eighth and/or tenth domain. The huge size, polymorphic variations in the number of filaggrin repeats and highly repetitive nature prevent the entire gene from being sequenced. However, the improvements in PCR strategy that involve long-range sequencing and multiple-alignment techniques that permit comprehensive sequencing of the entire *FLG* gene have recently been developed [4,13]. Smith *et al.* first identified the homozygous or compound heterozygous *FLG* mutations R501X and 2282del4 as the cause of moderate or severe IV in 15 kindreds [4]. Those investigators also demonstrated that IV is a semi-dominant condition with incomplete penetrance (~90% in homozygotes). Homozygotes or compound heterozygotes had a severe form of IV, whereas heterozygotes displayed mild or no IV phenotype.

A fewer number of *FLG* repeat domains might be associated with the dry-skin phenotype [22]. Individuals with an absence of the 12-repeat profilaggrin allele (i.e., with allelotypes 10, 10; 10, 11; or 11, 11) were at least four-times more likely to report skin dryness than those who carried one or two 12-repeat alleles (i.e., 10, 12; 11, 12; or 12, 12 allelotypes) [22]. The genotype and phenotype correlation in *FLG* mutations is still lacking. *FLG* mutations at any site were reported to result in similarly severe deficiency of profilaggrin/filaggrin processing [13]. Currently, it is hypothesized that the profilaggrin C-terminal region is essential for proper profilaggrin processing. The hypothesis is supported by the finding of nonsense mutation p.Lys4022X in the C-terminal incomplete filaggrin repeat. In the epidermis of patients carrying this mutation, levels of profilaggrin/filaggrin peptides are remarkably reduced, even though *FLG* mRNA expression is not reduced significantly and expresses mRNA-inclusive messages derived from both the wild-type alleles and the mutant alleles [23]. All the truncation mutations are now generally regarded as leading to serious loss of filaggrin peptides, resulting in absence of genotype/phenotype correlations with respect to *FLG* mutations in IV or AD.

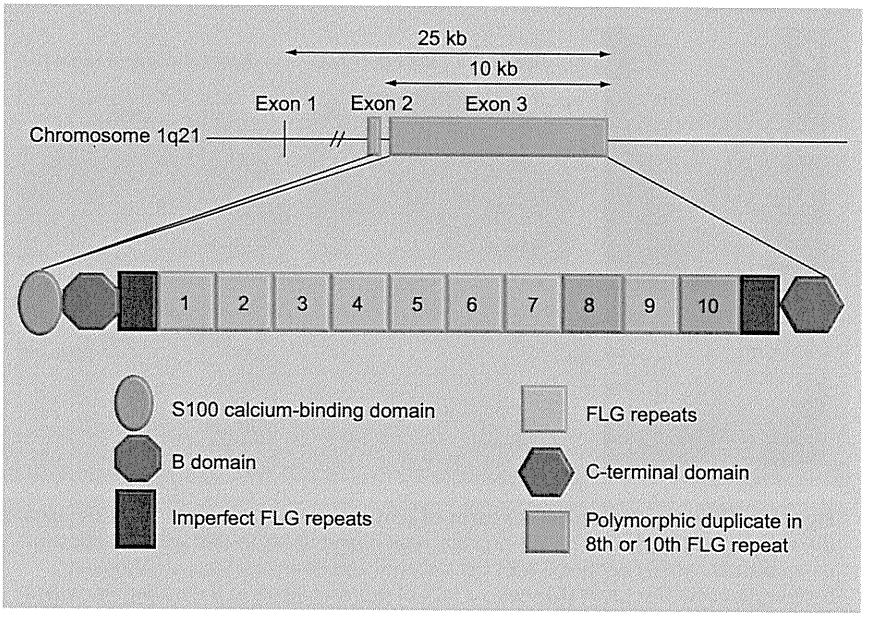

Figure 2. Profilaggrin and filaggrin gene structure. The *FLG* gene, which is within the epidermal differentiation complex on chromosome 1q21, spans approximately 25 kb of genomic DNA and comprises three exons and two introns. Profilaggrin contains a S100 calcium-binding domain, a B-domain and two imperfect filaggrin-repeat domains flanking ten essentially identical filaggrin repeat domains, as well as a C-terminal domain. There exist polymorphic variations in the number of filaggrin repeats. Some individuals have duplication of the eighth and/or tenth filaggrin repeat.
Modified from [28,79].

Prevalent filaggrin mutations: distinct in each race

Since the establishment of sequencing methods for the entire *FLG* coding region in 2006 [4,13,24], approximately 40 loss-of-function *FLG* mutations have been identified in IV and/or AD [5,21] (Figure 4). The *FLG* mutations were initially identified in European families [4,24,25]. Using this methodology, we identified two novel *FLG* mutations (3321delA and S2554X) in four Japanese families with IV [26]. Subsequently, six additional *FLG* mutations in Japanese have been identified [26–31]. The study was repeated for other Asian populations, including Chinese [32], Taiwanese [28] and Korean populations [33]. Only two mutations (R501X and E2422X) were reported in both European and Asian populations [31,32]. Further haplotype analysis of the European-specific mutation R501X in the Japanese family showed that the mutation was not inherited from a European ancestor, but recurred *de novo* in Japan [31]. In Asian populations, 3321delA was found in all four East Asian populations [26,28,31–33], and Q2417X was reported in both Chinese and Taiwanese populations [28,32]. These results revealed the differences in filaggrin population genetics between Europe and Asia (Figure 4).

Filaggrin mutations: a major predisposing factor for AD

Atopic dermatitis is a common chronic pruritic inflammatory skin disease with high prevalence in developed countries, and it is responsible for a notable share of morbidity and health service costs [34,35]. A systematic review estimated the annual costs of treating AD in the USA at US$364 million–3.8 billion [36]. The costs will likely increase in proportion to the increasing prevalence of the disease [36].

The clinical manifestations of AD vary with age [35,37]. In infancy, the lesions are generally more acute and usually present on the face and scalp. Serous exudates or crusted erosions frequently appear secondary to scratching. During childhood, AD lesions involve flexures, nape and the dorsal aspects of the limbs. In adolescence and adulthood, lichenified plaques usually affect flexures, head and neck.

Atopic dermatitis has been regarded as a genetically complex disorder with a strong environmental component [2]. There are two

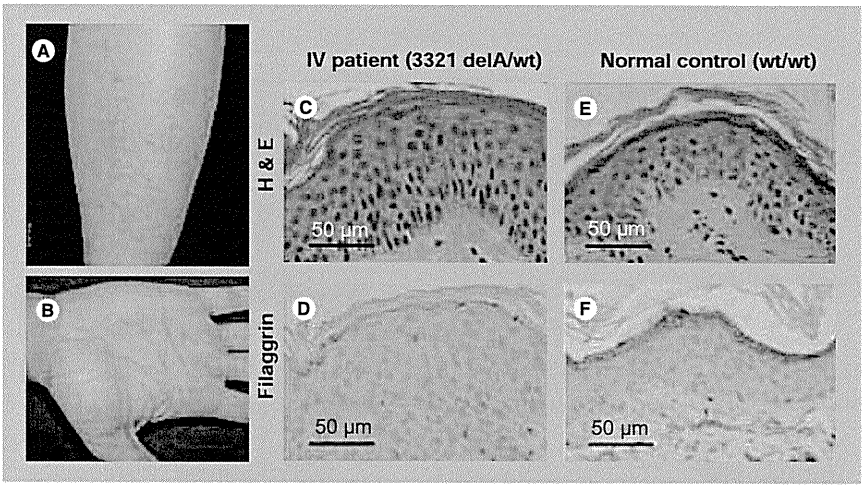

Figure 3. Clinicopathological features of ichthyosis vulgaris. (A) Ichthyosis vulgaris (IV) with dry and scaly skin on the pretibial region, and IV associated with **(B)** apparent palmoplantar hyperlinearity. **(C & E)** Hematoxylin and eosin staining. An IV patient heterozygous for 3321delA **(C)** shows a lack of granular layers in the epidermis, where only a small amount of basophilic substance, resembling keratohyalin, is occasionally present. In contrast, normal control skin **(E)** has abundant keratohyalin granules in the granular layers. **(D & F)** In immunohistochemical staining, a 3321delA heterozygote **(E)** shows a marked reduction in staining for filaggrin, whereas normal control skin **(F)** stains strongly.

proposed hypotheses explaining the mechanism [35]. One suggests that the primary defect is immuno-aberration, evidenced by serum IgE elevation and eosinophilia; thus, skin barrier dysfunction is a consequence of local inflammation. The other proposes that AD originates from an intrinsic defect of epithelial cells that leads to barrier dysfunction; thus, the immunologic aspects are epiphenomena. A main hallmark of AD is xerosis. Transepidermal water loss, the measurement of skin barrier function, was reported to increase in AD patients due to skin barrier defect [38,39]. Significant correlations were observed between penetration rates of a hydrophilic dye and elevated IgE levels in patients with severe AD [40]. Taken together, these findings strongly support the hypothesis that patients with AD have a skin barrier defect.

Before 2006, despite considerable efforts to elucidate genes associated with AD susceptibility, no gene with strong, reproducible effect was identified [41]. There were three clues suggesting that *FLG* mutation plays an important role in the pathogenesis of AD. First, to dermatologists, it has been well understood that AD often occurs in IV patients, although the precise mechanisms of this co-occurrence remain unknown [42–44]. Second, the linkage of AD to a chromosome locus on 1q21 where *FLG* resides was also demonstrated [45]. Third, the skin in patients with AD also demonstrates decreased filaggrin expression at both the mRNA and the protein levels [46,47]. In addition, it has been long proposed that the permeability barrier abnormality in AD is not just an epiphenomenon, but rather is an important driver of disease activity [48], and that the severity of the permeability barrier abnormality precisely parallels the AD severity [39,49]. Therefore, the two loss-of-function mutations in *FLG* found initially in IV were soon applied in the genetic investigation of families with AD [24].

