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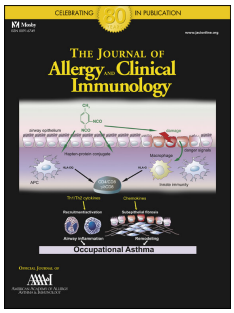

Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the atopic march

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PII: S0091-6749(16)30498-5

DOI: 10.1016/j.jaci.2016.06.002

Reference: YMAI 12181

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 15 February 2016

Revised Date: 8 June 2016

Accepted Date: 13 June 2016

Please cite this article as: Egawa G, Kabashima K, Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the atopic march, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.06.002.

Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the atopic march

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Supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Disclosure of potential conflict of interest:
The authors declare that they have no relevant conflicts of interest.

Key words:
atopic dermatitis, barrier function, stratum corneum, corneocytes, cornified envelope, tight junction, filaggrin, lipid

Abbreviations used:
AD: atopic dermatitis, ARCI: autosomal recessive congenital ichthyosis, CE: cornified envelope, FLG:filaggrin gene, KLK: kallikrein, NMF: natural moisturizing factor, PCA: pyrrolidine carboxylic acid, SC: stratum corneum, SG: stratum granulosum, SPR: small proline-rich protein, TG: transgultaminase, TJ: tight junction, UCA: urocanic acid

Abstract

Atopic dermatitis (AD) is the most common inflammatory skin disease in the industrialized world, and has multiple etiologies. Over the past decade, data from both experimental models and patients have highlighted the primary pathogenic role of skin barrier deficiency in AD. Increased access of environmental agents into the skin results in chronic inflammation and contributes to the systemic “atopic (allergic) march”. In addition, persistent skin inflammation further attenuates skin barrier function, resulting in a positive feedback loop between the skin epithelium and the immune system that drives pathology. Understanding the mechanisms of skin barrier maintenance is essential for improving management of AD and limiting downstream atopic manifestations. In this article, we review the latest developments in our understanding of the pathomechanisms of skin barrier deficiency, with a particular focus on the formation of the stratum corneum, the outermost layer of the skin, that contributes significantly to skin barrier function.

The skin covers the entire body and protects us from the external environment. When this barrier is impaired, external toxins are able to penetrate the skin and induce inflammation. Over the last decade, numerous studies have demonstrated that skin barrier dysfunction is a critical component of atopic dermatitis (AD).[1-3] In particular, inherited defects in epidermal barrier proteins facilitate the interaction of external antigens with skin-resident immune cells, driving local inflammation that can also lead to systemic immune responses. This is the "outside-in" hypothesis of AD pathogenesis, and it helps to explain the increased risk AD sufferers have of developing food allergies, asthma and allergic rhinitis later in life, the progression to which is known as the "atopic (allergic) march".[4] In addition, it is now evident that this secondary immunologic activation results in further attenuation of the skin barrier, which further exacerbates inflammation and allergic sensitization to environmental allergens.[5] These observations suggest that maintaining the skin barrier function is important for both the effective management of AD and preventing the development of subsequent allergic diseases.

In this article, we summarize how the physical barrier of the skin is organized and review its link to AD pathogenesis. This article does not cover chemical and biological skin barriers (such as the skin acid mantle, antimicrobial peptides and bacterial flora) or the immune cell-mediated skin barrier function. Reviews covering these aspects can be found elsewhere.[6-8]

DEVELOPMENT OF THE STRATUM CORNEUM

The barrier function of the skin is largely dependent on the stratum corneum (SC), the outermost layer of the epidermis (**Fig 1A and B**). The SC is formed during the course of a tightly regulated processes of keratinocyte differentiation called cornification.[9]

Cornification is achieved by keratinocytes passing through four cell layers of the epidermis: the stratum basale, the stratum spinosum, the stratum granulosum (SG), and the SC (**Fig 1B**). In the SG, keratinocytes start to produce two membrane-circumscribed granules: keratohyalin granules and lamellar bodies. Keratohyalin granules contain intracellular components of the SC (such as filaggrin [FLG], loricrin, and keratin filaments), whereas lamellar bodies contain extracellular components (such as lipids, corneodesmosin and kallikreins). In the SC, keratinocytes become flattened and denucleated (which are then called corneocytes), while their membranes are replaced by a specific barrier structure known as the cornified envelope (CE) (**Fig 1C and D**). At the transition from the SG to the SC, lamellar bodies are secreted into the intercellular space between the corneocytes and fill with lipids. These structures are often described

as bricks (corneocytes) and mortar (intercellular lipids), which together provide a highly hydrophobic barrier against the environment.

Below, we describe the formation of the SC barrier in terms of the following five categories, and review their link to AD pathogenesis: 1) FLG metabolism; 2) the cornified envelope; 3) intercellular lipids; 4) the corneodesmosome; and 5) corneocyte desquamation. The genes involved in each process are listed in **Table 1**.

