a chalazion although neither several incisions nor antibiotics improved it. The initial skin biopsy was performed at the ophthalmology clinic, and the lesion was diagnosed as PG from the histopathological findings. Systemic prednisolone (initial dose: 1 mg/kg/day) improved the lesion; however, the skin lesion recurred when the prednisolone dose was reduced to 0.2 mg/kg/day. The patient was referred to our department for further consultation.

At the initial examination, an ulcer with surrounding erythema was observed (fig. 3a). Skin biopsy specimens showed a dermal abscess containing dense aggregates of plasma cells (fig. 3c, d). Light microscopic observations did not show giant cells, ballooning degeneration or reticular degeneration. Gram, PAS, Grocott and Ziehl-Neelsen stains, and culture of skin tissue failed to identify infectious diseases due to bacteria, mycobacteria, atypical mycobacteria and fungi. Using a laboratory examination and endoscopy, no systemic complications were detected. Anti-proteinase 3, anti-myeloperoxidase antibodies or atypical anti-neutrophil cytoplasmic antibodies were not detected. Orbital MRI showed hypointensity areas on T_2-weighted image and hyperintensity areas on T_1-weighted image, suggesting fibrosis in the right lachrymal gland and eyelid (fig. 3b). From the clinical features and histopathological findings, the lesion was also diagnosed as PG in our department. We started high-dose systemic prednisolone (1.2 mg/kg/day), and it improved not only the lesion of the eyelid but also the intraorbital involvement (fig. 4c). The systemic prednisolone was then gradually tapered for 15 months. In order to obtain adequate ocular surface protection, an eyelid repair was performed 12 months after the cessation of systemic prednisolone (fig. 4a, b). No recurrence of skin ulcers or ocular involvement was observed without systemic steroid administration for 5 years.

Discussion

Here, we report 2 cases with PG of the eyelid and review well-documented reports from the literature [2–11]. We summarise the clinical information of the 15 PG cases of the eyelid including the present 2 patients in table 1. It seems to be difficult to make an early diagnosis of PG of the eyelid. In fact, 5 cases (33%) out of 15 were initially misdiagnosed as bacterial infections, chalazia or adnexal tumours (table 1). In 7 cases (47%) it took more than 1 month to be diagnosed as PG. Two cases (13%) were treated by surgical operation, resulting in enlargement of the PG lesions. Seven cases (47%) showed extracutaneous PG lesions including the lachrymal gland, orbit, sclera and uvea as well as internal organ involvement [2, 8, 9]. The eyelids are indispensable for protection of the eye, especially the cornea. Destruction of the eyelid often leads to serious visual disability. Eyelid defects due to PG caused corneal perforation in 4 cases (27%) including the present case 1 [5, 9]. In 3 patients (20%), the affected eyes were required to be enucleated [5, 9]. One patient had defective ocular motility due to severe fibrosis involving the orbital cavity [8].

In the present case 1, surgical intervention made the skin lesion worse. A new distinct PG lesion appeared along the postoperative wounds, affecting the right upper eyelid and cheek. Despite the systemic treatment with immunosuppressive agents, the defect in the right upper eyelid remained, leading to a continuous corneal exposure and perforation of the cornea. Eventually, the right eye had to be enucleated. In contrast, the visual function in case 2 was preserved owing to early diagnosis and immunosuppressive therapy.

Although there has been no report which described the usefulness of MRI, our 2 cases suggest that MRI is effective in detecting PG lesions within intraorbital tissues. In case 1, MRI showed marked homogeneous hyperintensity on fat-saturated T_2-weighted image and hypointensity on T_1-weighted image in the right lachrymal gland, indicating acute inflammation. In case 2, MRI revealed marked hypointensity on T_2-weighted image and hyperintensity on T_1-weighted image in the right lachrymal gland, suggesting the presence of fibrosis. MRI also demonstrated improvements after immunosuppressive therapy in both cases. The present 2 cases suggest that MRI is a powerful tool for evaluating the extent of subcutaneous PG lesion involvement.

In conclusion, our cases and the review of the literature indicate that successful management of PG and the preservation of visual acuity depend on early diagnosis and the induction of an adequate immunosuppressive therapy.

References

1 Ruocco E, Sangiuliano S, Gravina AG, Miranda A, Nicoletti G: Pyoderma gangrenosum: an updated review. J Eur Acad Dermatol Venereol 2009;23:1008–1017.
2 Happle R, Schiffer HP, Kovary PM: Ocular involvement in pyoderma gangrenosum. Arch Dermatol 1977;113:1612.
3 Browning DJ, Proia AD, Sanfilippo FP: Pyoderma gangrenosum involving the eyelid. Arch Opthalmol 1985;103:551–552.
4 Mensing H: Clofazimine in dermatitis ulcerosa (pyoderma gangrenosum). Dermatologica 1988;177:232–236.

