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## Epidermal Lipids: Key Mediators of Atopic Dermatitis Pathogenesis

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### Abstract

Skin barrier keeps the “inside-in” and the “outside-out” forming a protective blanket against external insults. Epidermal lipids such as ceramides, fatty acids, triglycerides and cholesterol are integral components in driving the formation and maintenance of epidermal permeability barrier (EPB). A breach in this lipid barrier sets the platform for the subsequent onset and progression of atopic dermatitis (AD). Such lipids are also important in the normal functioning of different organisms, both plants and animals and in diseases including cancer. A double increase in AD rate in the recent years and the chronic nature of this disorder emphasizes the need of this review to shed light on the multifaceted role of the diverse types of lipids in mediating AD pathogenesis.

### Keywords

Atopic Dermatitis (AD); Epidermal Permeability Barrier (EPB); Lipid Gene Regulation; Cutaneous Lipid Metabolism

## Beyond the Brick Wall: The Stratum Corneum and Epidermal Barrier Functions

The skin is the frontier between organisms and environment controlling two key functions: 1) maintaining a robust external barrier resilient against environmental stressors and 2) maintaining a water-tight internal barrier that prevents **trans-epidermal water loss**

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(TEWL) (see glossary), as well as prevent the colonization of pathogens into the skin and underlying tissues. Together, these two account for the role of the skin as a versatile *double barrier* with dual external-internal protection [1–3]. Besides being an indispensable barrier, the skin is also involved in the implementation of chief regulatory functions in association with the fundamental organs of the body. Such functions include the ability of the skin to serve as a common platform for signal exchange between internal organs and the environment via the cutaneous neuroendocrine system upon conditions such as immunostimulation or exposure to solar radiation through production of neuropeptides, biogenic amines, melatonin, opioids, cannabinoids, secosteroids as well as growth factors and cytokines to mediate their divergent effector functions [4, 5].

Although the essential barrier functionality is mainly localized to the outermost layer of the skin that is the epidermis or the stratum corneum (SC), sebaceous glands embedded in the deeper layers of the skin are also endowed in part with the responsibility of maintained lubrication and delayed dehydration of the skin along with photoprotection and antimicrobial action thereby serving as another crucial mediator of effective barrier functionality [6]. In this review, we restrict our discussion to the role played by the epidermal lipids in constituting effective epidermal barrier functionality.

The SC, resembling a brick-wall consists of protein enriched corneocytes (analogous to bricks) embedded in intercellular matrix of non-polar lipids (mortar component) present in between the granular and the cornified layers [1–3]. Corneocytes as “bricks” represent the cellular complement of SC structure and contain a variety of enzymes, water, and keratin filaments, which gives rise to the mechanical strength of the SC [3, 7, 8]. They are encased in a cross-linked layer of proteins such as filaggrin, loricrin, and involucrin [3, 9–11] which anchor the cells with the lipid rich extracellular matrix, the “mortar” [12]. This highly ordered, hydrophobic lipid scaffolding/matrix is composed of approximately 50% ceramides (CER), 15% free fatty acids (FFAs) and the rest 25% comprising predominantly cholesterol and a small percentage of triacylglycerol (TAG) species, with FFAs and long chain bases liberated from sebaceous TAG and epidermal CER, respectively, in SC serving as potent antimicrobial agents [13–15]. The epidermal permeability barrier (EPB) formation involves proper expression of proteins involved in epidermal terminal differentiation and proper cornified envelope crosslinking [12, 16–19].

Improper EPB formation contributes to TEWL and triggers onset of inflammatory skin disease e.g. Atopic Dermatitis (AD) [20–23]. This validates the crucial role of SC in maintaining dual barrier functionality. Also, SC has potent antioxidant properties to prevent oxidative damage to the skin by the secretion of enzymes such as catalase as well as non-enzymatic molecules such as vitamin E, glutathione and uric acid [24].

Indeed, many of the skin diseases such as lamellar ichthyosis (LI), psoriasis, Netherton’s syndrome (NTS), and AD (see Box 1) are all characterized by defective or weakened epidermal barrier functionality [25]. There are various factors and hypotheses governing the final outcome of AD (for details, refer to Box 2). In the following section we have elucidated the role of the key genes involved in the formation of a proper EPB, how their deficiency leads to improper barrier formation and finally how a disrupted barrier results in enhanced

Th2 inflammatory response which can thereby act as a positive feedback loop to further worsen the integrity of the barrier leading to the chronic nature of AD.

## Key Genes Involved in EPB Formation and the Th2 Inflammatory Response in AD due to Breaches in the Brick Wall

Firstly, any alteration in the expression of the genes involved in lipid biosynthesis and metabolism pathways of the crucial lipids constituting the EPB results in destroyed barrier functionality and hence AD. Transcriptional regulatory proteins COUP-TF-interacting protein 1 and 2 (CTIP1 and CTIP2) are involved in regulation of epidermal lipid metabolism [26, 27]. A study by Li et al., 2017 in murine models showed altered expression level of several of those genes in *Ctip1*<sup>-/-</sup> mice. Expression of genes such as 3-Ketodihydrosphingosine Reductase (*Kdsr*), UDP-Glucose Ceramide Glucosyltransferase (*Ugcg*), N-acylsphingosine Amidohydrolase 1 (*Asah1*) and Ceramide Synthase 6 (*Cers6*) were found to be downregulated by approximately 0.6-fold, while expression of Sphingomyelin Synthase 2 (*Sgms2*), ELOVL Fatty Acid Elongase 4 (*Elov14*), Sphingomyelin Synthase 1 (*Sgms1*) and Sphingosine-1-phosphate phosphatase 1 (*Sgpp1*) were found to be downregulated by 0.5-fold as compared to control. On the other hand, genes such as Sphingosine Kinase 1 (*Sphk1*) and Sphingosine Kinase 2 (*Sphk2*) were found to exhibit an upregulated expression by approximately 1-fold, Sphingomyelin Phosphodiesterase (*Smpd1*) by 0.5-fold, Serine Palmitoyl transferase Long Chain Base Subunit 3 (*Sptlc3*) and Ceramide Synthase 2 (*Cers2*) by 0.3-fold in *Ctip1*<sup>-/-</sup> [26]. Another study by Wang et al., 2013 showed CTIP2 as a regulator of genes such as *Ugcg*, *Asah1*, *Cers6* and *Sgms2* with *Ugcg* expression found to be downregulated by 0.2-fold, *Asah1* by 0.1-fold and *Cers6* by 0.25-fold in *Ctip2*<sup>-/-</sup> mice at E16.5. *Sgms2* however was found to be expressed at a higher level by 0.5-fold in *Ctip2*<sup>-/-</sup> mice at E16.5 [27]. Thus, the above studies established the central role played by CTIP1 and CTIP2 in mediating the formation of EPB in adult mouse skin. (All fold changes are taken to the nearest approximation).