Palmer *et al.* first reported that decreased or absent *FLG* expression due to loss-of-function mutations leads to impaired barrier function that manifests as AD [24]. They found that AD manifested in heterozygous carriers of two null *FLG* mutations (R501X and 2282del4) with a relative risk (odds ratio [OR]) for AD of 3.1, suggesting a causal relationship. Thereafter, numerous studies established *FLG* as a major genetic predisposing factor for AD [13,50–56]. Baurecht *et al.* performed a meta-analysis of nine studies of *FLG* mutations and AD, focusing on the mutations prevalent in Europeans (R501X or 2282del4) [41]. They found an overall OR of 4.09 (95% CI: 2.64–6.33) from the case–control studies and a summary OR of 2.06 (95% CI: 1.76–2.42) from the family studies [41]. The strong association between *FLG* mutations and AD was a milestone in the genetic study of the complex allergic disorders. The *FLG* gene is the most likely candidate as a predisposing gene for AD so far. Based on the information of population-specific *FLG* mutations, many cohort studies on *FLG* mutations in AD were performed, and approximately 25–50% of AD patients were revealed to harbor *FLG* mutations as a predisposing factor [5].

One factor affecting the frequency of *FLG* mutation is the number of identified mutations among specific population. For example, we first identified two null mutations (p.Ser2554X and c.3321delA) among 11 patients from seven Japanese IV families, but only 5.6% of 143 AD patients carried either or both of these *FLG* mutations [26]. We identified two additional novel *FLG* mutations (S2889X and S3296X) in seven Japanese families with IV [27], and more than 20.6% of patients in 102 AD cases carried either or both of these *FLG* mutations [27]. Eight *FLG* variants have been identified in the Japanese population, including six that are prevalent, and we found that approximately 27% of the patients in our Japanese AD case series carry at least one of these eight *FLG* mutations and that these variants are also carried by 3.7% of Japanese general control individuals [23]. Thus, information on population genetics of *FLG* mutation is essential for global *FLG* mutation screening in AD patients.

As mentioned above, every population is likely to have a unique set of *FLG* mutations. *FLG* mutation screening in one population using the *FLG* mutations reported in other populations may result in false-negatives. For example, Ching *et al.* found that the *FLG* mutations that are prevalent in Caucasian and non-Chinese Asian populations are rarely found in childhood AD among the Chinese [57]. It is therefore important to identify novel *FLG* mutations in different populations by sequencing this important AD candidate gene in order to establish global population genetic maps that will facilitate research into that gene's pathogenetic roles for AD.

Flaky tail (ft/ft) is a spontaneous autosomal-recessive mutation

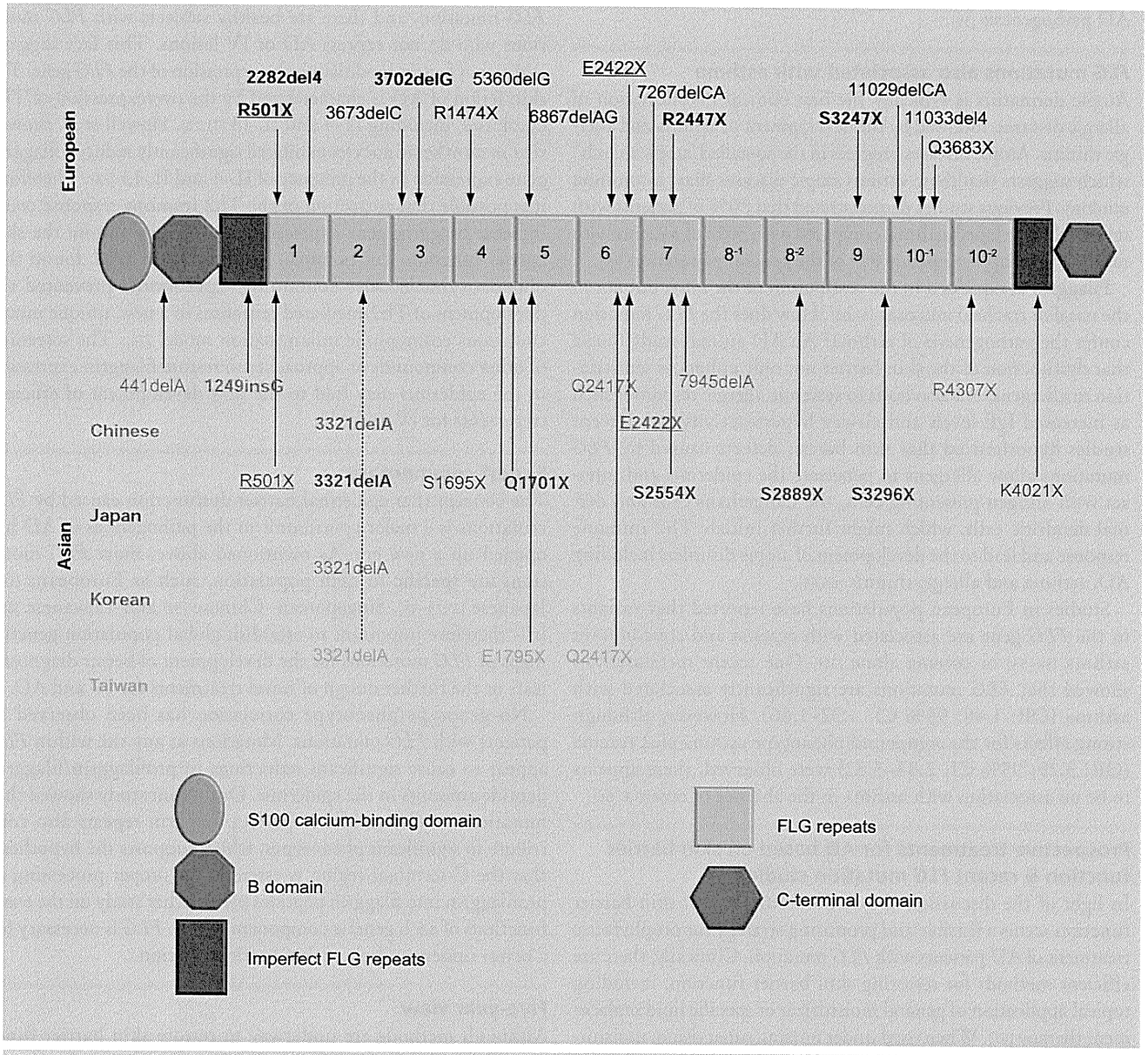

Figure 4. Locations of reported FLG mutations in the profilaggrin peptide. Several of these mutations are rare, but a number of recurrent mutations have been identified (bold text). FLG mutations appear to differ between the European and Asian populations. Only two mutations (R501X and E2422X) were reported in both European and Asian populations (underlined text). We further analyzed FLG mutations in Asian populations (Chinese, marked blue; Japanese, marked red; Korean, marked purple; and Taiwanese, marked green). 3321delA was found in all four East Asian populations, and Q2417X was reported in the Chinese and Taiwanese populations. The duplication of the eighth and tenth filaggrin repeats is represented as 8–1, 8–2, 10–1 and 10–2.
Modified with permission from [5,28].

in mice that results in dry, flaky skin and annular tail in the neonatal period. Presland *et al.* demonstrated that ft/ft mice express a lower-molecular-weight form of profilaggrin (220 kDa) instead of the normal high-molecular profilaggrin (~500 kDa). In addition, the abnormal profilaggrin is not proteolytically processed into profilaggrin intermediates or into filaggrin. The absence of filaggrin and, in particular, the hygroscopic filaggrin-derived amino acids that function in epidermal hydration, underlies the dry, scaly skin characteristic of ft/ft mice. This animal model provides a tool for understanding the role of filaggrin in normal epidermal function [58]. Recently, Fallon *et al.* demonstrated that topical application of allergen to flaky-tail (ft/ft) mice results in cutaneous inflammatory infiltrates and enhanced cutaneous allergen priming, resulting in development of allergen-specific antibody and cytokine responses mimicking human AD. These data provide experimental evidence for the barrier hypothesis of

AD pathogenesis [59].

FLG mutations also associated with asthma

Atopic dermatitis is typically the first clinical manifestation of allergic diseases, followed by the development of asthma and allergic rhinitis. Atopic diseases progress in the so-called 'atopic march', which suggests that these various atopic diseases share a common etiology. Previous studies demonstrated that 70% of patients with severe AD developed asthma, compared with 30% of patients with mild AD and approximately 8% of the general population [60].

Filaggrin is expressed in the vestibulum of the nose, but not in the nasal or tracheal mucosae [61,62]. How does the *FLG* mutation confer the pathogenesis of asthma? An AD animal study found that dysfunction of the skin barrier not only enhances sensitization to allergens, but also leads to systemic allergic responses such as increased IgE levels and airway hyperreactivity [63]. Recent studies hypothesized that skin barrier defects caused by *FLG* mutations allow allergens to penetrate the epidermis and interact with antigen-presenting cells, the Langerhans cells and dermal dendritic cells, which might further initiate Th2 immune response and lead to the development of atopic disorders including AD, asthma and allergic rhinitis [64,65].