5 Newman WD, Frank HJ: Pyoderma gangrenosum of the orbit. Eye 1993;7:89–94.
6 Tirpitz C, Buchwald HJ, Lang GK, Adler G, Reinshagen M: Simultaneous onset of pyoderma gangrenosum and bitemporal abscesses of the upper eyelids during a flare of ulcerative colitis. Inflamm Bowel Dis 1998;4:98–100.
7 Sidwell RU, Petel NN, Francis N, Staughton RCD: Pyoderma gangrenosum of the eyelid and acute rhinosinusitis. Clin Dermatol 2001;26:680–682.
8 Miserocchi E, Modorati G, Foster CS, Brancato R: Ocular and extracutaneous involvement in pyoderma gangrenosum. Ophthalmology 2002;109:1941–1943.

9 Rose GE, Barnes EA, Uddin JM: Pyoderma gangrenosum of the ocular adnexa. Ophthalmology 2003;110:801–805.
10 Lindberg-Larsen R, Fogh K: Traumatic pyoderma gangrenosum of the face: pathergy development after bike accident. Dermatology 2009;218:272–274.
11 Procianoy F, Barbato MT, Osowski LE, Bocaccio FJL, Bakos L: Cicatricial ectropion correction in a patient with pyoderma gangrenosum. Arq Bras Oftalmol 2009;72:384–386.

Filaggrin gene defects and the risk of developing allergic disorders

Rinko Osawa[1], Masashi Akiyama[2], Hiroshi Shimizu[1]

1 Department of Dermatology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

2 Department of Dermatology, Nagoya University Graduate School of Medicine

Correspondence:

Rinko Osawa

Department of Dermatology,

Hokkaido University Graduate School of Medicine,

Sapporo, Japan

Tel: 81-11-716-1161

Fax: 81-11-706-7820

E-mail: r-osawa@fm2.seikyou.ne.jp

Word count:3317

Conflicts of Interest: None declared.

Summary

Filaggrin is a key protein that facilitates terminal differentiation of the epidermis and formation of the skin barrier. Mutations in the gene encoding filaggrin (*FLG*) have been identified as the cause of ichthyosis vulgaris (IV) and have been shown to be major predisposing factors for atopic dermatitis (AD). Approximately 40 loss-of-function *FLG* mutations have been identified in patients with ichthyosis vulgaris (IV) and/or atopic dermatitis (AD) in Europe and Asia. Major differences exist in the spectra of *FLG* mutations observed between different ancestral groups. Notably, prevalent *FLG* mutations are distinct between European and Asian populations. Many cohort studies on *FLG* mutations in AD have revealed that approximately 25-50% of AD patients harbour filaggrin mutations as a predisposing factor. In addition, *FLG* mutations are significantly associated with AD-associated asthma. The risk for developing allergic rhinitis is also significantly higher with a *FLG* mutation, both with and without accompanying AD. Recent studies have hypothesized that skin barrier defects caused by *FLG* mutations allows allergens to penetrate the epidermis and to interact with antigen-presenting cells, leading to the development of atopic disorders including asthma. The restoration of skin barrier function seems a feasible and promising strategy for prophylactic treatment of AD patients with *FLG* mutations.

Key words: filaggrin / *FLG* / atopic dermatitis / atopic eczema / ichthyosis vulgaris / asthma / allergic rhinitis

Introduction

Filaggrin, which is processed from profilaggrin, is a key protein that facilitates terminal differentiation of the epidermis and formation of the protective skin barrier. In the outer granular layer of the epidermis, filaggrin is associated with keratin intermediate filaments and it aids their packing into bundles. In terminally differentiated keratinocytes, filaggrin is crosslinked to the cornified cell envelope, which constitutes an insoluble barrier in the stratum corneum, protecting the organism against environmental agents and preventing epidermal water loss.[1] Mutations in the filaggrin gene (*FLG*, GenBank accession number NM_002016) have been identified as the underlying cause of the relatively common genetic keratinization disorder ichthyosis vulgaris (IV; OMIM 146700), which is clinically characterized by scaling, especially on the extensor limbs, and by palmoplantar hyperlinearity.[2-4] Although *FLG* is very difficult to analyse because of its large size (>12kb) 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.[5] Until now, around 40 *FLG* mutations have been reported, and the prevalent *FLG* mutations are distinct in each population.[6] Based on the information of population-specific *FLG* mutations, many cohort studies on *FLG* mutations in atopic dermatitis (AD) have been performed and approximately 25-50% of patients with AD were revealed to harbour *FLG* mutations as a predisposing factor.[7] In several studies, these mutations also demonstrated strong association with other allergic phenotypes, including asthma and allergic rhinitis.[8] This article gives an overview of *FLG* population genetics with respect to AD, asthma and allergic rhinitis.