Secondly, Filaggrin is another multifunctional protein that plays a key role in EPB formation. There are predominantly two pathways exploited by filaggrin deficiency to act as mediators of AD, (1) by disrupting the normal epidermal terminal differentiation events and, (2) by inducing enhanced expression of Thymic Stromal Lymphopoietin (TSLP) on the epidermis and subsequent aberrant Th2 response [28]. Filaggrin mutations also correlate with asthma and food allergies, independent of AD. Filaggrin generates trans-urocanic acid, the principal UV-B filter in epidermis, and hence, reduced filaggrin predisposes to development of non-melanoma skin cancers. This sums up the most crucial genes and related proteins involved in intact EPB formation.

Studies in the past have successfully established the link between type 2 immune responses and AD pathogenesis. Hatano et al., 2005 first described IL-4 mediated downregulation of ceramide synthesis and barrier function [29]. Berdyshev et al., 2018 showed that altered lipid profiles, notably those with shorter chain FFA species, are linked to IL-13-driven AD skin lesion development. **RNA-seq** analysis revealed that FFA elongases (ELOVL3 and 6) were inhibited by IL-4/IL-13 cytokines in a STAT6-dependent manner with IL-13 mediated

downregulated expression of *Elovl3* and *Elovl6* by 0.77-fold and 0.57-fold respectively in lesional skin of murine models as compared to control [13]. A parallel study showed that the presence of IL-4 depressed the levels of mRNA for sphingomyelinase (SMASE) by 50% and glucocerebrosidase (GBA) by 70% and upregulated the expression levels of acid ceramidase by 20% resulting in overall decreased levels of ceramides in human (*Homo sapiens*) **epidermal living skin equivalents (LSEs)** [30], which was found to be strongly associated with increased TEWL and AD phenotype by **tape stripping analysis** [3, 31]. In a separate study involving LSEs, a cocktail of Th2 cytokines (IL-4, IL-31, and IL-13) significantly reduced the gene expression levels of the epidermal lipid metabolic enzymes such as those coding for SMASE by 0.7 fold, for GBA by 0.6 fold, and for ELOVL1 by 0.5 fold with respect to control [32]. The **lipidomic** profiling done in each to analyse the corresponding changes in lipid profile showed significantly downregulated levels of CER [AS] and [NS] (see figure 2 for nomenclature of ceramide subclasses) synthesized respectively by a SMASE and GBA and long chain FFAs and CER species synthesized in part by elongases such as ELOVL1, which correlated significantly with the AD phenotype [32].

Overall, these studies indicated the role played by various regulatory proteins in the formation of EPB, their regulation as well as how immune irregularities (in regard to ‘inside-out’ hypothesis) such as type 2 hyperactivation serves as an important factor in progression of AD. Extensive research in this area has the potential to offer beneficial therapeutic targets.

## Alteration of the Ceramide Composition in AD

Ceramide synthesis occurs through four major pathways in the epidermis: (1) *de novo* pathway, (2) sphingomyelinase pathway, (3) salvage pathway, and (4) exogenous ceramide recycling pathway [26] (see Fig. 3) (for details refer to Box 3). Till date, twelve classes of ceramide have been identified [33] (see Fig. 2). Ceramides were one of the first lipids to be connected with skin barrier function deficiencies and AD. DiNardo first cited this observation [34]. Later studies showed that deficient barrier function is associated with a general decrease of the so-called ultra-long chain ceramides, those more than 26 carbons in length and an increase in short-chain ceramides [13]. This study was further validated by the decreased expression in the mRNA level of the elongases in patients bearing lesions of AD. Consistently, **genetic ablation** of either *Elovl1* or *Elovl4* in mice resulted in decreased levels of ceramides with very-long acyl chains with CER (C30-C36) being reduced by almost 45% in null mice and 5% in heterozygous mice as compared to wild type mice, thereby resulting in increased TEWL and death soon after birth [35, 36].

The correlation between the chain length of ceramides and development of AD was first elucidated by Ishikawa et al., 2010 [37]. A reduced average chain length of ceramides in AD can be attributed to increases in C34 CER as well as a reduction in the ester linked omega hydroxy (CER[EO]) ultra-long chain subclasses. A human study showed within the C34 CER subgroup, subclasses [NH], [NS], [AS], and [AH] were found to be largely increased with [NH] and [NS] comprising 0.6% and 0.78% respectively of the total CER content in comparison to control in which both [NH] and [NS] comprises 0.18% of the total CER content. [AS] and [AH] in AD subjects were also found to comprise 2% and 0.5% respectively of the total CER content in contrast to healthy subjects where [AS] comprised

0.4% and [AH] comprised 0.2% of the total CER content [38]. Similar trends were also observed in mice skin with germline deletion of *Ctip2* (highly expressed in AD skin) and its homolog *Ctip1* [26, 27].

Ceramides, especially CER[NS] and CER[EOS], have been seen to be related to the packing of corneocytes in the SC. A study showed that after topical application of a formulation containing either CER[EOS] or CER[NS] to a skin barrier repair model, a higher fraction of lipids was found to be involved in assuming an orthorhombic organization compared to formulations without CER justifying the crucial role of CER[EOS] and CER[NS] in intact EPB formation [39].

ABHD5/CGI-58 is a protein involved in the regulation of an epidermal-specific lipase that liberates linoleic acid from a pool of triglycerides that is enriched in linoleic acid, making it available for transesterification to generate **acyl-ceramide** crucial to barrier formation (catalysed by PNLPA1). Mutation in the gene coding for ABHD5/CGI-58 results in Chanarin-Dorfman syndrome, characterized by dry scaly skin at birth similar to AD [40]. This further underscores the importance of epidermal CER and TAG metabolism in the generation of proper EPB and how their deficiency leads to AD.