FILAGGRIN METABOLISM

FLG and its metabolites are critical for normal cornification (**Fig 2**).[10, 11] In the SG, FLG is produced as a polymer (profilaggrin) of 10-12 linked repeats of FLG monomer, stored in keratohyalin granules. At the transition from the SG to the SC, profilaggrin is cleaved to generate FLG monomers by proteases such as CAP1/Prss8 and SASPase/ASPRV1.[12, 13] FLG monomers bind to keratin filaments and this keratin-FLG bundle is a fundamental structure within the corneocyte. At the upper layer of the SC, FLG becomes dissociated from the keratin filaments. In this process, the citrullination of FLG and keratin1 by peptidylarginine deiminase is considered essential.[14] The released FLG monomers are degraded to free amino acids, including glutamine, arginine and histidine, which are then converted into urocanic acid (UCA) and pyrrolidine carboxylic acid (PCA). This process is mediated by the proteases caspase14, calpain1 and bleomycin hydrolase.[15, 16] UCA is an important ultraviolet-absorbing chromophore in the SC and contributes to maintaining the acidic pH of the skin.[17] In contrast, PCA is a major constituent of natural moisturizing factors (NMFs), which are responsible for retaining water in the SC. Therefore, FLG and its metabolites assume a manifold role in the barrier function of the SC.

Gene targeting studies have revealed that FLG-deficient mice exhibit reduced SC barrier function with enhanced susceptibility to environmental sensitization.[18] Further, on a proallergic BALB/c background, FLG-deficient mice develop spontaneous dermatitis.[19] Likewise, the mice that have defect in profilaggrin processing (CAP1-deficient mice[12]/ SASPase-deficient mice[13]) or filaggrin processing (CASP14-deficient mice[15]) exhibit impaired skin barrier and/or dehydration of the SC, suggesting that the FLG metabolic process is also important for the development of intact SC barrier.

In humans, loss-of-function mutations in the *FLG* gene are associated with the development of AD as well as with ichthyosis vulgaris, a skin disorder with similarly dry and scaly skin.[1, 20] The prevalence of *FLG* mutations in AD patients ranges from

25-50% in Northern European and Asian populations.[21, 22] In addition, genome-wide association studies (GWAS) of individuals with European, African, Japanese and Latino ancestry have identified more than 30 risk loci for AD to date (**Supplementary Table 1**), and among them, the mutation in *FLG* has proven to be the consistent risk factor.[23] These observations indicate a major contribution of FLG-deficiency in AD pathogenesis. Manifestations of AD might also be influenced by FLG metabolic processes, since the mutations in *ASPRV1*, encoding SASPase, have been linked to the development of human AD.[13]

Although mutations in *FLG* are common in Northern European and Asian subjects, *FLG* mutations are less common in Southern Europe[24] and are even absent in some African countries.[25, 26] A recent study showed that the expression of another skin barrier protein, FLG2, is reduced in the epidermis of AD patients.[27] Further, a nonsense mutation in the *FLG2* gene was shown to be associated with persistent AD in patients of African ancestry.[28] The biological function of FLG2 remains to be elucidated, but its structure, pattern of expression, and biological properties are very similar to FLG. Therefore, FLG2 likely also plays an important role in skin barrier integrity. We must also note the possibility that FLG deficiency might be compensable under a tropical climate.[29]

FORMATION OF CORNIFIED ENVELOPE

The cornified envelope (CE) is a specific barrier structure formed beneath the cell membrane of corneocytes (**Fig 1D**).[30] The CE consists of highly crosslinked insoluble proteins anchored by extracellular lipids. This structure acts as a vital physical barrier to the SC.

The assembly of the CE starts in the upper layer of the stratum spinosum. In response to elevated intracellular Ca^{2+}, keratinocytes produce envoplakin, periplakin and involucrin. Envoplakin and periplakin form heterodimers that, together with involucrin, accumulate beneath the plasma membrane.[31] These three proteins become crosslinked to each other by transglutaminase (TG) 1 and TG5.[32] Involucrin acts as a scaffold of the CE, while the plakin dimers are binding sites for keratin filaments, enabling them to be combined with desmosomal proteins. Importantly, since plakin proteins are tightly crosslinked to the involucrin scaffold, desmosomes and keratin filaments are rigidly linked in the CE, thereby conferring mechanical stability to the corneocyte.

In the SG, loricrin and small proline-rich (SPR) proteins are produced. These proteins are crosslinked by TG3 and translocate to the cell periphery, where they are crosslinked to the involucrin scaffold by TG1 and TG5.[33] This process is repeated many times over,

resulting in a heavily reinforced CE in which up to 80% of the protein consists of loricrin. TG1 also combines extracellular ceramide lipids onto the involucrin scaffold until, eventually, ceramides replace the lipid bilayer of the plasma membrane.[34] This process is described in greater detail below.

Despite the ubiquitous presence of involucrin, envoplakin and periplakin in the CE (**Fig 1D**), single knockout mice of these genes do not show any obvious skin abnormalities.[35-37] Mice that lack all three of these proteins exhibit abnormal CE formation with reduced lipid content and decreased mechanical integrity, but skin barrier function remains intact, possibly compensated for by reduced desquamation of corneocytes.[38] Similarly, loricrin-deficient mice exhibit only a subtle phenotype, with shiny skin at birth and reduced CE stability.[39] These studies suggest that CE proteins are redundant, and indicate the existence of strong compensatory mechanisms. In accordance with this notion, no mutations in the genes of CE components have been linked to AD pathogenesis thus far. In contrast, the CE is abnormal or even absent with TG1-deficiency, in which severe ichthyosiform erythroderma (autosomal recessive congenital ichthyosis [ARCI]-1) develops.[40] In addition, TG5 deficiency causes peeling skin syndrome 2, which presents as superficial acral skin peeling, occurring at the junction between the SG and the SC.[41] These phenotypes indicate the non-redundant role of TGs in the formation of CE; however, there does not appear to be any association between genetic mutations in TGs and AD susceptibility.[42]

FORMATION OF INTERCELLULAR LIPID LAMELLAE

The intercellular lipids (the “mortar”) are an integral component of the SC barrier. They consist of a heterogeneous mixture of ceramides, free fatty acids and cholesterol in a roughly 1:1:1 molar ratio. These lipids are produced in the SG and stored in lamellar bodies, which are subsequently secreted into the extracellular space during the transition to the SC.