Studies in European populations have reported that variants in the *FLG* gene are associated with eczema and concomitant asthma [50–54] or eczema alone [25]. One recent meta-analysis showed that *FLG* mutations are significantly associated with asthma (OR: 1.48; 95% CI: 1.32–1.66). However, although strong effects for the compound phenotype asthma plus eczema (OR: 3.29; 95% CI: 2.84–3.82) were observed, there appears to be no association with asthma in the absence of eczema [66].

Prospective treatments for AD based on skin barrier function & recent *FLG* mutation studies

In light of the discussion above, the restoration of skin barrier function seems a feasible and promising strategy for prophylactic treatment of AD patients with *FLG* mutation. Clinically, there are efficient methods for restoring skin barrier function, including topical application of general moisturizer or specific lipid replacement therapy [67]. When used under nursing supervision, moisturizers have been shown to alleviate the need for topical steroids [68]. In addition, the topical application of ceramide dominant lipid replacement therapy has been proven effective in improving skin barrier defects and reducing AD severity significantly in childhood AD patients [39]. Most *FLG* mutations are caused by premature termination codons, which account for numerous genetic disorders, such as thalassemia and cystic fibrosis. Recently, several pharmaceuticals targeting nonsense mutations in genetic diseases have been developed [69]. For example, PTC124, a small molecule designed to induce ribosomes to selectively read through premature stop codons during mRNA translation, has been proven effective in restoring the function of the *CFTR* gene, whose mutation accounts for some cases of cystic fibrosis [70]. Skin diseases, such as IV and AD, might be even more feasible targets through topical application of similar pharmacological agents.

A large number of patients with severe AD do not have the *FLG* mutation, and there are healthy subjects with *FLG* mutations who do not express AD or IV lesions. This fact suggests additional factors modulating the expression of the *FLG* gene. The skin lesion of AD is characterized by the overexpression of Th2 cytokines, including IL-4 and IL-13 [71,72]. Howell *et al.* showed that *in vitro* keratinocytes exhibited significantly reduced filaggrin gene expression in the presence of IL-4 and IL-13 [73]. Therefore, it is possible that correction of the Th2 immune response could increase filaggrin gene expression and thereby restore the skin barrier function. For example, Kootiratrakarn *et al.* found that oligodeoxynucleotides containing CpG motifs prevented the development of Th2-mediated responses in a new, unique mouse cutaneous eosinophilic inflammation model [74]. The screening of other compounds or approaches to restore filaggrin expression in the epidermis may lead to the new development of efficient treatments for IV and AD.

Expert commentary

The concept that epidermal barrier dysfunction caused by *FLG* mutations is a major contributor to the pathogenesis of AD has opened up a new era. As mentioned above, most *FLG* mutations are specific to each population, such as Europeans [13], Japanese [27,29–30], Singaporean–Chinese [32] and Taiwanese [28]. It is therefore important to establish global population genetics maps of *FLG* mutations for the development of better diagnostic tests or the further design of novel treatments for IV and AD.

No genotype/phenotype correlation has been observed in patients with *FLG* mutations. Mutations at any site within *FLG* appear to cause significant reductions in profilaggrin/filaggrin peptide amounts in the epidermis. Our recent study showed that mutations in C-terminal imperfect filaggrin repeats also contribute to significant phenotypes, which supports the hypothesis that the C-terminal region is essential for proper processing of profilaggrin into filaggrin peptides [29]. Further study on the exact functions of each genetic component within *FLG* is necessary for a better understanding of skin barrier function.

Five-year view

Although methods are underway to restore skin barrier function, the concept of *FLG* mutation has not yet translated into therapeutic advances. Two therapeutic strategies focusing on *FLG* mutation were proposed, and related research is well underway in McLean's laboratory [75]. One strategy is to upregulate *FLG* gene expression by small molecules acting on pathways controlling *FLG* gene expression, and the other strategy is to read through premature termination codon mutations by interfering 'nonsense-mediated decay', which is a cellular mechanism of mRNA surveillance that functions to detect nonsense mutations [76]. We expect therapeutic modalities focusing on *FLG* mutation, especially topical agents, to evolve in coming years.

Atopic dermatitis is a genetically complex disorder complicated by a strong environmental component [2], so developing diagnostic criteria and classification is challenging. Although various validated sets of diagnostic criteria have been developed over the past few decades, there is disagreement about these [77].

Brenninkmeijer *et al.* performed a methodological review of 27 validation studies of various diagnostic criteria for AD [78]. Two frequently quoted criteria focusing on clinical presentation showed variable sensitivity and specificity. Hanifin and Rajka diagnostic criteria sensitivity and specificity ranged from 87.9 to 96.0% and from 77.6 to 93.8%, respectively. The UK diagnostic criteria showed sensitivity and specificity ranging from 10 to 100% and 89.3 to 99.1%, respectively [78]. The *FLG* mutation study is expected to have a major impact on the diagnostic criteria. In addition, we expect that in the future, classification of AD may be based on the presence or absence of *FLG* mutations. Such disease classification and treatment focusing on *FLG* mutation will complement each other.

Financial & competing interests disclosure

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan to M Akiyama (Kiban B 20390304) and by a grant from Ministry of Health, Labour and Welfare of Japan (Health and Labour Sciences Research Grants; Research on intractable diseases) to H Shimizu. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Filaggrin, processed from profilaggrin, is a key structural protein that facilitates terminal differentiation of the epidermis and formation of the skin barrier. Filaggrin aggregates keratin filaments in apoptosed keratinocytes into bundles and promotes the flattening of dead-cell remnants.
- *FLG* spans approximately 25 kb of genomic DNA and resides on human chromosome 1q21, within the so-called epidermal-differentiation complex.
- *FLG* comprises three exons and two introns. Exon 3 is extremely large (>12 kb) and encodes most of the profilaggrin polypeptides with almost completely homologous 10, 11 or 12 repeats.
- Ichthyosis vulgaris is a semi-dominant condition with incomplete penetrance (~90% in homozygotes). Homozygotes and compound heterozygotes have a severe form of ichthyosis vulgaris, whereas heterozygotes display mild or no phenotypic abnormality.
- There exist differences in filaggrin population genetics between Europe and Asia. Only two identical mutations (R501X and E2422X) were reported in both European and Asian populations.
- Approximately 25–50% of atopic dermatitis (AD) patients were revealed to harbor filaggrin mutations as a predisposing factor.
- A meta-analysis study showed that *FLG* mutations are significantly associated with asthma accompanied by AD (odds ratio: 1.48; 95% CI: 1.32–1.66).
- The restoration of skin barrier function seems a feasible and promising strategy for prophylactic treatment of AD patients with *FLG* mutations.

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REVIEW ARTICLE

BJD
British Journal of Dermatology

FLG mutations in ichthyosis vulgaris and atopic eczema: spectrum of mutations and population genetics

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Summary

Filaggrin is a key protein involved in skin barrier function. Mutations in the gene encoding filaggrin (*FLG*) have been identified as the cause of ichthyosis vulgaris and have been shown to be major predisposing factors for atopic eczema (AE), initially in European populations. Subsequently, *FLG* mutations were identified in Japanese, Chinese, Taiwanese and Korean populations. It was demonstrated that *FLG* mutations are closely associated with AE in the Japanese population. Notably, the same *FLG* mutations identified in the European population were rarely found in Asians. These results exemplify differences in filaggrin population genetics between Europe and Asia. For mutation screening, background information needs to be obtained on prevalent *FLG* mutations for each geographical population. It is therefore important to establish the global population genetics maps for *FLG* mutations. Mutations at any site within *FLG*, even mutations in C-terminal imperfect filaggrin repeats, cause significant reductions in amounts of profilaggrin/filaggrin peptide in patient epidermis as the C-terminal region is essential for proper processing of profilaggrin into filaggrin. Thus, no genotype–phenotype correlation has been observed in patients with *FLG* mutations. A restoration of the barrier function seems a feasible and promising strategy for treatment and prevention in individuals with filaggrin deficiency.

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Accepted for publication
2 October 2009

Key words
atopic eczema, filaggrin, FLG, ichthyosis, population genetics

Conflicts of interest
None declared.

DOI 10.1111/j.1365-2133.2009.09582.x

Mutations in *FLG*, the gene encoding profilaggrin/filaggrin, have been identified as the underlying cause of ichthyosis vulgaris (IV; OMIM 146700)[1] and have also been shown to predispose patients to atopic eczema (AE).[2] Although *FLG* is very difficult to analyse because of its large size (> 12 kb) and highly repetitive nature, a polymerase chain reaction (PCR) strategy that permits routine and comprehensive sequencing of the entire coding region has recently been developed.[3] Using this method and the information from other identified mutant alleles, filaggrin mutation searches have been carried out in a variety of geographical populations including European and Asian populations. Based on the information of population-specific *FLG* mutations, many cohort studies of AE for *FLG* mutations have been performed and approximately 25–50% of patients with AE were revealed to harbour *FLG* mutations as a predisposing factor.

Skin barrier defects caused by *FLG* mutations are thought to play a crucial role in the pathogenesis of atopic disorders including AE, asthma and allergic rhinitis. Recently, it was demonstrated that mice deficient in filaggrin expression show enhanced transfer of antigens through the epidermis, thus providing compelling experimental proof for the barrier hypothesis in AE pathogenesis.[4] This review provides an overview of *FLG* population genetics because the information is essential for global *FLG* mutation screening in patients with AE.