## Variation in the FFA Content in AD

FFAs are an integral part of the SC lipid matrix. The epidermis is dynamically engaged in FFA biosynthesis and processing. The disruption of the permeability barrier results in a rapid and marked increase in fatty acid synthesis within the epidermal keratinocytes to restore the integrity of EPB [41, 42]. Barrier disruption increases the activities and mRNA levels of both of the key enzymes required for *de novo* fatty acid synthesis, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) [43]. Fatty acids being an important constituent serving towards EPB formation, reduced expression of fatty acid synthesizing enzymes such as elongases, increased expression of desaturases had been found to correlate with the phenotype of shortened fatty acyl chain length and increased level of unsaturated FFAs in lesions associated with AD. Studies by Malecheid et al., 2002 in human models reported an apparent reduction in FFA chain length in AD patients with short chain FFAs comprising 7.7% and 7.6% of the total FFA fraction in lesional and non - lesional AD skin, respectively, as compared to healthy subjects where the short chain FFAs comprised only 5.4% of the total FFA fraction [44]. FFAs are in general toxic for living cells. Therefore, they are not stored as FFAs in lamellar bodies. they are rather stored as phospholipids and these phospholipids get metabolized at the SG-SC interface with FA as one of their products. Studies have shown phospholipids normally are degraded completely, however they do persist in AD [45] validating the importance of phospholipid metabolism at SG-SC interface for potential release of FFAs in mediating proper barrier formation and how the lack of proper metabolism of phospholipids and their subsequent accumulation serves as a hallmark of AD.

In addition to a well-documented reduction in FFA chain length, there also appears to be distinct alterations in unsaturated FFA levels in AD. Recent reports have observed increase in unsaturated FFA levels including **monounsaturated fatty acid species (MUFAs)** in human AD skin from 20% in control to almost 30% [46]. Li et al., 2017 however reported a

large decrease in the MUFAs C16:1 and C18:1, which correlated to increased susceptibility to *Staphylococcus aureus* infection and barrier dysfunctions [47]. Thus, while a study in human models by Van smeden et al., 2014 showed an increase in the level of MUFAs in lesional atopic eczema patients as compared to non-lesional atopic eczema and healthy subjects, a 2017 human study by Li et al., showed that the decrease in the level of MUFAs serve as a driving factor leading to increased colonization by *S. aureus* and resulting impaired EPB. Given the contradictory nature of the two observations, a more extensive study needs to be performed to determine the role of MUFAs in AD. A parallel study by Mojumdar et al., 2014 using *in-vitro* constituted model SC membrane system mimicking human SC lipid composition and organization showed that increased prevalence of MUFAs disrupt the EPB by promoting hexagonal packing conformation as opposed to the stable orthorhombic packing conformation [48].

Experimental studies in the past have shown the crucial role of epidermal elongases in mediating the lipid composition changes characteristic of AD and have been listed below. Park et al., 2012 demonstrated that the protein levels ELOVL1 and ELOVL4 were significantly reduced in an Oxazolone induced murine model of AD by western blot analysis. Reduced expression level of elongases such as ELOVL1 and ELOVL4 impairs elongation of FA beyond C22 and hence the C22 and C16 CER levels showed an increase of 0.2 and 3-fold respectively with respect to control, while the C24 and C26 CER levels showed a decrease of 0.25 and 0.75-fold respectively with respect to control as observed from liquid chromatography-mass spectrometric (LC-MS) analysis [49]. Further study by Sassa et al., 2013 showed the part played by ELOVL1 and ELOVL4 in the production of less than C<sub>26</sub>-CoA and more than C<sub>28</sub>-CoA respectively thus establishing the role of epidermal ELOVL1 in connecting the synthesis of long or very long chain fatty acids (LCFA/VLCFAs) to ultra-long chain fatty acids (ULCFAs) crucial to barrier formation [50]. Enzymatic changes underlying FFA saturation levels have also been characterized in AD. Stearoyl CoA desaturase 1 (SCD-1) is responsible for the formation of unsaturated FFAs 16:1 and 18:1 with other SCDs introducing increasing levels of desaturation. A study showed that the mRNA expression level of Fatty acid desaturase 2 (*FADS2*) was double the expression observed in control [51]. Detailed quantification of protein level of *FADS2* and *SCD1* could help ascertain their role in AD.

RNA-seq analysis showed that type 2 inflammatory cytokines IL-4 and IL-13 inhibit the expression of *ELOVL3* and *ELOVL6* in human keratinocyte culture [13]. Besides, genetic factors influencing epidermal barrier homeostasis may also contribute to altered FFA metabolism. *Ctip1* and *Ctip2* are two such genes know to play important roles in EPB formation [26, 27]. Li et al., 2017 showed that germline deletion of the *Ctip1* in a murine model reduced the gene expression level of *Elov14* by 1-fold and ultimately reduced long chain FFA species in the SC [26]. Overall, FFA metabolism appears to be regulated by Th2-dependent inflammatory cytokines and genes related to barrier formation and maintenance. Current AD management strategies aim at employing anti-inflammatory or immunosuppressive agents to influence downstream SC FFA composition. Future strategies involving direct supplementation of deficient FFA species may serve useful in disease management (see Clinician's Corner).



## Changes in Cholesterol Content in AD

The epidermis is an active site of cholesterol synthesis [52]. There are two primary types of cholesterol found in the SC, cholesterol and cholesterol-3-sulfate. Past studies have shown that cholesterol synthesis is vital to maintaining the homeostasis of the human skin barrier [12]. Disruption to this balance can lead to TEWL and thereby AD.

There are also certain derivatives of cholesterol that are known to play an important role concerning EPB homeostasis. Oxysterols derived either via cholesterol oxidization through enzymatic processes, or as by-products of the cholesterol biosynthetic pathway play an important role in inducing epidermal differentiation and inhibiting proliferation for formation of intact epidermal barrier [53].

*CYP11A1* is yet another gene expressed in the skin involved in production of various derivatives of cholesterol. It is important for local glucocorticoid synthesis to attenuate the inflammatory response [54], and also plays a role in activation of vitamin D through noncanonical pathway [55–57] or activation of 7-dehydrocholesterol or lumisterols [57–59]. These issues serve as an important aspect owing to their ability to attenuate inflammatory responses and thereby can also contribute to the maintenance of an intact barrier. Lastly, there are various potential targets for sterols, lipids and secosteroids regulation including a wide range of nuclear receptors [60] or Vitamin D receptors [61] or retinoic orphan acid receptors [62, 63] or aryl hydrocarbon receptor [64].