In the ceramide fraction alone, over 300 distinct species have been identified in human SC.[43] Among them, omega-hydroxyceramide is indispensable, as it is conjugated to the involucrin scaffold by TG1 and covers the surface of each corneocyte (**Fig 1D**). Using this ceramide as a template, multiple sheets of lipid lamellae are formed in the intercellular space between corneocytes.[44]

Several defects in ceramide-processing enzymes have been linked to the etiology of barrier-deficient skin diseases. 12R-lipoxygenase (encoded by the *ALOX12B* gene) and epidermal lipoxygenase-3 (encoded by the *ALOXE3* gene) are both essential for the

generation of omega-hydroxyceramide.[45] Defects in these enzymes causes congenital ichthyosis (ARCI-2, and ARCI-3, respectively).[46] The skin manifestations of ARCI-2 and ARCI-3 are less severe than those of ARCI-1, probably because the protein layer of the CE is still formed in these diseases.

The transmembranal transport of lamellar bodies is conducted by a lipid transporter called ATP-binding cassette subfamily A member 12 (ABCA12).[47] Mutations of this gene result in moderate (ARCI-4A) to severe (ARCI-4B, also known as harlequin ichthyosis) congenital ichthyosis, suggesting that the contents of lamellar bodies play an essential role in cornification. Recently, transmembrane protein 79/mattrin (Tmem79/Matt) was identified to be involved in the secretion of lamellar body contents.[48, 49] Tmem79 is a five-transmembrane protein that is localized to lamellar bodies, and Tmem79-deficient mice exhibit spontaneous dermatitis with elevated serum IgE, which resembles human AD. Further, a meta-analysis of AD patients revealed that a missense mutation of the *TMEM79* gene has a small, but significant, association with AD.[49] This suggests that abnormalities in lamellar body function, and/or intercellular lipid layer dysformation, contributes to barrier deficiency in some AD patients.

STRUCTURE OF CORNEODESMOSOME

The adhesion of corneocytes to one another is dependent on the desmosome apparatus, called the corneodesmosome (**Fig 1C and D**). The desmosome is composed of three protein families: desmosomal cadherin, armadillo proteins, and plakins. In the corneodesmosome, desmoglein 1 and desmocollin 1 (members of the cadherin family) interact with plakoglobin and plakophilins (armadillo proteins), which attach to envoplakin and periplakin (**Fig 1D**). As described above, envoplakin and periplakin heterodimers are crosslinked to the involucrin scaffold to bind keratin filaments. The corneodesmosin is another important modulator of corneodesmosomal adhesion.[50] It is stored in the lamellar bodies and secreted into the intracellular space of the SC to interact with cadherin proteins and support their adhesion.

Abnormalities of the corneodesmosome make the skin prone to hyper-desquamation (peeling) of the corneocytes, which may lead to skin barrier defects and inflammation. A recent study revealed that the homozygous mutation of desmoglein 1 results in severe dermatitis (erythroderma) accompanied by palmoplantar keratoderma, hypotrichosis, and increased serum IgE (EPKHE, also known as severe dermatitis, multiple allergies, and metabolic wasting [SAM] syndrome).[51] Importantly, EPKHE patients often have multiple food allergies. In contrast, deficiency in corneodesmosin causes peeling skin

syndrome 1, which is characterized by dermatitis, severe pruritus, food allergies, repeated episodes of angioedema and urticaria, asthma, and increased serum IgE.[52] Since these corneodesmosome-deficiency-oriented diseases share several clinical features of AD, this deficiency may also contribute to AD pathogenesis; however, this remains to be demonstrated.

CONREOCYTE DESQUAMATION

At the surface layer of the SC, corneocytes are constantly shed. This phenomenon is called desquamation and it is an important aspect of SC homeostasis. Corneocyte desquamation is mainly regulated by a proteolytic cascade of kallikrein (KLK)-related peptidases, such as KLK5, KLK7 and KLK14.[53] The activity of these proteases is pH-dependent and is enhanced when the pH in the SC is elevated. Their activity is also strictly regulated by a cocktail of protease inhibitors, including lymphoepithelial Kazal-type 5 serine protease inhibitor (LEKTI) encoded by serine protease inhibitor Kazal-type 5 (*SPINK5*).[54] KLKs and LEKTI are stored in lamellar bodies and secreted into the intercellular space at the SG-SC interface.