Filaggrin is an indispensable component for the skin barrier

Filaggrin is initially synthesized as profilaggrin, a > 400-kDa, highly phosphorylated, histidine-rich polypeptide, which comprises a S100 calcium-binding domain, a B-domain and two imperfect filaggrin-repeat domains flanking 10–12 essentially identical filaggrin repeats, as well as a C-terminal domain.[3,5] During the keratinization of epidermal keratinocytes, keratohyaline granule degradation products subsequently occupy the cytoplasm of keratinized cells in the stratum corneum and play important roles in skin barrier function.[6] Keratohyaline granules in the granular layer of the epidermis are predominantly composed of profilaggrin polyproteins.[7–9]

Upon keratinocyte terminal differentiation, profilaggrin is dephosphorylated and cleaved into 10–12 essentially identical 37-kDa filaggrin peptide units. The liberated filaggrin subsequently and highly efficiently aggregates the keratin filament cytoskeleton,[7] causing the collapse of the granular cells into

flattened squames. The collapsed cytoskeleton is crosslinked by transglutaminases to bind it to the cornified cell envelope. Filaggrin degradation products also contribute to moisture retention in the cornified layers as a natural moisturizing factor.[6,10] Thus, filaggrin is a key epidermal protein essential for the formation of a normal, intact, protective and correctly moisturized skin barrier.[6,11]

Filaggrin deficiency caused by *FLG* mutations results in ichthyosis vulgaris

IV is a common inherited skin disorder exhibiting scaling and dry skin typically on the flexor limbs and lower abdomen, associated with palmoplantar hyperlinearity.[1,11,12] Histologically, IV is characterized by a decrease in the size and number or complete absence of keratohyaline granules in the upper epidermis.[12] Marked reduction in epidermal keratinocyte filaggrin due to *FLG* loss-of-function mutations was identified as the cause of IV.[1] Loss or reduction of filaggrin expression correlates with excessively dry skin and impaired barrier function, which variously manifests as IV.

Filaggrin mutations are a major predisposing factor for atopic eczema in Europe

AE is among the most common diseases in children from developed countries. Despite considerable efforts to elucidate AE susceptibility genes and to clarify the genetic background of atopic disorders, until recently no strong and reproducible genetic factor has been identified.[13] It has long been proposed that a permeability barrier abnormality in AE is not just an epiphenomenon but is rather an important driver of disease activity[14] because the level of the permeability barrier abnormality precisely parallels AE severity[15,16] and both clinically uninvolved skin regions and skin sites cleared of inflammation for as long as 5 years continue to show significant barrier abnormalities.[17]

As mentioned above, filaggrin is a major epidermal moisturizing factor and significantly contributes to the skin barrier function. For a long time, we as dermatologists have realized that AE often occurs in patients with IV,[18–20] although the pathophysiological mechanisms of this co-occurrence have not been fully clarified. Linkage of AE to the chromosome locus 1q21, containing the epidermal differentiation complex where *FLG* resides, has also been reported.[21] In addition, decreased filaggrin expression has been reported in the skin of patients with AE at both mRNA and protein levels.[22,23] Palmer *et al.*[2] initially reported that decreased or absent *FLG* expression due to loss-of-function mutations leads to impaired barrier function which manifests as AE.

Subsequently, it was confirmed that the strong effect of *FLG* mutations on AE risk exceeds that of any other candidate predisposing gene for AE identified so far.[24] A correlation between *FLG* mutations and eczema is one of the most robust genotype–phenotype linkages in complex trait genetics and several case–control association studies have been reported to date.[3,24–30] These studies have established *FLG* as a major genetic factor predisposing for AE, although they showed considerable differences in study design and strength of the genetic effect.

Henderson *et al.*[31] sought to determine the natural history and course of atopic diseases conferred by the two most common *FLG* mutations in a large, population-based birth cohort study in the U.K. and reported that eczema associated with these *FLG* mutations presents in early life and is more persistent. The risk of asthma was remarkably high in the context of eczema and firm associations were confirmed with sensitization to multiple allergens including grass, house dust mite and cat dander.

Prevalent filaggrin mutations are distinct in each population

To date, it has generally been considered that *FLG* mutations are a significant predisposing factor for AE in Europeans, Asians and quite possibly most other races worldwide to differing degrees.

Mutations in *FLG* were initially identified in European families. After the establishment of sequencing methods for the entire *FLG* coding region,[1–3] to date approximately 40 loss-of-function *FLG* mutations have been identified in IV and/or AE.[32,33]

Major differences exist in the spectra of *FLG* mutations observed between certain globally distinct ancestral groups. In the European population, the genetic spectrum of *FLG* mutations is complicated, with up to six recurrent mutations and several other family-specific mutations, and the two mutations R501X and 2282del4 are the most prevalent in the U.K. population (Fig. 1).[3]

From 2006 to date, to establish baseline *FLG* mutation data in the Japanese population, we performed *FLG* mutation searches in more than 30 Japanese families with IV. We carried out comprehensive sequencing of the entire *FLG* coding region using an overlapping PCR strategy and identified four Japanese population-specific mutations in *FLG*, c.3321delA, p.Ser2554X, p.Ser2889X and p.Ser3296X.[34,35] Two *FLG* mutations among them, p.Ser2889X and p.Ser3296X, were reported later by another Japanese group independently using shotgun methods.[36] In 2009, we reported two additional novel *FLG* mutations, p.Ser1695X and p.Gln1701X, in the Japanese population.[37] Furthermore, we studied 19 newly recruited Japanese patients with AE and identified a novel *FLG* nonsense mutation c.12069A>T (p.Lys4021X) in one patient with AE without any other known Japanese *FLG* mutation (Fig. 1).[33] In addition, one of the common European mutations p.Arg501X was reported in a Japanese family, although the mutant allele with p.Arg501X reported in the Japanese family was shown to be on a different haplotype from the common European variant of the same residue.[38] Thus, the Japanese p.Arg501X mutation was thought to arise separately.[38] This p.Arg501X mutation is a CpG mutation and can arise commonly as well as being present in Europeans as an

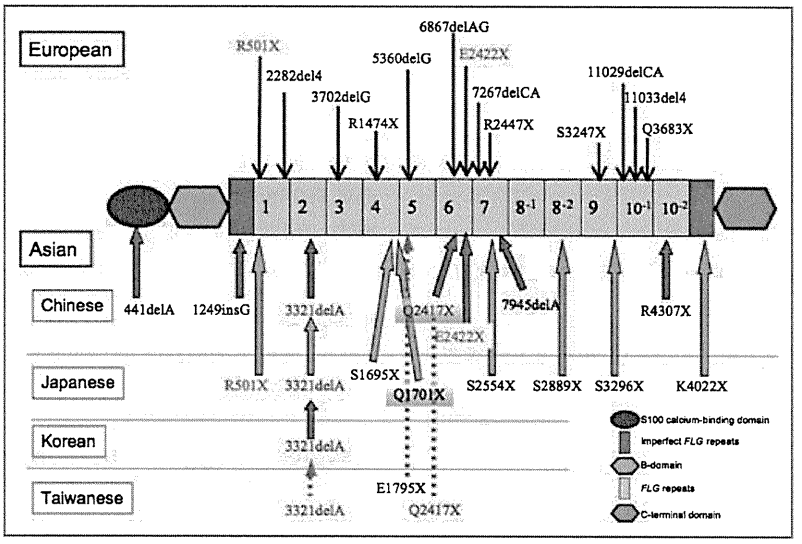

Fig 1. Reported *FLG* mutations shown in a scheme of profilaggrin peptide. Profilaggrin contains 10–12 highly homologous filaggrin-repeat domains. Note that *FLG* mutations in the European and the Asian populations appear to be unique in each population. Only two mutations shown in green (R501X and E2422X) were reported in both European and Asian populations. 3321delA, shown in red, was found in all the four East Asian populations. Q2417X, shown in blue, was reported in both Chinese and Taiwanese populations. Mutations are distributed widely in the profilaggrin sequence and the mutation p.Lys4022X (K4022X) we reported recently[29] is the most distal mutation located in the C-terminal incomplete filaggrin repeat. Some individuals have duplication of the 8th and/or 10th filaggrin repeat(s). Duplicated filaggrin repeats are represented as 8^{-1}, 8^{-2}, 10^{-1} and 10^{-2}.

ancient, ancestral mutation. In total, there are at least eight *FLG* variants in the Japanese population.

A Japanese AE case–control study for the eight *FLG* mutations demonstrated that about 27% of the patients in our Japanese AE case series carry one or more of the eight *FLG* mutations and that these variants are also carried by 3·7% of Japanese control individuals.[33] It was thus confirmed that *FLG* mutations are significantly associated with AE in the Japanese population (Fig. 2).

In other Asian populations, for example the Singaporean Chinese population, it was reported that *FLG* mutations are again different from those found in Europeans and Japanese.[39] In total, six *FLG* mutations, five previously unreported mutations and one known mutation, were found in eight Singaporean Chinese patients with IV.[39] The known mutation was previously identified in a single patient with IV from the Netherlands[3] and, in fact, the patient had Chinese ancestry.[39]

Examining the Taiwanese population, we examined 12 individuals from four unrelated Taiwanese families with IV and identified three *FLG* mutations.[40] One mutation, E1795X, was a previously unidentified *FLG* mutation which might be Taiwanese specific. Interestingly, another *FLG* mutation, 3321delA, is prevalent both in the Japanese population[34] and the Chinese population.[3] This mutation 3321delA was also reported in a Korean patient with IV.[41] The other mutation, Q2417X, was found in the Singaporean Chinese population. No *FLG* mutation identified in the European population was found in the Taiwanese population. The present findings suggest that the Taiwanese population, as an East Asian group, shares *FLG* mutations with both the Japanese and the Chinese populations. These results exemplify differences in filaggrin population genetics between Europe and Asia (Fig. 1).