The gene expression level of several enzymes involved in cholesterol biosynthetic pathway were found to increase in upper and lower epidermis following acute barrier disruption in a study concerning murine models. HMG-CoA Reductase (*Hmgcr*), HMG-CoA Synthase (*Hmgcs*), Farnesyl Pyrophosphate Synthase (*Fdps*), Squalene Synthase (*Fdft1*) were found to increase by approximately 1-fold, 1.5-fold, 0.8-fold and 0.9-fold respectively with respect to control upon acute barrier disruption thereby resulting in enhanced epidermal cholesterol synthesis, mediated via Sterol Regulatory Element Binding Protein (SREBP-1) and (SREBP-2) [43] (For details, see Fig. 4). These observations demonstrated the role of cholesterol in maintaining the integrity of EPB.

Li et al., 2017 found elevated levels of cholesterol-3-sulfate in AD patients and potentially identified it as a contributor to barrier disruption [47]. Recent studies in normal human epidermal keratinocytes (NHEK) have shown cholesterol-3-sulfate as an inducer of the skin barrier protein pro-filaggrin expression in the epidermal keratinocytes [65]. 10 $\mu$ M and 40 $\mu$ M of cholesterol-3-sulfate downregulated the mRNA expression level of pro-filaggrin by 0.66 fold and 0.86 fold, respectively, with respect to control in ROR $\alpha$  knocked down cells justifying the role of cholesterol-3-sulfate in inducing pro-filaggrin expression through increased expression of ROR $\alpha$ . However, these observations could not justify the role of cholesterol-3-sulfate as an effective barrier disruptor because increased expression of pro-filaggrin and its conversion to mature filaggrin protein are normally associated with maintained integrity of EPB [66, 67]. Possibility is such that even though increase in cholesterol-3-sulfate in AD patients have been found to be correlated with increased pro-filaggrin expression, maybe the pro-filaggrin protein fail to undergo maturation to the

filaggrin protein, and hence barrier breach results. Increased expression of cholesterol-3-sulfate in human AD skin positively correlated to colonization by *S. aureus* thereby further compounding the severity of the AD disease [47].

## Impact of Modified Triglyceride Composition on AD Development

The epidermis is an active site of triglyceride synthesis, but their role in EPB homeostasis is poorly defined. Diacylglycerol acyltransferase (*Dgat2*) expressed in the epidermis plays a crucial role in the acylation of the linoleoyl CoA containing diacylglycerol (DAG) to TAG [68]. *Dgat2* knockout mice showed markedly reduced content of linoleic acid-containing TAGs by a fold change of 0.97 with respect to control and hence reduced synthesis of acylceramides in the skin leading to disrupted EPB and demise soon after birth [68].

Other studies in murine models have also shown an additional role of triglycerides in regulating epidermal proliferation and barrier integrity through controlled calcium influx into epidermal keratinocytes [69].

Radner and Fischer noted that mutations in the gene coding for ABHD5/CGI-58 protein were linked with the degradation of triglycerides in the human skin [2] as is commonly observed in case of neutral lipid storage disorder. Another gene potentially connected with alteration of triglyceride levels in AD skin is filaggrin with a general increase in the number of triglycerides present in AD patients with enhanced filaggrin expression [66, 67, 70]. More specifically, Li et al., 2017 discovered that certain groups of triglycerides, namely 46:1, 48:1, 48:2, 50:1, 50:2, and 50:3 as well as 46:2 and 56:2, were altered in populations of AD patients compromised by *S. aureus* colonization [47]. Besides, increased TEWL was associated with decreased levels of triglyceride 46:2. A parallel study showed an increase in the triglyceride levels in group 52:2 by a fold change of 2.33 and a decrease in group 52:5 by a fold change of 0.5 in the skin of mice lacking transcription factor CTIP1/BCL11A that control lipid metabolism and barrier functions [26]. Thus, the accumulation of long-chain triglycerides in the SC could impact barrier functions and influence AD pathogenesis.

## Concluding remarks

Strategies to counteract AD is an area of extensive research. Initial treatment options included application of prescribed emollients and administration of immunosuppressants and anti-inflammatory drugs such as corticosteroids. However, they were non-specific and exhibited transient efficacy. Topical application of lipids as a treatment strategy could be a promising alternative in mild and moderate AD. These involve the use of either non-physiological lipids such as petrolatum, beeswax, etc. or alternatively lipids similar to the intrinsic pool of lipids present in the lamellar bodies. However, their mechanism of action are still unknown [71]. Yet other therapeutic strategies include using anti-IL-4/13 receptor antibody, anti-IL-13 antibodies in order to reduce the systemic Th2 inflammation reported in severe AD [72]. Additional studies using Omics approach to identify new potential candidate genes associated with the development of various endotypes of AD are necessary [73] (see Outstanding Questions). Addressing how filaggrin mutations affects epidermal lipid metabolism or the role of cholesterol-3-sulfate as a barrier disruptor, can help us gain



further insight into the factors governing the pathogenesis of AD and thereby enable us develop better therapies against AD.

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## Glossary

### Acyl-ceramide

Acyl-ceramide contain a long chain base and an N-acylated ultralong acyl chain with an additional hydrophobic linoleic acid and it serves an important role in the organization of lipid lamellae and skin barrier formation

### Epidermal Living Skin Equivalents (LSEs)

Co-culture of primary human keratinocyte and fibroblast cells within a collagen gel at the air liquid interface for almost ten days result in a model closely resembling the fully stratified human epidermis

### Genetic ablation

It involves modification of the original DNA sequence in order to disrupt production of a specific type of gene. It is used for knockout studies and is instrumental in determining the function of a particular type of gene

### HPA axis

The hypothalamic-pituitary- adrenal (HPA) axis is also referred to as the pivotal stress response system. This response is characterized by hypothalamic release of corticotropin-releasing factor (CRF). CRF in turn stimulates the release of adrenocorticotrophic hormone (ACTH). ACTH upon binding to receptors on the adrenal cortex stimulates release of cortisol. In response to stressors, cortisol continues to be released for several hours in the blood stream up to a certain blood concentration when protection is achieved and the cortisol then exerts negative feedback to the hypothalamic release of CRF and the pituitary release of ACTH (negative feedback) to bring back systemic homeostasis