In AD patients, the skin surface pH is increased, at least in part due to the decreased production of UCA derived from FLG (**Fig. 2**).[55] As such, KLK activity is often enhanced in the AD skin. This condition is thought to induce an adverse effect on the SC barrier through multiple mechanisms (**Fig 3**). Firstly, KLKs cleave corneodesmosomal cadherins to promote corneocyte desquamation. Secondly, KLKs activate protease-activated receptor (PAR)-2, a G-protein-coupled receptor on keratinocytes. Upon activation, PAR-2 signals lead to suppression of lamellar body secretion via the downregulation of lipid-processing enzymes.[56] Finally, activated KLKs increase the generation of interleukin (IL)-1α and IL-1β, whose preforms are abundantly stored in the cytosol of corneocytes. Indeed, IL-1 cytokines are increased in the SC of AD patients and their enhanced production is associated with FLG deficiency.[57]

Two genetic polymorphisms that result in increased KLK activity have been linked to AD pathogenesis: gain-of-function mutations in *KLK7*, and loss-of-function mutations in *SPINK5*. A 4bp insertion polymorphism of *KLK7* was first reported to be associated with AD in the UK,[58] however this was not replicated in a French study.[59] *SPINK5* is known as the gene responsible for Netherton syndrome, in which patients display a broad range of allergic manifestations, such as AD-like dermatitis, food allergies, asthma, hay fever and markedly elevated serum IgE levels.[60] A significant association

between polymorphisms in *SPINK5* and AD has been found in the UK and Asian populations,[61-63] but not in the French population.[59] Further, a single nucleotide polymorphism in the gene encoding PAR-2 (*F2RL1*) has been associated with AD in the Korean population.[64] This mutation is thought to increase the stability of *F2RL1* mRNA transcripts. These studies suggest that the congenital mutations in protease activity in SC are linked to AD pathogenesis in specified populations.

TIGHT JUNCTION IN AD PATHOGENESIS

In addition to the SC, tight junctions (TJs) are structures that are essential to the integrity of the skin barrier. In the skin, TJs seal adjacent keratinocytes in the SG (**Fig 1B**) and act as a barrier for water and solutes.[65] TJs are composed of transmembrane proteins, particularly the claudin and occludin families, and several cytosolic scaffold proteins, including zonulae occludens (ZOs). The indispensable role of TJs in skin homeostasis was first demonstrated using claudin1-deficient mice, which die within 24 hours of birth from severe dehydration.[66] Importantly, these mice had no abnormalities in the production of SC components. A recent study using an AD model in mice showed that the expression of TJ proteins was suppressed during skin inflammation but was not affected by FLG deficiency.[67]

In humans, *CLDN1* (encoding Claudin 1) expression is reduced in non-lesional skin of AD patients, and an association between *CLDN1* polymorphisms and AD susceptibility has been reported.[68] These observations suggest that an impairment in TJs contributes to the barrier dysfunction observed in AD patients. Since most of the skin is covered with the SC, TJs appear to act as a second line of defense against external pathogens; however, TJs are likely to act as the primary barrier structure in skin appendages lacking SC, such as hair follicles and sweat glands (**Fig 1A**). Indeed, it is well known that hair follicles are important "shunt routes" into the skin for drugs and chemicals.[69] In accordance with this notion, widespread eruptive infections with *herpes simplex virus* or *molluscum contagiosum virus*, which enter the body through hair follicles, often occur as a complication of AD.[70, 71] These observations suggest that such skin appendages are the "security holes" of the skin, particularly in AD patients with TJ deficiency.

IMMUNOLOGICAL MODULATION OF SKIN INTEGRITY

Accumulating evidence suggests that immune cells influence skin integrity through the

304 production of cytokines.[72, 73] Although the complex inflammatory cascade that drives
305 AD skin lesions remains incompletely understood, multiple lines of evidence strongly
306 suggest that AD immunopathogenesis is driven by a Th2 cell-skewed immune
307 response.[74] This is further supported by recent clinical trial data demonstrating that
308 blocking the signalling from the IL-4 and IL-13, the two major 'type 2' cytokines,
309 ameliorates AD**.**[75] Previous studies have shown that IL-4 and IL-13 downregulate the
310 production of: 1) FLG and keratins; 2) the CE components (loricrin and involucrin); 3)
311 cell adhesion molecules (desmogleins, ZO-1); and 4) ceramide lipids. IL-31, another
312 Th2 cell-derived cytokine, also downregulates FLG expression.[76] Interestingly, a recent
313 study has shown that IL-33, an alarmin that is abundantly produced in the epidermis of
314 AD patients, has the potency to downregulate FLG expression as well.[77]
315 The physiological role for this adverse skin response to type 2 cytokines remains
316 unclear, but may have evolved to facilitate anti-parasite responses and/or wound healing.
317 However, in the context of AD, this 'type 2' immune response creates an exacerbation
318 loop between the inherited barrier deficiency and immune dysregulation, resulting in the
319 chronic, persistent skin inflammation that can only be alleviated by
320 immunosuppression.
321
322
323 **BARRIER DEFICIENCY AND THE DEVELOPMENT OF ALLERGIC**
324 **DISEASES**
325 It is now evident that epicutaneous antigens are strong sensitizer of allergic disorders.
326 Mouse studies have demonstrated that food allergy and asthma can be induced via
327 epicutaneous sensitization and are enhanced under disrupted skin barrier.[78-80] In human,
328 sequential acquisition of allergic diseases (atopic march) are frequently observed in both
329 AD and some genodermatoses, such as Netherton syndrome (mutation in *SPINK5*),[81]
330 peeling skin syndrome 1 (*Corneodesmosin*)[82] and SAM syndrome (*Desmoglein1*)[51]
331 (**Table 1**, asterisks), which strongly suggests that skin barrier deficiency contributes to
332 the development of atopic march. Eosinophilic esophagitis is another chronic immune
333 disorder that is associated with hypersensitivity to food, and has recently been linked to
334 the mutations in *Calpain 14* (*CAPN14*), a protease specifically expressed in the
335 esophagus.[83] An in-vitro expriment showed that overactivation of CAPN14 results in
336 loss of Desmoglein1. [84] These studies demonstrate that barrier deficiency in mucosal
337 epithelium may also contribute to the induction of allergic disorders. Importantly, recent
338 clinical trials have shown that epicutaneus antigen exposure induces sensitization while
339 oral antigen consumption induces immune tolerance.[85, 86]