As mentioned above, most *FLG* mutations are specific to each population, such as European,[3] Japanese,[33,35,37] Singaporean Chinese[39] and Taiwanese.[40] Major differences exist in the spectra of *FLG* mutations observed between different ancestral groups. Prevalent *FLG* mutations are distinct in both the European and the Asian populations. In addition, there is a need for assessing the ancestral admixture in geographical regions in order to know precisely the spectrum and preferential occurrence of *FLG* mutations in different populations.

Every population is likely to have a unique set of *FLG* mutations. Population differences highlighted by *FLG* mutations make it difficult to perform world-wide screening for *FLG* mutations in patients with AE. We cannot perform *FLG* mutation screening in one population using the *FLG* mutations reported in other populations. For example, we cannot use the prevalent European *FLG* mutations when we perform screening of Asian patients with AE. For the mutation screening, we have to obtain information on prevalent *FLG* mutations in each population. It is therefore important to establish global population genetic maps for patient *FLG* mutations.

No genotype–phenotype correlation in *FLG* mutations so far

Genotype–phenotype correlation in *FLG* mutations is lacking. *FLG* truncation mutations at any site within the profilaggrin

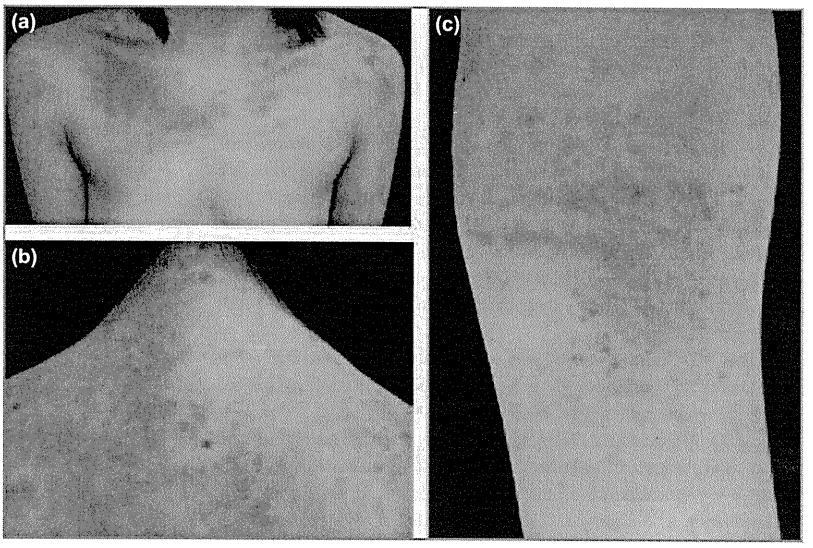

Fig 2. Clinical features of a patient with atopic eczema with compound heterozygous *FLG* mutations. (a) Erythematous lesions and reddish papules with scratch marks and lichenification are seen on the chest (a), back (b) and arm (c). Mutation screening revealed that the patient is a compound heterozygote for *FLG* mutations c.3321delA and p.Ser2554X.

peptide were reported uniformly to result in severe deficiency of profilaggrin/filaggrin processing.[3] It has been hypothesized that the C-terminal region of profilaggrin is essential for proper processing of profilaggrin to filaggrin and, in due course, truncation at any site of profilaggrin results in abolishment of filaggrin/profilaggrin peptides.[3] The nonsense mutation p.Lys4022X that we identified most recently[33] is in the C-terminal incomplete filaggrin repeat and is the most distal mutation among those previously reported. In the epidermis of the patients carrying this mutation, *FLG* mRNA expression including messages derived from the mutant alleles was not significantly reduced. However, histopathologically the size of keratohyaline granules in the granular layers decreased and immunohistochemically profilaggrin/filaggrin peptides were remarkably reduced in the patients' epidermis.[33] These observations further support the hypothesis that the profilaggrin C-terminal region is essential for proper profilaggrin processing. In this context, it is now generally considered that all the truncation mutations lead to serious loss of profilaggrin/filaggrin peptides, resulting in a lack of genotype–phenotype correlations as regards *FLG* mutations in IV or AE.

Novel skin barrier-oriented care and prevention approach to atopic eczema

The concept of epidermal barrier dysfunction caused by *FLG* mutations as a major contributor to the pathogenesis of AE has opened up a new era over the past few years. It is now believed that, at least in a subset of patients with AE, the skin barrier defect is the primary event that initiates disease pathogenesis, allowing the entrance of numerous antigens into the epidermis.[42] Thus, restoration of barrier function seems a feasible and promising strategy for prophylactic treatment of AE in an individual with a filaggrin deficiency.

The range of clinically valuable methods to restore skin barrier function in individuals harbouring *FLG* mutations includes general moisturization measures, or specific lipid replacement therapy. Moisturizers have already been widely used in AE[43] and have been shown to reduce topical steroid use by a specialist dermatology nurse.[44] Lipid replacement therapy is well under development as a triple-lipid, ceramide-dominant, barrier repair therapy for AE, that is provided in an acidic formulation.[43]

One clinical study supports the efficacy of targeted, ceramide-dominant lipid replacement therapy in AE.[15] In the study, topical application of a ceramide-dominant, physiological lipid-based emollient improved skin barrier defects and reduced AE severity significantly in the majority of patients.

Regarding the association between filaggrin deficiency and sensitization to specific antigens: during early life allergen exposure may increase the risks of AE, but the protective effect of reduction in allergen exposure remains uncertain. According to the population-based, longitudinal birth cohort study by Henderson *et al.*,[31] eczema associated with *FLG* mutations presents in early life and is more persistent. In addition, a strong association of *FLG* mutations was identified with sensitization to grass, house dust mite and cat dander. Our study revealed that AE disease severity and specific IgE for house dust, mite allergen and cat dander were significantly correlated in *FLG* mutation-related patients with AE.[45]

In this context, if we select patients with *FLG* mutations and perform early intervention to reinforce/improve their skin barrier function and reduce sensitization to allergens, we may achieve a significant prophylactic effect against AE development. Further studies are required to clarify the preventive effect of early intervention to AE in filaggrin-deficient, high-risk children.

Acknowledgments

This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan to M.A. (Kiban B 20390304).

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Journal of Dermatological Science 58 (2010) 1–7

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.elsevier.com/jds

Invited review article

Extrinsic and intrinsic types of atopic dermatitis

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ARTICLE INFO

Article history:
Received 27 January 2010
Received in revised form 8 February 2010
Accepted 9 February 2010

Keywords:
Atopic dermatitis
Extrinsic
Intrinsic
IgE
Metal

ABSTRACT

Atopic dermatitis (AD) can be categorized into the extrinsic and intrinsic types. Extrinsic or allergic AD shows high total serum IgE levels and the presence of specific IgE for environmental and food allergens, whereas intrinsic or non-allergic AD exhibits normal total IgE values and the absence of specific IgE. While extrinsic AD is the classical type with high prevalence, the incidence of intrinsic AD is approximately 20% with female predominance. The clinical features of intrinsic AD include relative late onset, milder severity, and Dennie-Morgan folds, but no ichthyosis vulgris or palmar hyperlinearity. The skin barrier is perturbed in the extrinsic, but not intrinsic type. Filaggrin gene mutations are not a feature of intrinsic AD. The intrinsic type is immunologically characterized by the lower expression of interleukin (IL) -4, IL-5, and IL-13, and the higher expression of interferon-γ. It is suggested that intrinsic AD patients are not sensitized with protein allergens, which induce Th2 responses, but with other antigens, and metals might be one of the candidates of such antigens.

Contents

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doi:10.1016/j.jdermsci.2010.02.008

1. History of extrinsic and intrinsic atopic dermatitis (AD)

AD is a clinically defined, chronic-intermittent, genetically predisposed, eczematous dermatitis that starts at infancy or early childhood. Although a large number of clinical, laboratory and experimental studies have been performed, the pathophysiology of AD remains to be elucidated, because AD has a variety of aspects in the causes and pathogenesis.

The clinical phenotype of AD has been classified into the extrinsic and intrinsic types [1]. Historically, this dichotomy was first used for asthma. The terminology of extrinsic or allergic asthma was first introduced by Rackeman in 1947 and referred to the triggering role of allergens in asthma. By symmetry, he described intrinsic or non-allergic asthma as a disease characterized by later onset in life, female predominance, higher degree of severity, and more frequent association with nasosinusal polyposis. As intrinsic asthmatic patients was not improved by conventional treatments, this author considered intrinsic asthma to be caused by a non-allergic, unknown phenomenon [2].

In AD, the extrinsic and intrinsic types began to be adopted in the late 1980s [3]. They are also called the allergic (or classical) and non-allergic types. Since there is still no sufficient consensus whether the intrinsic type is a distinct entity, some researchers denominate it atopiform dermatitis [4]. However, the classification into the extrinsic and intrinsic AD has been widely used especially since the millennium. Recently, various kinds of clinical studies have been performed under this dichotomy in many countries, including Germany [1,5,6], Netherland [4], Hungary [7], Italy [8,9] and other European countries, and Asian countries such as Korea [10,11], and Japan [12].