### Lipidomics

It involves complete characterization of the entire lipid content of a particular species as well as large scale and comprehensive study of the various pathways involved in lipid metabolism and function as well as factors governing regulation of genes involved in those pathways

### Mono-Unsaturated Fatty Acid (MUFA)

Long Chain Fatty Acids bearing double bond at a single position within the chain. For example, C16:1 represents fatty acid with a chain length of 16 carbons and bearing a single double bond

### RNA-Seq

It is used to determine the presence and quantity of total cellular RNA (mRNA, rRNA and tRNA) present in the biological sample. It helps decipher the varying expression pattern of

the genes in tissue, time and context dependent manner and hence allows proper determination of the diseased state versus the control conditions

### **Sphingolipids**

This class of lipids comprise fatty acid derivatives of sphingosine (an amino alcohol) or other sphingoid bases, serving as essential component of biological membranes. The simplest form of sphingolipid is ceramide

### **Tape-Stripping**

Tape Stripping constitutes an essential methodology in the field of dermatological research to determine the physiology of the stratum corneum. Adhesive films are pressed to the surface of the skin and thereafter can be used for further investigations

### **TEWL**

Trans-epidermal water loss (TEWL) refers to the water naturally lost through the epidermal layer of the animal skin into the surrounding atmosphere via diffusive and evaporative processes

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**Box 1:****Diseases of Defective Epidermal Barrier Function**

Diseases such as AD, Lamellar ichthyosis (LI), psoriasis and Netherton syndrome (NTS) are all characterized by defective or weakened epidermal barrier functionality.

**Atopic Dermatitis**

Atopic Dermatitis, also known as eczema. It is a chronic, genetically inherited, multifactorial inflammatory skin disease characterized by dry, itchy and reddened skin [74].

**Lamellar Ichthyosis**

Lamellar Ichthyosis is a genetically inherited autosomal recessive condition that mainly affects the skin. It becomes apparent at birth and persists throughout life. People suffering from this condition show impaired permeability barrier function. Infants suffering are generally born with glossy, waxy layer of skin which undergoes shedding after the first two weeks of birth. Adults on the other hand show large, dark, plate-like scales covering majority of their skin along with hair loss, reddened skin, decreased sweating among other symptoms [75].

**Psoriasis**

Psoriasis is an autoimmune condition that is chronic in nature. It is caused by rapid buildup of skin cells that results in scaling on the skin surface. Symptoms include flaking, inflammation, and thick, white, silvery, or red patches of skin. Some common therapeutic strategies include topical application of steroid creams or administration of oral medication [76].

**Netherton Syndrome**

Netherton syndrome is an inherited autosomal recessive trait characterized by scaly skin, hair anomalies, increased susceptibility to atopic eczema (resulting in dry reddened and scaly skin) along with elevated serum IgE levels. It occurs due to mutation in the gene SPINK5 coding for a protein that acts as a brake on the activity of certain proteases in the skin. Excessive action of proteases such as kallikrein (KLK5/KLK7) results in few outer layers of skin and hence impaired permeability barrier function [77].

**Box 2:****Major Factors and Hypotheses Governing Atopic Dermatitis**

AD usually occurs as a part of “atopic triad” (including eczema, rhinitis and asthma) and is a multifactorial ailment associated with barrier disruption and severe immune irregularities leading to systemic inflammation [23, 74, 78–81]. Controversy remains regarding the primary cause of AD- (1) immune irregularities leading to chronic inflammation or (2) epidermal barrier breach. The “inside-out” hypothesis states that cutaneous inflammation precedes barrier disruption resulting in an impaired EPB. The “outside-in” hypothesis on the other hand states that skin barrier disruption results in increased cutaneous and systemic Th2 responses with TSLP being the potential activator of type 2 responses [82]. While some cytokines such as TNF- $\alpha$ , IFN- $\Gamma$  and IL-17 are indicators of chronic inflammation, others like IL-1 $\alpha/\beta$ , GM-CSF or IL-6 are homeostatic signals that can eventually reach the deeper layers of the skin versus the Th2 cytokines [83]. Inflammatory Th2 cells also predispose patients to the development of atopic triad. Recently a new “inside-outside-inside” hypothesis is suggested, which states that primary immune irregularities elicit secondary barrier disruption effects which can further result in activation of the type 2 immune responses [70, 84]. However, the pathways that are instrumental in driving AD remains to be identified.

Various factors operate through either of the hypotheses to promote the onset of AD as well as their progression. The two major factors include skin pH and stress. Elevated level of pH in the AD skin had been found to be correlated with increased activity of peptidases such as KLK5/KLK7 and concomitant PAR2 activation [85]. Following PAR2 activation, expression of inflammatory mediators predominantly TSLP promotes the recruitment of the Th2 cells to the site of inflammation [85, 86]. The production of cytokines such as IL-4, IL-5, IL-10 and IL-13 by the Th2 cells then serve as further determinants of AD progression [87]. PAR2 activity also gets regulated by NFK- $\beta$  pathway [88]. Environmental stress as well as emotional stress has also been shown to mediate its effect on the clinical manifestation of AD via neuroendocrine signalling pathways involved in aggravating or attenuating inflammatory responses through local production of CRF (Corticotropin Releasing Factor) and POMC peptides [89]. CRF or CRH (Corticotropin Releasing Hormone) acts as a major regulator of the **HPA (Hypothalamic-Pituitary-Adrenal) axis** and a coordinator of stress response. Neuroendocrine signalling is also known to show its effect on the inflammatory pathway through glucocorticoid, serotonin and melatonin production. Thus, the abundant expression of a cutaneous serotonergic/melatonergic system(s) can help counteract external and internal stresses to preserve the vital skin barrier function [90, 91].