In the presence of barrier defects in the SC, foreign antigens readily penetrate into the epidermis and activate innate immune receptors and pattern recognition receptors. This results in the production of Th2-promoting cytokines, such as IL-33, IL-25 and thymic stromal lymphoproteins (TSLP), which are produced by skin resident cells. Animal studies have demonstrated an essential role for TSLP in the epicutaneous induction of food allergy with AD-like skin lesions. Increased TSLP in the epidermis elicits the accumulation of basophils into the skin that promote Th2-cytokine responses.[80] In addition, TSLP signaling on epidermal Langerhans cells may be important for IgE production during the epicutaneous sensitization to food allergens.[87]

CONCLUSION –TOWARD THE BETTER MANAGEMENT OF AD

Skin barrier deficiency and excessive immune responses are two sides of the same coin in AD pathogenesis, and the inflammatory response is both precipitated by and maintained by barrier dysfunction. Thus, while therapeutic intervention in AD typically targets the inflammation though the use of immunosuppressive drugs, it is the maintenance of skin barrier function that is the key to effective management of AD. Recently, two groups investigated whether protecting the skin barrier with a moisturizer during the neonatal period prevents the development of AD.[88, 89] They reported that moisturizer treatment at an early stage of life resulted in 32 to 50% less AD prevalence. These results suggest that reinforcing the skin barrier function in the neonatal period is a promising strategy for the prevention of AD and epicutaneous sensitization to environmental allergens.

FLG replacement therapies have also been proposed. These include: 1) Use of "read-through" drugs, which may enable keratinocytes to skip the nonsense mutation of the *FLG* gene; 2) drugs that enhance FLG production; and 3) topical application of FLG metabolites, such as UCA and PCA.[90] Read-through drugs, such as gentamicin and PTC124 (Ataluren), are currently being trialed for other genetic diseases.[91, 92] A number of drugs have been proposed to enhance FLG production *in vitro*, or in animal models, including agonists of peroxisome proliferator-activated receptors (PPARs),[93] a serine-rich diet,[94] apigenin,[95] JTC801,[96] JTE-052,[97] and urea.[98] However, the efficacy of these strategies in AD remains to be tested, and may only apply to patients with heterozygous, but not homozygous, *FLG* mutations. Intensive research efforts to identify promising candidates that enhance skin barrier function is ongoing and is expected to lead to better management of AD in the near future.

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FIGURE LEGENDS:

FIG 1: Barrier structures of the skin. **A**, The skin consists of three layers: the epidermis, the dermis, and subcutaneous adipose tissue. Red arrowheads identify the pores of hair follicles and sweat glands. **B**, The structure of the epidermis. The red line represents tight junctions. **C**, The "bricks and mortar" structure of the SC. **D**, The structures of the CE and corneodesmosome.

FIG 2: Schema of the FLG metabolic process. In the SG, profilaggrins are stored in keratohyalin granules and then cleaved into FLG monomers. FLG monomers bind to keratin filaments in corneocytes. At the upper layer of the SC, FLG monomers are released from keratins and cleaved into free amino acids, followed by conversion into PCA and UCA. Asterisks denote the genes whose mutations have been linked to the AD pathogenesis.

FIG 3: Kallikrein (KLK) function in the SC. 1) KLKs cleave corneodesmosomal cadherins to promote desquamation. 2) KLKs activate PAR2 to regulate lipid synthesis and immune responses. 3) KLK cleavage of IL-1 preforms. IL-1 preforms are stored in the cytosol of corneocytes and escape into the intercellular space upon damage. Asterisks denote the genes whose mutations have been linked to the AD pathogenesis.

TABLE 1: A list of genes involved in the CE formation process. The genes that their mutations have been linked to AD pathogenesis are shown in bold. Asterisks denote the diseases that may represent AD-like dermatitis. In the column of human associated disease, the modes of inheritances (AD; autosomal dominant, AR; autosomal recessive) are shown.

Supplementary TABLE 1: A list of "AD-associated" loci that are identified by GWAS. This table is modified from the data shown in ref. 23. We should note that some of these loci are still unwarranted (see ref. 23 for detail).