Skin barrier

The skin serves numerous functions, the most obvious being its primary protective or barrier function. The large surface area of the skin puts it in constant contact with environmental pollutants, irritants, and allergens, and the horny layer of skin forms the major protective barrier between the body and the environment.

The terminal differentiation of keratinocytes (Fig. 1) results in the formation of an impenetrable barrier (the horny layer) that is the uppermost layer of the epidermis. The successive stages of keratinocytic differentiation in the epidermal layers are the basal cell, spinous cell, and granular cell layers. When spinous cells differentiate into granular cells, they begin to accumulate keratinocyte-specific proteins involved in terminal differentiation of the horny layer.

The skin barrier of the horny layer shows three key features: (i) intercellular lipid layers, (ii) the cornified cell envelope and (iii) the keratin filament network and keratohyaline granules.[9] Genetic defects in these components may result in various cutaneous disorders, such as ichthyosis, which is characterized by dry, thickened, scaly or flaky skin. The word "ichthyosis" is from the Ancient Greek, *ichthys*, meaning "fish".

The keratin filament network is an important basic structure for maintaining the integrity and dimensions of the cornified cell, and the degraded products of the keratohyalin granules, filaggrins, aggregate the keratin filaments in apoptosed keratinocytes into bundles and promote the flattening of dead-cell remnants.[10-13]

Abnormalities in the barrier function of the horny layer have been

hypothesized as permitting epicutaneous allergen exposure in atopic and asthmatic patients. Furthermore, these alterations may, in part, help to explain the recent dramatic increase in atopic and asthmatic disorders in humans living in industrialized nations.

Filaggrin expression and function

The term 'filaggrin' is derived from filament aggregation protein. A giant inactive precursor, profilaggrin is a large, complex, highly phosphorylated polypeptide that is the main constituent of the keratohyalin granules that are visible in the granular cell layer of the epidermis (Fig. 1). The profilaggrin/filaggrin gene (*FLG*) resides on chromosome 1q21 and consists of three exons (Fig. 2). Exon 3 is extremely large (>12kb) and encodes most of the profilaggrin polypeptides with almost completely homologous 10, 11 or 12 repeats. Filaggrin is initially synthesized as profilaggrin, a >400-kDa, highly phosphorylated, histidine-rich polypeptide that comprises an S100 calcium-binding domain, a B-domain and two imperfect filaggrin-repeat domains flanking 10 to 12 essentially identical filaggrin repeats, as well as a C-terminal domain (Fig. 2).[14,15] On terminal differentiation of keratinocytes, profilaggrin is dephosphorylated and cleaved into 10 to 12 essentially identical 37-kd filaggrin peptides. As mentioned above, the liberated filaggrin subsequently and highly efficiently aggregates the keratin filament, which causes the keratinocytes to collapse in the stratum corneum.[10,13] The collapsed crytoskeleton is crosslinked by transglutaminases to bind it to the cornified cell envelope. Filaggrin is subsequently degraded into amino acids that act to retain epidermal moisture.[13,16] Thus, filaggrin is a key protein during terminal differentiation

and it is essential for the formation of a normal, intact, protective, and correctly moisturized skin barrier.[9,13]

Filaggrin deficiency caused by *FLG* mutations results in ichthyosis vulgaris (IV)

IV (OMIM 146700) is a common semidominant inherited skin disorder, estimated to affect 1 in 250 individuals. The onset is early childhood. It is characterized by generalized dry and scaly skin prominent on the extensor surfaces of limbs and on the lower abdomen, and it is associated with palmoplantar hyperlinearity (Fig. 3a,b).[2,17] The symptoms subside during the summer and aggravate during the winter, when the skin tends to dry. Histologically, a decrease in the size and number, or a complete absence, of keratohyalin granules in the epidermis is characteristic of IV. (Fig. 3c-f).[2,18] An association between IV and profilaggrin has long been suspected, but the gene that encodes profilaggrin, *FLG*, proved technically challenging to sequence. *FLG* resides on human chromosome 1q21 within the so-called epidermal-differentiation complex (EDC). The EDC is a dense cluster of genes involved in the terminal differentiation of the epidermis and the formation of the stratum corneum, the outermost dead cell compartment of the skin, where the main skin barrier function resides.