**Box 3:****Major Pathways Involved in Sphingolipid Biosynthesis**

Ceramide comprise almost 50% of the total lipid component constituting SC [14]. The first and rate-limiting step in the *de novo* synthesis of ceramides is catalysed by the serine palmitoyl transferase (SPT) enzyme complex, which catalyses the condensation of palmitoyl-CoA and L-serine in ER to form 3-Ketosphinganine finally giving rise to ceramide. *De novo* synthesis of ceramide primarily occurs in the ER followed by its transfer to the Golgi apparatus by either vesicular trafficking or the ceramide transfer protein (CERT). They then get further metabolized to other **sphingolipids**, such as sphingomyelin and complex glycosphingolipids [92]. Hydrolysis of sphingomyelins forms ceramides in the sphingomyelinase pathway [93]. The salvage pathway involves constitutive breakdown of sphingolipids and glycosphingolipids in the late endosomes and the lysosomes, with the final aim of producing sphingosine. Ceramide can also undergo hydrolysis by acid ceramidases present in the lysosome to form sphingosine and FFA. The long-chain sphingoid bases upon release from the lysosome then re-enter pathways for synthesis of ceramide and/or sphingosine-1-phosphate catalysed by ceramide synthase family members accounting for almost 50% to 90% of sphingolipid biosynthesis [94]. Finally, the exogenous recycling pathway allows short chain ceramides to create sphingosine thereby leading to the synthesis of endogenous long chain ceramide species. *De novo* pathway is the predominant pathway while others serving as secondary ones, since ceramides need to be first incorporated in the derivatised compounds by prior functioning of this pathway [95]. The ceramide species thereafter undergo acylation with linoleoyl-CoA to form acyl-ceramides. Acyl-ceramides are glycosylated in the Golgi and with other lipids are packed into lamellar bodies [12]. The lamellar bodies, containing glycosylated ceramides, acyl ceramides, cholesterol, and VLCFAs, are secreted to the extracellular space of SC, where the ceramides become covalently linked to structural skin proteins such as involucrin, filaggrin, Small Proline Rich Proteins (SPR) to form the EPB [12, 14, 52] (see Fig. 1).

Though ceramides dominate by weight, they are no more important than other key lipids. Application of one or two of these lipids such as a mixture of only ceramide species or ceramide along with FFA to perturbed skin delays barrier recovery; only equimolar mixtures of all the crucial lipids allows normal recovery as measured by TEWL. This shows the crucial role of all four lipids in EPB formation irrespective of their relative abundance within the SC [96]. Drugs such as Epiceram serve as the most representative example of the above observation [97].

### Clinician's Corner

- Defects in epidermal lipid metabolism are strongly associated with AD and manifest in readily detectable changes in epidermal lipid composition. Strategies designed to restore barrier functions at the molecular level may be more effective than those designed to reduce inflammation such as corticosteroids. That approach can involve supplementing the skin with lipid-rich formulations designed to restore proper SC lipid composition and ultimately strengthen EPB integrity.
- Preventative reinforcement of the EPB may prove useful in AD management. Treatment of neonates with topical moisturizers has demonstrated a marked reduction in AD prevalence [98]. Such studies underscore the shifting paradigm of EPB integrity, rather than aberrant Th2-mediated inflammation, being the most important determinant in AD development. Accordingly, future treatment strategies should be designed to reflect this paradigm shift.
- Topical application of lipids can improve EPB homeostasis and has been useful against AD. Such strategies involve treatment with non-physiologic or physiologic lipid species. Although non-physiologic lipid treatment quickly restores barrier function, they can also inhibit normal barrier repair mechanism and thus not correct the underlying abnormality. Appropriate molar mixes of physiologic lipids, i.e. those containing CER, FFA, CHOL and TAG, can improve EPB homeostasis. Incomplete or misbalanced mixtures of lipids can negatively affect EPB. This underscores the need to develop more personalized and precise lipid formulations [96].
- Therapies designed to modulate epidermal lipid enzyme activity could be employed to restore proper healthy lipid composition. This is especially relevant to changes in AD profile involving increased amounts of lipid classes (*e.g.* increased CER-34, CER-NS, and CER-AS) where levels cannot simply be supplemented by topical lipid application. Topical application of acetyl CoA carboxylase activator and/or fatty acid synthase activator can also increase the rate of de-novo fatty acid synthesis resulting in rapid recovery of barrier functions. Similarly, application of activators involved in stimulating various enzymes involved in CER, FFA, CHOL and TAG synthesis pathway can result in rapid restoration of the EPB integrity.
- Lipidomic and biophysical techniques have been used to rigorously evaluate the effects of a topical formulation on epidermal lipid composition. Such knowledge of AD biomarkers may allow the development of personalized topical treatments optimized to remediate a patient's lipidomic profile [99]. Given the heterogeneity in AD phenotype, such personalized approaches may represent the most effective means of treating AD.

- Finally, it should be also noted that treating immune abnormality improves, but does not normalize barrier function, favoring ‘outside-in’ model - disease inevitably rebounds.