TABLE 1

	Gene	Gene symbol	Function	Associated human disease	Knockout mice phenotype	Reference
FLG metabolism	**Filaggrin**	FLG	Keratin filaments aggregation	Ichthyosis vulgaris [AD]	Skin barrier deficiency Spontaneous dermatitis	1, 18-20
	Filaggrin2	FLG2	Similar to FLG?			28
	Cap1/Prss8	PRSS8	Cleave proFLG to FLG		Skin barrier deficiency	12
	SASpase	ASPRV1	Cleave proFLG to FLG		SC dehydration	13
	Peptidylarginine deiminase	PADI	Citrullilation of FLG			14
	Caspase14	CASP14	FLG metabolism		Skin barrier deficiency	15
	Calpain1	CAPN1	FLG metabolism			16
	Bleomycin hydrolase	BLMH	FLG metabolism		Penetrant ring-tail dermatitis	16
Formation of Cornified envelope	Involucrin	IVL	Scaffold of CE		No skin phenotype	35
	Envoplakin	EVPL	Plakin family		No skin phenotype	36
	Periplakin	PPL	Plakin family		No skin phenotype	37
	Loricrin	LOR	Reinforce CE		Shiny skin	39
	Small proline-rich protein	SPRR	Reinforce CE			99
	Transglutaminase 1	TGM1	Crosslink CE proteins	ARCI-1 [AR]	Skin barrier deficiency	40, 100
	Transglutaminase 3	TGM3	Crosslink CE proteins		Skin barrier deficiency	101
	Transglutaminase 5	TGM5	Crosslink CE proteins	Peeling skin syndrome 2 [AR]		41
Intercellular lipid-lamellae formation	12R-lipoxygenase	ALOX12B	Ceramide processing	ARCI-2 [AR]	Skin barrier deficiency Neonatal death	46, 102
	Epidermal lipoxygenase 3	ALOX3E	Ceramide processing	ARCI-3 [AR]	Skin barrier deficiency Neonatal death	46, 103
	ATP-binding cassette subfamily A member 12	ABCA12	Transport of lamellar body	ARCI -4A/-4B [AR] (Harlequin ichthyosis)	Skin barrier deficiency	47, 104
	Tmem79/mattrin	TMEM79	Secretion of lamellar bodies		Spontaneous dermatitis	48, 49
Corneodesmosome	Desmoglein1	DSG1	Cadherin family	SAM syndrome* [AR]		51
	Desmocollin1	DCN1	Cadherin family		Skin barrier deficiency	105
	Plakoglobin	JUP	Armadillo family	Naxos disease [AR]	Embryonic lethal	106
	Plakophilin	PKP	Armadillo family	Skin fragility syndrome [AR]	PKP3-deficient mice develop dermatitis	107
	(Envoplakin)	EVPL	Plakin family		No skin phenotype	36
	(Periplakin)	PPL	Plakin family		No skin phenotype	37
	Corneodesmosin	CDSN	Support the corneodesmosome adhesion	Peeling skin syndrome 1* [AR]	Skin barrier deficiency Neonatal death	82
Corneocyte desquamation	Kallikrein5	KLK5	Serine protease		Skin inflammation *(when overexpressed)	108
	Kallikrein7	KLK7	Serine protease		Skin inflammation *(when overexpressed)	109
	Kallikrein14	KLK14	Serine protease			110
	Lympho-epithelial Kazal-type-related inhibitor (LEKTI)	SPINK5	Serine protease inhibitor	Netherton syndrome* [AR]	Neonatal death due to dehydration	61-63, 111
	Protease-activated receptor 2	PAR2	Receptor on keratinocytes		Altered skin immune response	64