The initiation codon of the *FLG* gene is located in exon 2, although the bulk of the profilaggrin polyprotein in encoded by exon 3 (Fig. 2). Sequencing of exon 3 is problematic, not only because of its size (>12 kb) but also because it consists of between 10 and 12 tandemly arranged filaggrin repeat units. Some individuals have duplication of the 8th and/or 10th domain. The huge size, polymorphic variations in the number of

filaggrin repeats, and highly repetitive nature prevent sequencing of the entire gene. Despite these difficulties, the improvement of PCR strategy by the use of long-range sequencing and multiple alignment techniques that permit comprehensive sequencing of the entire *FLG* gene have recently been developed.[14,17] In 2006, two null mutations, R501X and 2282del4, in the *FLG* gene were first identified in patients with moderate or severe IV in 15 kindreds from Scottish, Irish, and European-American populations.[17] To date, approximately 40 loss-of-function *FLG* mutations have been identified in IV and/or AD in European populations and Asian populations (Fig. 4).[6,19] In addition, IV was found to exhibit semidominance, with incomplete penetrance (~90% in homozygotes). The homozygotes or compound heterozygotes had a severe form of IV, while the heterozygotes displayed mild or no phenotype.

The genotype/phenotype correlation in *FLG* mutations has not been clarified. *FLG* truncation mutations at any site within the profilaggrin peptide were reported uniformly to result in severe deficiency of profilaggrin/filaggrin processing.[14] Currently, it has been hypothesized that the profilaggrin C-terminal region is essential for proper processing of profilaggrin to filaggrin and, eventually, truncation at any site of profilaggrin results in abolishment of filaggrin/profilaggrin peptides. The hypothesis is supported by the finding of the nonsense mutation K4022X in the C-terminal incomplete filaggrin repeat. In the epidermis of patients carrying this mutation, profilaggrin/filaggrin peptides were remarkably reduced, even though *FLG* mRNA expression was not reduced significantly and the expressed mRNA included messages derived from both the wild-type alleles and the mutant alleles.[20] Histopathologically,

however, the size of keratohyaline granules in the granular layers was decreased and immunohistochemically profilaggrin/filaggrin peptides were remarkably reduced in the patients' epidermis. These observations further support the hypothesis that the profilaggrin C-terminal region is essential for proper profilaggrin processing. It is now generally considered that all the truncation mutations lead to serious loss of filaggrin peptides, resulting in the absence of genotype/phenotype correlations with regard to *FLG* mutations in IV and AD.

Prevalent filaggrin mutations are distinct in each race

To date, approximately 40 loss-of-function *FLG* mutations have been identified in IV and/or AD in European populations and Asian populations (Fig. 4).[6,19] Mutations in *FLG* were initially identified in European families.[17,21,22] To establish baseline *FLG* mutation data for the Japanese population, we performed *FLG* mutation searches in more than 30 Japanese families with IV, after sequencing methods for the entire *FLG* coding region had been established. 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*: 3321delA, S2554X, S2889X, and S3296X.[23,24] In 2009, we reported two additional novel *FLG* mutations, S1695X and Q1701X, in the Japanese population.[25] Futhermore, we studied 19 newly recruited Japanese patients with AD and identified a novel *FLG* nonsense mutation, K4022X, in one patient with AD without any other known Japanese *FLG* mutation.[20] In addition, one of the common European mutations, R501X, was reported in a Japanese family.[26] The study was repeated in other Asian populations, including

Chinese,[27] Taiwanese[28] and Korean populations.[29] Only two identical mutations (R501X and E2422X) were reported in both European and Asian populations.[26,27] 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 occurred de novo in Japan.[26] Among Asian populations, 3321delA was found in all four East Asian populations[23,26-29] and Q2417X was reported in Chinese and Taiwanese populations.[27,28] These results have revealed the differences in filaggrin population genetics between Europe and Asia (Fig. 4). As mentioned above, most *FLG* mutations are specific to a population, such as Europeans, Japanese, Singaporeans, Chinese, and Taiwanese. 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 to assess 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. For mutation screening, we have to obtain information on prevalent *FLG* mutations in each population.

