Ishikawa et al. 2010; Janssens et al. 2012)very-long-chain FFA (Macheleidt et al. 2002).

Based on evidence accumulated to date, the mechanism underlying the lipid abnormalities for the SC in AD skin can most probably be explained by a combination of events, as follows: (1) the lower level of total CER would be caused by both the upregulation of GSDase and the reduced *de novo* synthesis of CER in the epidermis of AD skin, (2) the altered CER composition might be ascribed to changes in activities of enzymes relevant to the production of CER in the SC although this remains to be clarified, and (3) CER species with shorter chain lengths might originate from downregulated elongases although that also remains to be elucidated.

Are the Lipid Abnormalities Primary or Secondary to the Development of AD?

Traditionally, it was thought that the primary cause of AD was an immunological abnormality that led to the secondary barrier dysfunction (inside–outside view of AD pathogenesis). Many reports on the pathogenesis of AD focused on the primary role of abnormalities in the immune system, as reviewed by Leung (2006) and Ong and Leund (2006). In fact, therapy for AD was largely directed toward ameliorating Th2-mediated inflammation and/or pruritus using steroids or immunomodulators

in spite of concerns about their side effects. A new paradigm of the outside–inside view (or outside–inside–outside), however has proposed that the primary inherited and acquired barrier abnormalities are followed by immune system activation, which further exacerbates the barrier function with a vicious cycle (Elias 2008; Cork et al. 2009; Elias and Schmuth 2009; Elias and Wakefield 2011).

Elias and Schmuth (2009) insist on the probability of the outside–-inside view because specific replacement therapy, which targets the prominent lipid abnormalities that account for the barrier abnormality in AD, corrects not only the barrier impairment but also comprises an effective anti-inflammatory therapy for AD. Topical application of a CER or pseudoceramide-dominant physiological lipid-base barrier repair emulsion has demonstrated clinical efficacies to improve the impaired barrier function in AD skin as well as to ameliorate AD symptoms (Mao-Qiang et al. 1996; Berardesca et al. 2001; Chamlin et al. 2002; Jensen and Elias 2006; Madaan 2008; Bikowski 2009; Park et al. 2010; Kircik et al. 2011). The availabilities of skin care products containing lipids/oils for AD skin have also been evidenced in other cases. Topical application of emollients, moisturizers, or creams containing lipids/oils, such as CER (Hon et al. 2013), pseudoceramide (Hon et al. 2011), petrolatum (Matsumoto et al. 2007), and paraffin oil and vegetable oil (Patzelt et al. 2012), efficiently improved the impaired skin conditions. Those clinical efficacies could be considered in part to be caused by the lipids/oils which provide an exogenous barrier to water loss from the inside and to the penetration of foreign material from the outside. This corroborates the validity of the outside–inside view as well as the important role of CER. However, the outside–inside view and the role of CER are still speculative. Before getting a true answer for the question, the further detailed studies are required.

The current conclusion to answer the question “are the lipid abnormalities primary or secondary to the development of AD?” is that it has not been fully elucidated whether the impaired barrier function due to the lipid abnormalities is primary or secondary to AD. Recent studies showing the availabilities of lipids/oils therapy to treat AD skin certainly suggest that the outside–inside view might be more likely and that CER might be a key. However, no technologies seem to have reached a scientifically convincing proof from the viewpoint of the mechanism underlying the clinical efficacies to AD skin, although there have been some reports of technologies that can upregulate levels of endogenous CER in *in vivo* and *in vitro* studies (Rawlings et al. 1996; Tanno et al. 2000; Ishikawa et al. 2012). We need to wait some time to get a true answer which would be obtained from our future studies.

Summary

It is well known that lipid abnormalities occur in the SC of lesional AD skin, as seen in the lower level of total CER, the altered CER composition and the CER species with shorter chain lengths compared to control healthy skin. It is also known that

the lipid abnormalities cause an altered structure of the lipid bilayer, which further causes the impaired barrier function of AD skin, as seen in the higher values of TEWL. The reason for the lower level of total CER is likely to involve a mechanism where GSDase is upregulated and the *de novo* synthesis of CER is deactivated in the epidermis of AD skin. Based on accumulated evidence showing the clinical efficacies of lipids/oils therapy for patients with AD, the outside–inside view seems more likely than the traditional inside–outside view. On the other hand, there are still several unknown factors as follows; the lipid abnormalities for the nonlesional SC of AD, the reasons why the composition of CER subclasses is altered and why the chain lengths of CER species become shorter in AD lesions, the mechanism underlying the development of AD, and its relationship to the lipid abnormalities, that is, whether the lipid abnormalities are primary or secondary for AD. Those unknown factors will be clarified by our future work, which should help improve the quality of life for patients who suffer from AD symptoms.

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