FIG 1

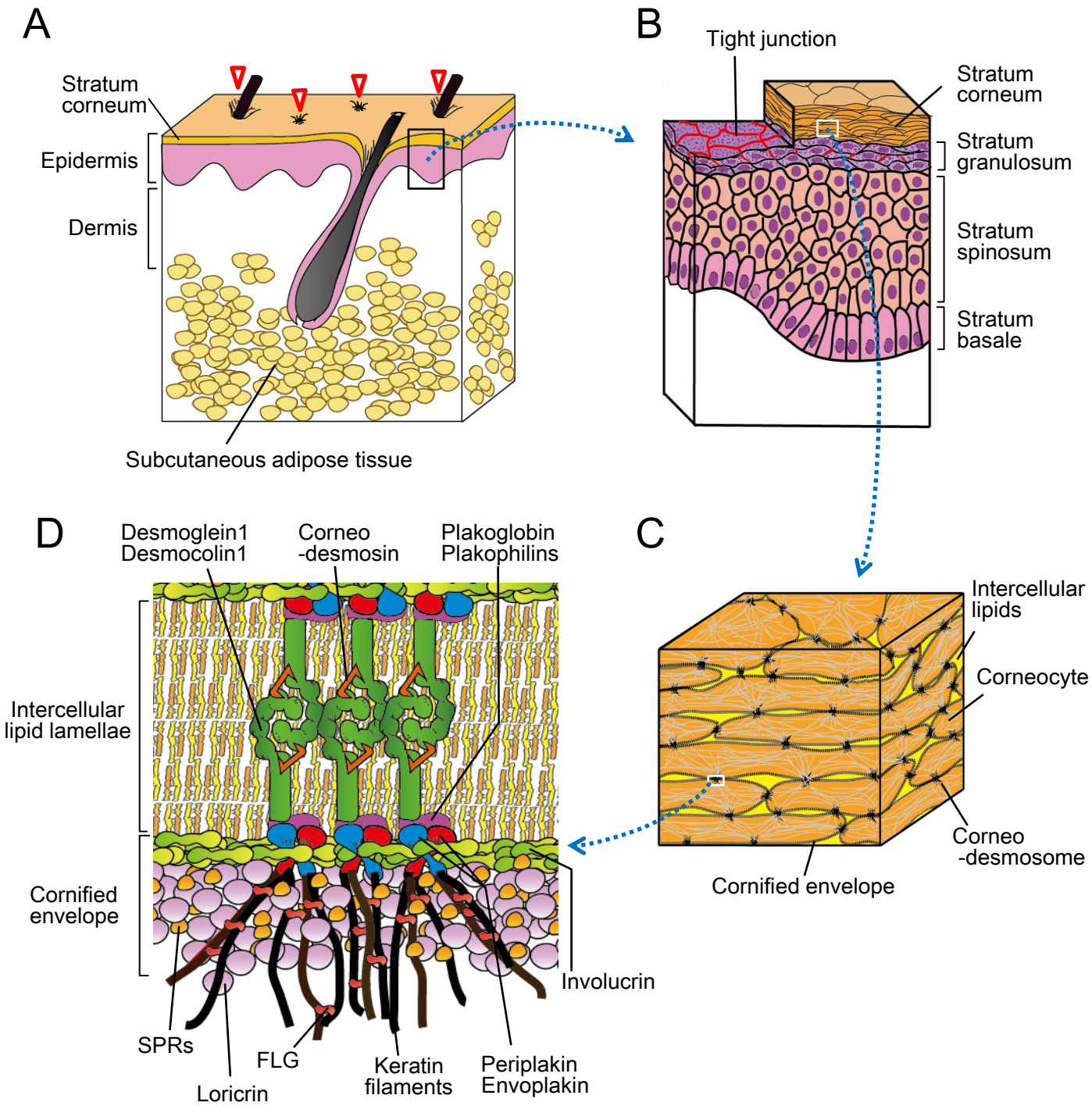

A
Stratum corneum
Epidermis
Dermis
Subcutaneous adipose tissue
B
Tight junction
Stratum corneum
Stratum granulosum
Stratum spinosum
Stratum basale
C
Intercellular lipids
Corneocyte
Corneo -desmosome
Cornified envelope
D
Desmoglein1
Desmocolin1
Corneo -desmosin
Plakoglobin
Plakophilins
Intercellular lipid lamellae
Cornified envelope
Involucrin
SPRs
FLG
Loricrin
Keratin filaments
Periplakin
Envoplakin

FIG 2

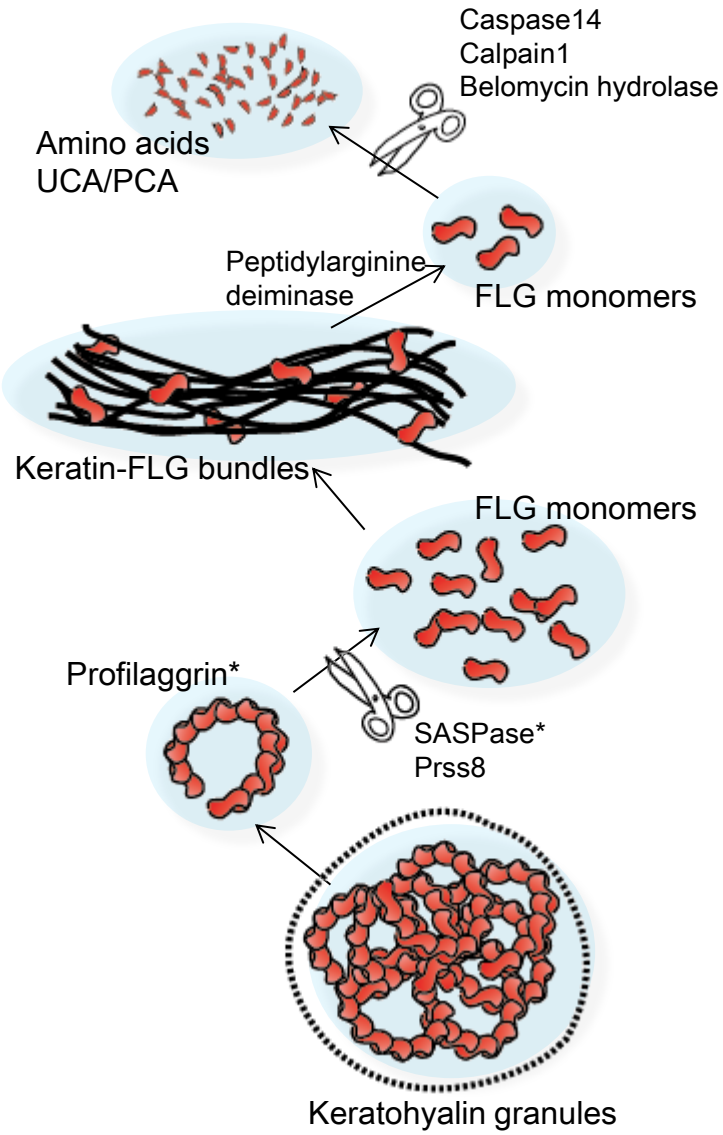
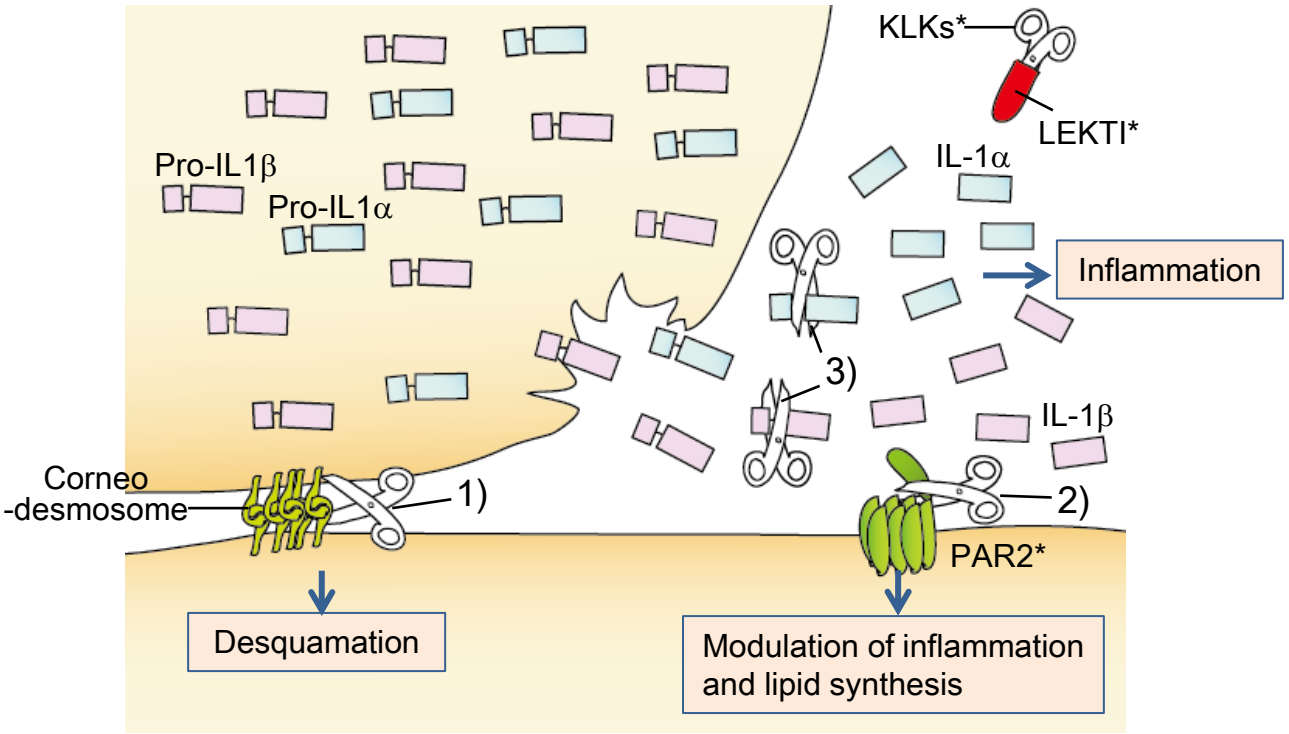