2. Definition

Extrinsic AD and intrinsic AD are defined according to IgE-mediated sensitization, namely the presence or absence of specific IgE for environmental allergens and food allergens [11,12,13]. According to the EAACI nomenclature task force, the term "atopic eczema/dermatitis syndrome (AEDS)" can be used to cover the different subtypes of AD. In this nomenclature, the intrinsic type is termed non-allergic AEDS, which shows normal IgE levels, no specific IgE, no association with respiratory diseases (bronchial asthma or allergic rhinitis), and negative skin-prick tests to common aeroallergens or food allergens [14]. Since total serum IgE values are significantly associated with the allergen-specific IgE status [15], total IgE can be regarded as a clinically useful parameter to differentiate between the extrinsic and intrinsic types in both adults [5,12] and children [15]. The reported mean values of total serum IgE in the intrinsic type are from 22.2 to 134 kU/l, or alternatively, IgE values less than 150 or 200 kU/l have been used for an indication of intrinsic AD [16]. Our study of Japanese patients also showed that the mean value of total serum IgE was 110.5 kU/l (11–219 kU/l) [12].

Among specific IgE antibodies, infantile AD patients are more allergic to food [11], while environmental antigens are common in adults. It should be careful that some allergens may not be useful to discriminate the two types. For example, IgE to *Malassezia sympodialis* was found in patients with the intrinsic type as well as the extrinsic type [17].

3. Prevalence of intrinsic AD

3.1. Incidence

Since extrinsic AD is the prototype of AD, its prevalence is well known. On the other hand, the frequency of intrinsic AD has been a matter of investigation. Schmid et al. [16] summarized the twelve reports that has been published from 1990 to 2000 and documented the clinical features of extrinsic and intrinsic AD. According to their review paper, the frequency of intrinsic AD was 10–45%. More recently, the incidence of extrinsic AD and intrinsic AD were reported as follows: 73% *vs* 27% [18] and 63% *vs* 37% [15] in German children, 88% *vs* 12% in Hungarian adults [7], 78.2% *vs* 21.8% in Dutch patients from 13 to 37 years of age [4], and approximately 80% *vs* 20% in Korean [19]. These data are in accordance with the empirical knowledge that about 20% of AD patients show normal IgE levels and lack of sensitization towards environmental allergens. Intrinsic AD is seen in various countries, but the prevalence may depend on local areas, as it was reported that intrinsic AD was higher in incidence in East Germany than West Germany, although the exact reason remains unclear [6].

3.2. Female predominance

The female predominance in intrinsic AD is well known and has been observed by a number of studies [1,4,16,20]. Our observation disclosed that 76.5% of AD patients were female [12]. More extremely, the 14 intrinsic AD patients enrolled in a study by another group were all female [20].

3.3. Adults and children

Several reports on the prevalence may provide an implication that the intrinsic type is seen at higher frequencies in children than adults [15]. A Korean group of AD investigators showed that the intrinsic type is more prevalent in infancy, and even the third group of the indeterminate type between the intrinsic and extrinsic ones can be identified in this younger generation [11]. A prospective birth cohort study followed for 5 years by a German group demonstrated that one third of child AD was the intrinsic one, and more common in female [21]. Another German group indicated the low prevalence of the intrinsic AD among adult patients [5]. They showed 6.9% patients fulfilled the criteria of intrinsic AD, and after follow-up, the incidence was declined to 5.4% because some patients developed respiratory allergies or IgE-mediated sensitizations. Taken together these observations, it is likely that the intrinsic type is more prevalent in children than adults.

4. Clinical features

The skin manifestations of the two types of AD are indistinguishable. As shown in Figs. 1–3, patients with the intrinsic type share the features with those with extrinsic type. However, Brenninkmeijer et al extensively studied the clinical features of intrinsic AD [4] and found that the Dennie-Morgan fold is significantly more present in the intrinsic type (Fig. 2). The later onset of disease and milder

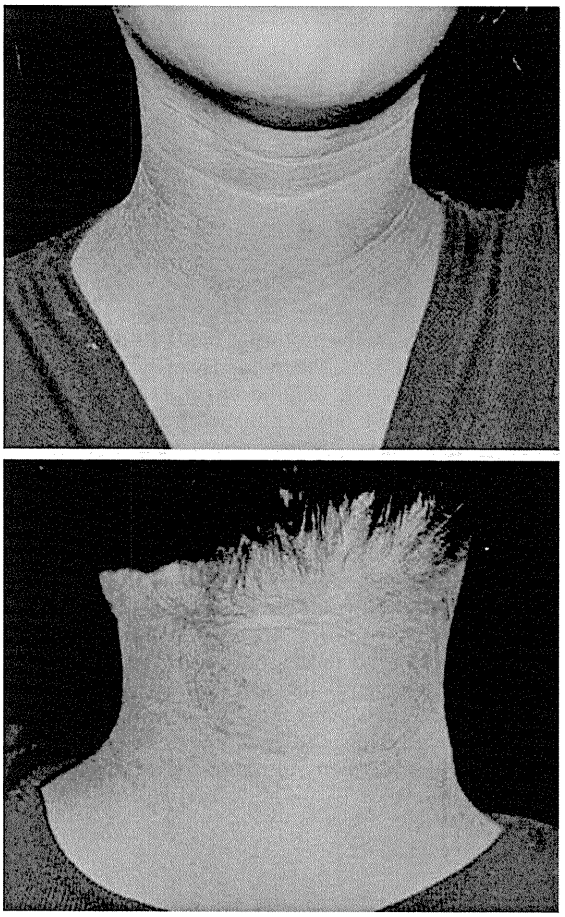

Fig. 1. Intrinsic AD. A 25-year-old female, with total serum IgE, 69 kU/l; and blood eosinophils, 10%. A lichenified eruption on the neck and upper chest (top) and nuchal area (bottom).

disease severity are also characteristics of intrinsic AD. The features that are negatively associated with intrinsic AD include personal or family history of atopy, recurrent conjunctivitis, palmar hyperlinearity, keratosis pilaris, pityriasis alba, non-specific hand or foot eczema, and influence of emotional or environmental factors [4]. As mentioned below, some of these non-associated features are considered to stem from the lack of barrier disruption and/or filaggrin gene mutations in intrinsic AD (Table 1).

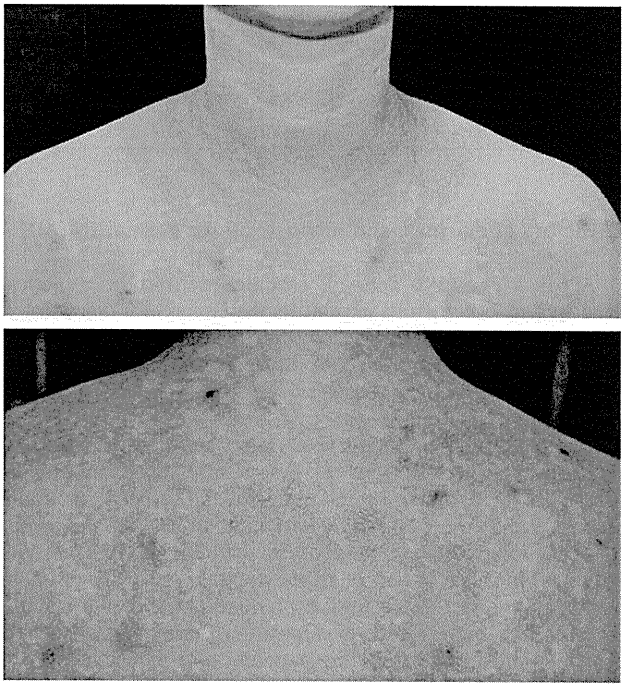

Fig. 3. Intrinsic AD. A 29-year-old female, with total serum IgE, 43 kU/l; and blood eosinophils, 11%. A lichenified eruption on the neck and upper chest (top) and upper back with scratches (bottom).

5. Skin barrier function

5.1. Barrier function of stratum corneum

The barrier function is usually assessed by transepidermal water loss (TEWL) and skin surface hydration (capacitance). The extrinsic AD patients showed increased TEWL and lower skin surface hydration, whereas the intrinsic patients showed no significant differences in TEWL or skin surface hydration as compared to control [19]. On the antecubital fossae, both types of AD patients showed higher TEWL and decreased capacitance. We examined the skin surface hydration and TEWL at the nonlesional forearm and lower leg of patients and normal volunteers in a comparison between the extrinsic and intrinsic types [12]. The level of skin surface hydration was significantly lower in extrinsic AD than in normal control subjects. On the other hand, there was

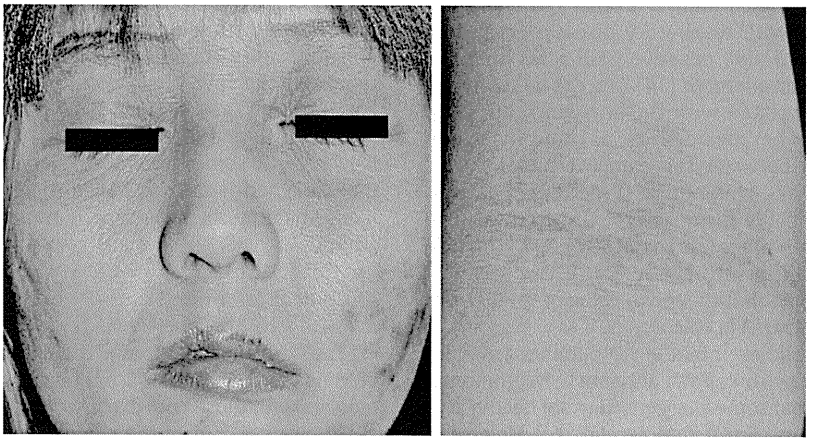

Fig. 2. Intrinsic AD. A 32-year-old female, with total serum IgE, 226 kU/l; and blood eosinophils, 18%. A lichenified eruption with Dennie-Morgan folds on the lower eyelids and pigmentation on the lips (left); and a pigmented and chronic lesion on the antecubital fossa (right).