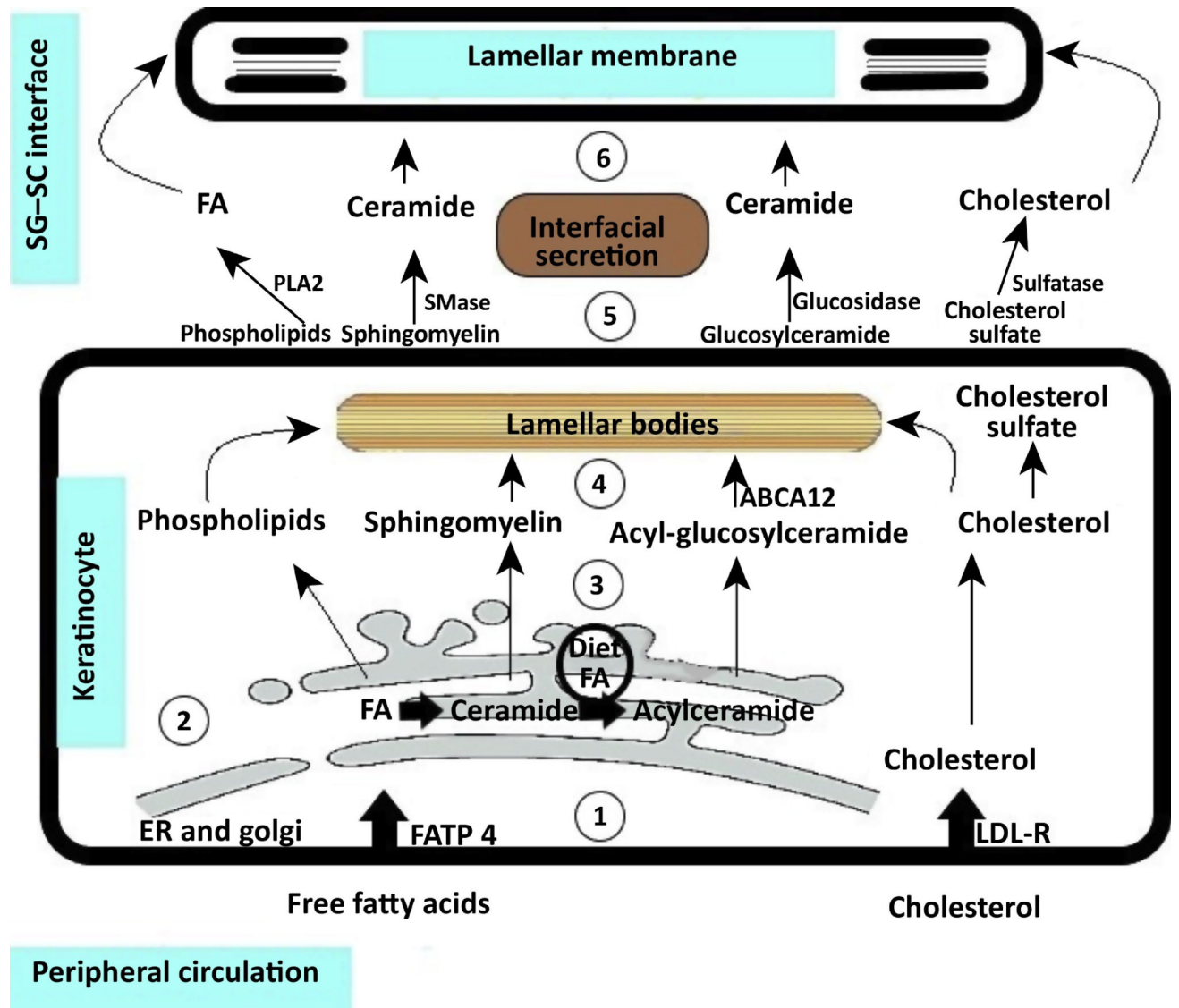
### Outstanding Questions

- How *FLG* mutations affects epidermal lipid metabolism? Multiple lines of evidence suggest *FLG* mutations do not correlate with SC lipid composition. However, there is some correlation with *FLG* metabolites, namely natural moisturizing factors, and SC lipid composition which suggests the presence of a common factor influencing both.
- Will therapies aimed at solely restoring barrier function improve antimicrobial defenses in AD? It is currently unknown whether simply reestablishing a healthy SC lipid profile is sufficient to prevent microbial infiltration.
- Could FLG replacement represent a legitimate AD management strategy? One such study suggested that topical application of FLG metabolites might be used to reduce AD symptoms. Although such therapies are likely to have an effect on SC lipid content/organization, it is thought that such a treatment would nonetheless improve overall barrier functions.
- It is unclear whether epidermal barrier abnormalities are drivers of AD or consequences of Th2 mediated inflammatory immune response. Likewise, it is not clear whether skin inflammation is a source of, or response to a damaged barrier.
- AD patients are characterized by elevated levels of cholesterol-3-sulfate, which was shown to be an inducer of pro-filaggrin gene. Increased level of pro-filaggrin should correlate with increased level of mature filaggrin and hence restore barrier integrity. However, that does not justify the role of cholesterol-3 sulfate as a barrier disruptor. How it directs the disruption of EPB as observed in AD? Is it via a filaggrin mediated pathway where even though there is increased level of pro-filaggrin, they fail to undergo maturation to filaggrin? Or it acts via a filaggrin-independent pathway to act as barrier disruptor? What specific types of inhibitor can help us target its role as a barrier disruptor and hence as a therapy for AD?



### Highlights

- An elaborate interaction of extrinsic and intrinsic factors plays an important role in the outcome of AD. Such factors include immune irregularities, genetic level changes, epigenetic alterations such as methylation and histone modifications, the microbiome, the environmental stimuli, the age as well as the ancestry of the subject.
- Changes in epidermal lipid metabolism are associated with AD and lipidomic tools such as LC-MS/MS have revealed dramatic changes in lipid composition with respect to AD skin in rodents and humans.
- Global analysis of the various biomarkers associated with the varied endotypes of AD involving genomics, epigenomics, proteomics, metabolomics, transcriptomics, lipidomics, microbiomics and exposomics can provide us with new biological insights into the etiology of AD as well as help us detect new AD associated genes and pathways.



Trends in Molecular Medicine

**Fig 1. Major lipids constituting epidermal permeability barrier (EPB).**

(1) The epidermis can uptake free fatty acids from the peripheral circulation via FATP4 as well as can uptake cholesterol from the peripheral circulation in the form of LDL via the LDL-Rs. (2) In the epidermis, free fatty acids play an important role in the synthesis of ceramides and acyl-ceramides in the endoplasmic reticulum (ER) followed by their conversion to phospholipids, sphingomyelin and acyl-glucosylceramide in the cytosol. The epidermis also serves as an active site for synthesis of cholesterol (3) The phospholipids such as sphingomyelin, acyl-glucosylceramide formed from ceramide and cholesterol inside epidermal keratinocytes of the granular layer undergo processing through the trans-golgi network. (4) They get packaged in the lamellar bodies (5) and get released into the extracellular space from the upper differentiated layers (Stratum granulosum) of the skin epidermis at the interface of the granular and the cornified layer. (6) The secreted lipids then contribute to the formation of the lamellar membrane and thus the EPB. Phospholipase A2