Caspase14
Calpain1
Belomycin hydrolase
Amino acids
UCA/PCA
Peptidylarginine
deiminase
FLG monomers
Keratin-FLG bundles
FLG monomers
Profilaggrin*
SASPase*
Prss8
Keratohyalin granules

FIG 3


KLKs*
LEKTI*
Pro-IL1β
Pro-IL1α
IL-1α
Inflammation
3)
IL-1β
Corneo
-desmosome
1)
2)
PAR2*
Desquamation
Modulation of inflammation
and lipid synthesis

Supplementary TABLE 1:

Variant	Nearest gene	OR	p-value
rs61813875	*FLG*	1.61	5.6E-29
rs10791824	*OVOL1*	1.12	2.1E-19
rs12188917	*RAD50/IL13*	1.14	4.0E-17
rs6419573	*IL18R1/IL18RAP*	1.11	1.5E-13
rs2212434	*C11orf30/LRRC32*	1.09	4.6E-13
rs4809219	*RTEL1/TNFRSF6B*	0.90	7.0E-13
rs2918307	*ADAMS10/ACTL9*	1.12	4.6E-12
rs2041733	*CLEC16A*	0.92	2.5E-11
rs12730935	*IL6R*	1.08	6.1E-11
rs2038255	*PPP2R3C*	1.11	1.8E-10
rs7127307	*ETS1*	0.93	3.9E-10
rs7512552	*C1orf51/MRPS21*	0.93	9.1E-10
rs6473227	*MIR5708/ZBTB10*	0.93	1.4E-09
rs6602364	*IL15RA/IL2RA*	1.08	1.5E-09
4:123243592	*KIAA1109 (IL2)*	1.08	4.2E-09
rs4713555	*HLA-DRB1*	0.91	5.4E-09
rs10214237	*IL7R/CAPSL*	0.93	2.9E-08
rs10199605	*LINC00299*	0.93	3.4E-08
rs4643526	*PUS10*	1.09	3.5E-08
rs12951971	*STAT3*	1.13	4.1E-08
rs7625909	*SFMBT1/RFT1*	1.07	4.9E-08
rs112111458	*CD207/VAX2*	0.91	1.4E-07
rs2592555	*PRR5L*	0.93	8.7E-07
rs2944542	*ZNF365*	0.94	1.2E-06
rs145809981	*MICB*	0.91	1.5E-06
rs16948048	*ZNF652*	1.05	1.7E-05
rs1249910	*CCDC80/CD200R1L*	0.98	1.4E-01
rs7701890	*TMEM232*	1.02	3.6E-01
rs6780220	*GLB1*	1.01	4.0E-01
rs4312054	*OR10A3/NLRP10*	1.00	7.4E-01
rs4733404	*CARD11*	1.00	8.1E-01