Table 1
Characteristics of intrinsic AD.

1. Definition
Normal total serum IgE values (mean total serum IgE, 22.2–134 kU/l [1])
Absence of specific IgE for environmental allergens and food allergens
2. Incidence
Percentage of intrinsic AD in total AD: 10–45% [1], 27% [2], 37% [3], 12% [4], 21.8% [5], 20% [6]
Female predominance (collectively 70–80%) [1,5,7,8]
3. Clinical features
Dennie-Morgan fold [5]
No ichthyosis vulgris or palmar hyperlinearity [5]
No non-specific hand or foot eczema [5]
Lower colonization of *Staphylococcus aureus* [9]
Relative late onset
Milder severity
4. Skin barrier
Normal barrier function [6,10]
No filaggrin mutation (presence of filaggrin mutations in extrinsic AD [24,25])
5. Immunological features
Lower expression of IL-4, IL-5, and IL-13 [11]
Higher expression of IFN-γ [12]
6. Contact allergens
High prevalence of metal allergy [39,40]

no significant difference in the hydration level between intrinsic AD and healthy control. The extrinsic type tended to be lower than the intrinsic type at both sites. Thus, the skin barrier function was impaired in extrinsic AD and preserved in intrinsic AD.

5.2. Pruritus perception and barrier function

The skin perception threshold of electric current stimuli is one of the indices of itch. We found that the electric current perception threshold was significantly correlated with the skin surface hydration and inversely with TEWL in intrinsic AD patients as well as healthy individuals. In contrast, extrinsic AD patients did not exhibit such a correlation. Therefore, intrinsic AD patients retain the normal barrier function and sensory reactivity to external pruritic stimuli [14].

5.3. Presence and lack of filaggrin mutations in extrinsic and intrinsic AD, respectively

The recent identification of loss-of-function mutations in filaggrin as a widely replicated major risk factor for eczema sheds new light on the mechanisms of AD [22].

These mutations also represent a strong genetic predisposing factor for atopic eczema, asthma and allergies in various countries [23]. Profilaggrin is the major component of the keratohyalin granules within epidermal granular cells. During epidermal terminal differentiation, the profilaggrin polyprotein is dephosphorylated and rapidly cleaved by serine proteases to form monomeric filaggrin, which is further degradated into natural moisturising factor. Recent human genetic studies strongly suggest that perturbation of skin barrier function as a result of reduction or complete loss of filaggrin expression leads to enhanced percutaneous transfer of allergens. Filaggrin is therefore in the frontline of defense, and protects the body from the entry of foreign environmental substances that can otherwise trigger aberrant immune responses. The association of the filaggrin mutations in particular with the extrinsic type of AD was observed [24,25]. Furthermore, filaggrin mutations are significantly associated with palmar hyperlinearity in patients with AD, which represents a shared feature of AD and ichthyosis vulgaris. This is in accordance with the finding that palmar hyperlinearity is negatively associated in the intrinsic type [4]. In our preliminary study, we found that typical cases of intrinsic AD had no mutation in filaggrin, whereas some of the extrinsic patients possessed filaggrin mutations. Although future studies are necessary, it is expected that barrier disruption, as represented by filaggrin gene mutations, is associated with extrinsic AD.

6. Immunological characteristics of circulating T cells and cytokines/chemokines

6.1. Systemic Th1/Th2 immunological state

AD is well known as a Th2-polarized disease. However, there have been reported some differences in systemic cytokine polarization between the two types of AD. As expected with elevation of total serum IgE, extrinsic AD patients show high levels of Th2 cytokines, IL-4, IL-5 and IL-13, and intrinsic AD is linked with much lower levels of IL-4 and IL-13 [8]. Along with the elevation of IL-5 [26,27], eosinophil counts [11] and eosinophil cationic protein levels [18] are increased in the extrinsic type of AD. On the other hand, there was a report demonstrating that both extrinsic and intrinsic patients showed increased production of IL-5 and IL-13 [28]. In that study, however, when peripheral blood mononuclear cells were stimulated with anti-CD3 antibody, extrinsic AD patients had a decreased capacity to produce IFN-γ and GM-CSF as compared to the intrinsic AD [28]. Accordingly, we found, in our preliminary study, that there was no significant difference in the percentages of IL-4^+ or IL-17^+ T cells between the extrinsic and intrinsic types, but that of IFN-γ^+ T cells was higher in the intrinsic type than the extrinsic type. Thus, there are some variations in these results of Th1 and Th2 cytokines, perhaps depending on the evaluation systems, i.e., measurements of cytokine protein amounts in either *in vivo* sera or *in vitro* culture supernatants, mRNA expression by lymphocytes, and intracellular cytokine staining in T cells. However, all the data can be interpreted to indicate that the extrinsic pathogenetic factors mount a Th2-skewing action, and that the intrinsic type shows less Th2-skewing state or relative overproduction of Th1 cytokine IFN-γ.

6.2. Chemokines and others

With regard to chemokines, patients of both types showed high serum amounts of CCL17/TARC and CCL22/MDC and high peripheral blood mononuclear cell expression of CCL17 and CCL22 at comparable levels [29]. Therefore, no difference was observed in the promoted production of chemokines attractive to Th2 cells. The blood levels of soluble receptors derived from lymphocytes correlate to the activity in various diseases. There is no significant difference in the elevated amounts of sCD23, sCD25, and sCD30 between the two types [30].

7. Immunological characteristics of skin lesions

7.1. T cells and cytokines

In skin lesions, $CD4^+$ T cells, $CD8^+$ T cells, and Langerhans cells are comparably increased in both extrinsic and intrinsic AD, but eosinophils infiltrate in the dermis more markedly in the extrinsic than the intrinsic type, and the extrinsic type exhibits more prominent deposition of eosinophil granular protein and higher staining for eotaxin [10,31]. Although the levels of mRNA expression for IL-5, IL-13, and IL-1β are higher in both types of AD patients than non-atopic subjects, extrinsic AD shows even higher levels than intrinsic AD [31]. The expression of IFN-γ, IL-12, and GM-CSF, IL-4, and IL-10 are elevated in both types without

differences between the extrinsic and intrinsic AD [31]. Thus, tissue eosinophilia and IL-5 expression may be a characteristic of the extrinsic type.

7.2. Dendritic cells (DC) and Langerhans cells (LC)

As to epidermal DC, the extrinsic type is characterized by a significantly high level of the expression of IgE high-affinity receptor (FCεR) on the $CD1a^+$ epidermal DC compared to the intrinsic type [1,32]. When the high-affinity/low-affinity expression ratio is used as a disease marker for AD, the values for intrinsic AD fall below the diagnostic cut-off level, suggesting that intrinsic AD can be distinguished by phenotyping of epidermal DC [1,32]. In accordance with these data from the lesional skin, the surface expression of the high- and low-affinity receptor for IgE and the IL-4Rα chain are significantly elevated in monocytes from patients with the extrinsic type [2]. As described below, it is possible that epidermal LC in the barrier-disrupted skin produce high amounts of Th2 and eosinophil chemokines, further suggesting that LC are activated in the extrinsic type.

8. Relationship between barrier status and skin immune reactions

8.1. Epidermal cytokine production in barrier-disrupted skin

The skin immune status is closely associated with the disordered condition of skin barrier (Fig. 4). Studies using a mouse model of contact hypersensitivity (CHS) have shown that CHS responses to hapten are increased when a hapten is applied to the barrier-damaged skin [33]. Barrier disruption of the skin is experimentally performed by extraction of epidermal lipids with acetone or removal of corneocytes by tape stripping. Both procedures can induce elevated CHS responses. Not only increased permeability of hapten through the epidermis but also altered immune functions of epidermal cells potentiate T-cell activation in acute barrier disruption [33]. Such augmentation of immune reactivity may be critical to elimination of environmental noxious agents that penetrate easily into the barrier-disrupted epidermis, and it is also closely related to the mechanism underlying extrinsic AD.

8.2. Epidermal chemokine production in barrier-disrupted skin

Regarding epidermal chemokines of the barrier-disrupted skin, the mRNA expression levels of Th1 chemokines (CXCL10, CXCL9 and CXCL11), Th2 chemokines (CCL17 and CCL22) and eosinophil chemoattractant (CCL5) are high in the epidermal cells from BALB/c mice. In particular, we found that CCL17, CCL22 and CCL5 were remarkably elevated in BALB/c mice [34]. Tape stripping induced dermal infiltration of eosinophils in BALB/c mice, and the late-phase reaction was increased with infiltration of Th2 cells as well as eosinophils, when challenged *via* the tape-stripped skin. Notably, Th1 chemokines (CXCL9 and CXCL10) and Th2 chemokines (CCL17 and CCL22) are derived mainly from keratinocytes and LC, respectively [35]. In this notion, one of the crucial actions of IFN-γ is upregulation of keratinocyte production of Th1 chemokines and downregulation of LC production of Th2 chemokines. Therefore, the barrier damage likely induces the infiltrates of Th2 cells and eosinophils in extrinsic AD, but their infiltrates are inhibited by IFN-γ in intrinsic AD.