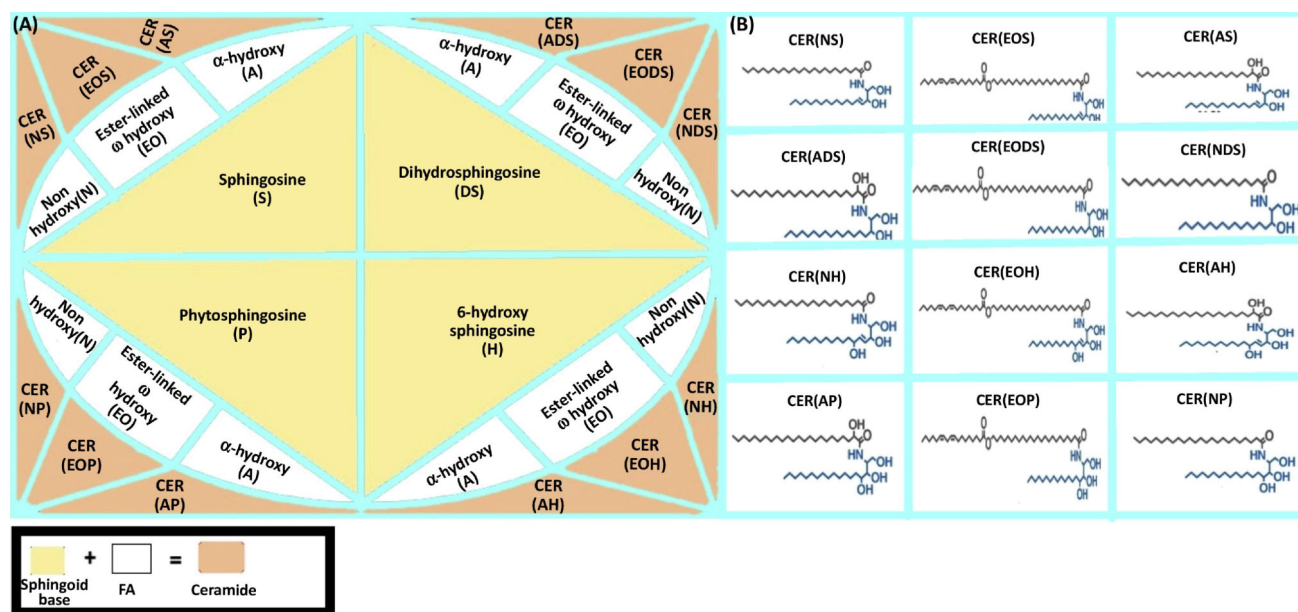
(PLA2); Sphingomyelinase (SMase); Low Density Lipoprotein (LDL); Low Density Lipoprotein Receptor (LDL-R); Fatty Acid Transport Protein 4(FATP 4); ATP Binding Cassette Sub-Family A Member 12 (ABCA12).

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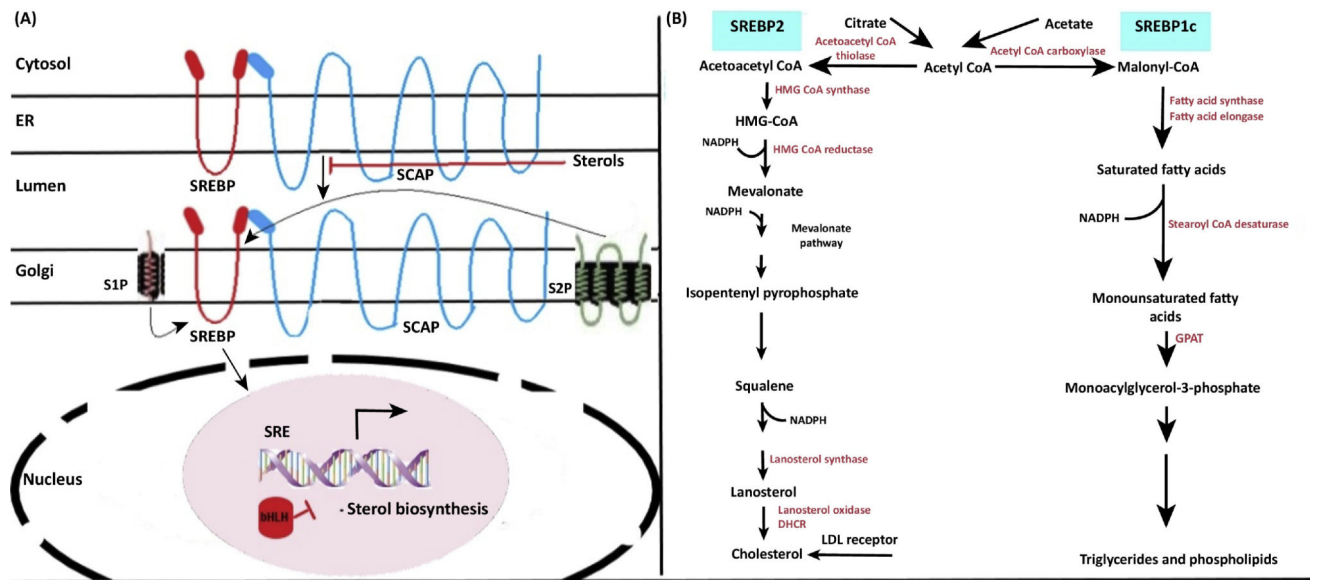
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**Fig 2. Classification of ceramides and their subclasses.**

Three types of fatty acid namely Non-hydroxy (N), Ester-linked  $\omega$ -hydroxy (EO) and  $\alpha$ -hydroxy (A) combine with four different types of sphingoid base namely Sphingosine (S), Dihydro-sphingosine (DS), Phyto-sphingosine (P) and 6-hydroxy sphingosine (H) to give rise to twelve classes of ceramides. The ceramide classes being abbreviated as CER(NDS), CER(NS), CER(NP), CER(NH), CER(EODS), CER(EOS), CER(EOP), CER(EOH), CER(ADS), CER(AS), CER(AP) and CER(AH). The structures representing different ceramide classes are depicted in the right panel.





Trends in Molecular Medicine

**Fig 4. SREBP mediated regulation of genes involved in synthesis of cholesterol, fatty acids and triglycerides.**

The left panel depicts sterol activated SCAP (SREBP Cleavage Activating Protein) mediated transport of SREBP (Sterol Regulatory Element Binding Protein) from ER (endoplasmic reticulum) to the Golgi Apparatus where two site specific proteases S1P (site-1-protease) and S2P (site-2-protease) stimulate the release of N-terminal bHLH domain from the membrane. The bHLH domain upon being directed to the nucleus bind to the sterol response element (SRE) upstream of the target genes thereby stimulating transcription of genes involved in sterol biosynthesis. Abundance of sterol moieties inhibits this process. The right panel shows the role of isoforms SREBP-2 and SREBP-1c in regulating the fatty acid biosynthetic pathway and cholesterologenesis respectively when expressed at normal cellular levels. Expression at levels higher than normal allows both the isoforms to govern activation of all the enzymes involved in both fatty acid biosynthesis as well as in synthesis of cholesterol. Glycerol-3-Phosphate Acyltransferase (GPAT); 7-Dehydrocholesterol Reductase (DHCR).