8.3. Implications for the difference between extrinsic and intrinsic AD

The above findings suggest that Th2 and eosinophil responses and resultant late-phase reaction are prone to take place in the skin with damaged barrier by the modulated function of LC. This may provide the mechanism of Th2-polarized immunophenotype of the extrinsic AD. On the contrary, LC are not stimulated to produce Th2 chemokines in the intrinsic type because of the presence of normal stratum corneum. Protein antigens penetrating the damaged barrier further induce the Th2-shifted response in the extrinsic AD, while non-protein antigens exert the Th1 response as well in the intrinsic AD (Fig. 4).

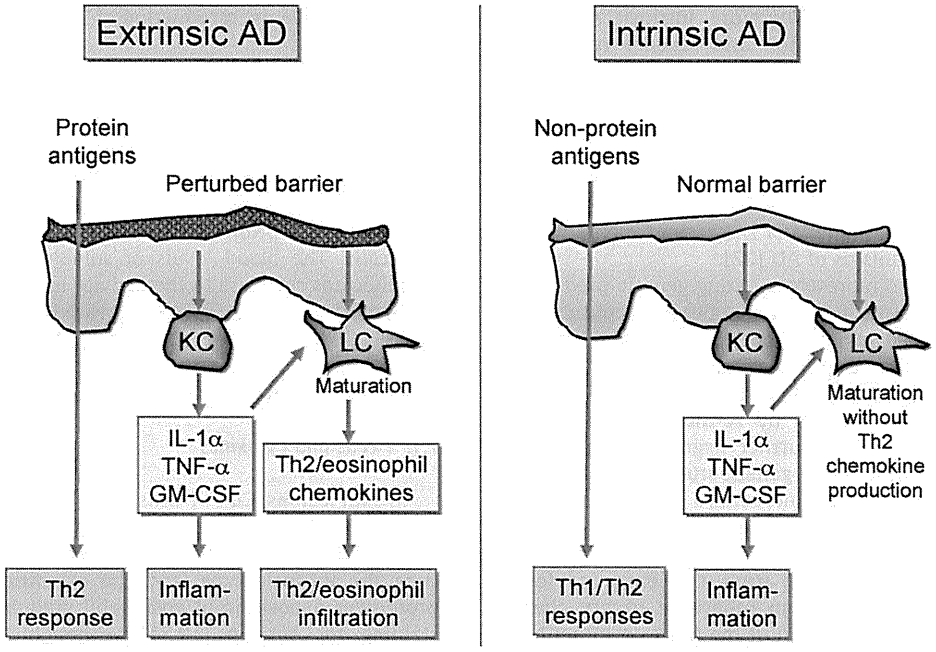

Fig. 4. Comparison between extrinsic and intrinsic AD in relation to the barrier and immune states.
Protein and non-protein antigens are causative in the extrinsic and intrinsic types, respectively. In both types, antigen application to the skin stimulates keratinocytes to produce cytokines, including IL-1α, TNF-α, and GM-CSF, which induce maturation of Langerhans cells (LC). In the perturbed skin of extrinsic AD, LC can produce CCL17/TARC, CCL22/MDC, and CCL5/RANTES, which promote infiltration of Th2 cells and eosinophils. On the other hand, LC of the intrinsic AD do not elaborate those chemokines.

It has been reported that Th2 cytokine IL-4 suppresses the enhancement of ceramide synthesis and cutaneous permeability barrier functions, which further aggravates the barrier [36]. This 'outside-to-inside, back to outside' paradigm [37] is applicable for the pathogenesis of extrinsic AD. A more recent observation suggests that neutralization of the normally acidic stratum corneum has deleterious consequences for permeability barrier homeostasis and stratum corneum integrity/cohesion attributable to serine proteases activation leading to deactivation/degradation of lipid-processing enzymes and corneodesmosomes [38]. Hyperacidification improves permeability barrier homeostasis, attributable to increased activities of two key membrane-localized, ceramide-generating hydrolytic enzymes, which correlate with accelerated extracellular maturation of stratum corneum lamellar membranes. Thus, the surface pH may be another important factor to differentiate between the extrinsic and intrinsic types.

9. Patch tests and metal allergy

9.1. Patch tests for mite antigens

An Italian group performed patch test with house dust mites at a concentration of 20% in petrolatum in the extrinsic and intrinsic types of adult male AD patients [9]. The patch test was positive in 47.4% of extrinsic AD and in 66.6% of intrinsic AD, and in 12.2% of healthy subjects [9]. Since that extrinsic AD patients usually have high levels of IgE specific for mites, the authors wondered the reason why the patch test was highly positive in the intrinsic AD. However, patch tests can reflect mostly the T-cell mediated contact sensitivity, and the IgE-high extrinsic nature does not promote the patch test reactions. Rather, given that IFN-γ is produced at a higher level in the intrinsic type than the extrinsic type, the higher frequency of positive reaction in the intrinsic type seems to be reasonable.

9.2. Patch tests for metals

It is known in patients with AD that the most frequent contact allergens are metals [39]. In 137 atopic children, 19.3% patients were positive to metals [39]. In 1965, Shanon reported that patients with metal allergy occasionally exhibit a skin manifestation indistinguishable from AD under the name of "pseudo-atopic dermatitis" [40,41], and chrome is the causative in their report [40]. Some patients with AD were improved by intake of metal-free diet and elimination of metals [41]. We found that patients with intrinsic AD showed positive patch tests to cobalt, chrome, and/or nickel at a higher percentage than extrinsic one, suggesting that systemic metal allergy is one of the potential causes of intrinsic AD. With regard to metals, our tentative observation with sweat demonstrated that a high incidence of sweat allergy in AD and a therapeutic effect of desensitization with sweat in the patients. Since sweat contains high concentrations of metals, this finding might be related to the pathogenetic role of metals in intrinsic AD.

10. Skin infections

Both extrinsic and intrinsic AD patients suffer from recurrent bacteria and viral infections [42]. A higher colonization of *Staphylococcus aureus* was observed in the extrinsic (71%) *vs* the intrinsic children (49%) [43]. The expression of human β-defensin-3, an anti-microbial peptide, is decreased in both types of AD as compared to normal skin and psoriatic skin [42]. Therefore, skin infection with microorganisms, in particular *S. aureus*, may be severer in the extrinsic type because of barrier perturbation, but it remains unclear whether or not the defense responses are different between the types.

11. Neurotrophins and neuropeptides

Neurotrophins, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are increased in both extrinsic and intrinsic AD, suggesting a similar pathophysiologic background implicating a neuroimmune network [27]. However, there is a significant correlation between BDNF and SCORAD only in the intrinsic type [27]. Maternal NGF levels were significantly higher in patients with both extrinsic and intrinsic AD than controls [30]. It is an issue to be elucidated whether these neurotrophines or neuropeptides such as substance P are different between the two types.

12. Animal models for intrinsic AD

A non-IgE-associated AD model was regarded as a mode of human intrinsic AD [44]. In an animal model of AD, IL-18 contributes the spontaneous development of AD-like skin lesions independently of IgE [45]. When the skin barrier was destroyed in mice and protein A from *S. aureus* was topically applied to the skin, the mice developed AD lesions with dermal infiltration of eosinophils and mast cells and showed an increase in serum levels of IL-18, but not IgE [46]. In this model, IL-18 might be important for the development of infection-associated AD by induction of IL-3 from IFN-γ- and IL-13-producing "super" Th1 cells. Since the intrinsic AD shows high levels of IFN-γ-producing cells [28] and normal levels of IgE, this mouse model resembles intrinsic AD and suggests that some intrinsic AD patients may be related to infection.

13. Conclusions

The causes and mechanisms of intrinsic AD remain unfully elucidated. However, as compared to extrinsic AD, the intrinsic type can be characterized by normal barrier function [12] and IFN-γ-producing potency [28]. These findings suggest that the intrinsic AD patients are not sensitized with protein allergens, which induce Th2 responses, but with other antigens. Metals might be one of the candidates as antigens [40].

Some dermatologists are still skeptical whether the distinction between the intrinsic and extrinsic types of AD is really meaningful. If the contactants and pathophysiology of intrinsic AD are clarified, we might eliminate the term of "intrinsic" from the whole spectrum of AD. However, we have been unable to elucidate them to date. Furthermore, as shown in Figs. 1–3, the clinical appearance of intrinsic AD resembles that of extrinsic AD, expect for a small group of intrinsic patients. In this context, it is reasonable to use "intrinsic" in clinical dermatology.

The extrinsic nature may be changed as the patients grow. Therefore, the classification into the extrinsic and intrinsic types is necessary at each stage of life, i.e., infancy, childhood, teenage, and adult for the allergological management of patients as to allergen avoidance, second allergy prevention, and immunotherapy [14]. However, the risk of an "atopy march" is significantly lower in children with the intrinsic type [14].

A German study demonstrated that the intrinsic type was associated with early daycare attendance [21]. In relation to the feasibility of AD in individuals, early daycare attendance is known as a factor related to the hygiene hypothesis as well as the number of older siblings of individuals. Therefore, intrinsic AD is different from extrinsic AD, whose development is depressed by Th1-inducing environmental factors. Again, it appears that the intrinsic type is not related to the pure Th2 dominant immunological state. Future studies on the intrinsic type of AD may clarify the pathophysiology of not only intrinsic AD, but also dermatitis of

unknown cause that have been called atopiform dermatitis [4] or pseudo-atopic dermatitis [40].

Acknowledgement

I thank Ms. Yukako Miyazaki for technical assistance.

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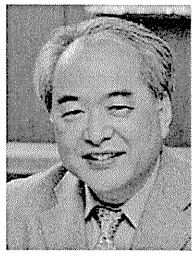

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