Filaggrin mutations confer strong genetic susceptibility to atopic dermatitis

AD, one of the most common skin disorders, affects 15-20% of children in the developed world. AD often presents with IV. AD is a pruritic skin disease that typically starts early in life. The onset is during the first 6 months of life in 45% of affected individuals, the first year of life in 60% of affected individuals, and before 5 years of age in at least 85% of affected

individuals.[30] The hallmark of the disease is a pruritic dermatitis that localizes in different areas depending on age. In infancy it tends to affect the face and extensors of the lower legs, and in childhood the flexural areas; in adulthood the eruption has a more diffuse distribution. Other important diagnostic indications include xerosis of the skin, early age of onset, and a chronic, relapsing course. The incidence and prevalence of AD decreases with increasing age. AD is thought to have various heterogeneous etiologic factors, including genetic predisposing factors and environmental factors. Despite considerable efforts to elucidate genes that confer susceptibility to AD and to clarify the genetic background of atopic disorders, until recently no strong and reproducible genetic factor has been identified.[31] Transepidermal water loss (TEWL) and SC hydration, which are measurements of skin barrier function, were reported to be increased in AD patients due to their skin barrier insufficiency.[32] Significant correlations were observed between penetration rates of a hydrophilic dye and elevated IgE levels in patients with severe AD.[33] In addition, percutaneous penetration of sodium lauryl sulphate was reported to be increased in uninvolved skin of patients with AD.[34] Taken together, these findings strongly support the hypothesis that patients with AD have a skin barrier defect. Three clues suggested that *FLG* mutations play an important role in AD pathogenesis. First, dermatologists have recognized that AD often occurs in patient with IV, although the pathophysiological mechanisms of this co-occurrence have not been fully clarified.[35-37] Second, the linkage of AD to the chromosome locus on 1q21, which contains the epidermal differentiation complex where *FLG* resides, has been reported.[38] Third, decreased filaggrin expression has been reported in the skin of patients with AD at both the mRNA and the protein levels.[39,40]

Palmer et al. first reported that decreased or absent *FLG* expression due to loss-of-function mutations leads to impaired barrier function which manifests as AD.[21] They found that AD was manifested in heterozygous carriers of two null *FLG* mutations, R501X and 2282del4, with a relative risk (odds ratio) for AD of 13.4, implying a causal relationship. Thereafter, about twenty case-control analyses and eight familial analyses investigated the association between filaggrin gene defects and AD. Most of the studies were on western European populations, but three case-control studies and one family study were on a Japanese population and one case-control study was on a North American population.[14,41-47] In the Japanese population, there are at least eight *FLG* mutations. We showed that about 27% of the patients in our Japanese AD case series carried one or more of these eight *FLG* mutations (OR: 9.94; 95%; CI: 3.77 - 26.2) and that these variants were also carried by 3.7% of the Japanese general control individuals.[20] Meta-analysis *FLG* mutation studies on AD, focusing on the European-prevalent mutations (R501X or 2282del4) found an overall OR of 4.78 (95%; CI: 3.31 - 6.92) from the case-control studies and a summary OR of 1.99 (95%; CI: 1.72 - 2.31) from the family studies.[8] The strong association between *FLG* mutations and AD marked a milestone in the genetic study of complex allergic disorders. It was confirmed that the strong effect of *FLG* mutations on AD risk exceeds that of any other candidate predisposing gene for AD identified so far. Based on the information of population-specific *FLG* mutations, many cohort studies of AD for *FLG* mutations were performed and approximately 25-50% of AD patients were revealed to harbour *FLG* mutations as a predisposing factor.[6]

As mentioned above, every population is likely to have a unique set of *FLG* mutations. Population differences highlighted by *FLG* mutations

make it difficult to perform worldwide screening for *FLG* mutations in patients with AD. 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 screen Asian patients with AE. For 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 *FLG* mutations.

FLG mutations and asthma

The clinical cause of atopic disorders has been described as an atopic or allergic march. It involves sensitisation to food or aeroallergens, or both, in early life, progressing to eczema and wheezing within the first two years of life, and often leading to chronic asthma, rhinitis, and other clinical manifestations of atopic allergy in later life. Previous studies showed that 70% of patients with severe AD developed asthma, compared with 30% of patients with mild AD, and approximately 8% of the general population.[48] Previous studies in European populations have reported that variants in the *FLG* gene are associated with eczema and concomitant asthma[41-45] or with eczema alone.[22] One recent meta-analysis study showed that *FLG* mutations are significantly associated with asthma (OR: 1.48; 95%; CI, 1.32 - 1.66). And strong effects for the compound phenotype of asthma plus eczema (OR: 3.29; 95%; CI, 2.84 - 3.82) were observed. In contrast, *FLG* mutations did not seem to be associated with asthma in the absence of eczema (OR: 1.11; 95%; CI: 0.88 - 1.41).[49]

To clarify whether *FLG* mutations are a predisposing factor for asthma in non-European populations, we studied 172 Japanese AE patients,137 Japanese asthma patients and 134 unrelated Japanese control individuals.

There is a statistically significant association between the eight *FLG* mutations and AE with asthma, and between the eight *FLG* mutations and AE without asthma. In the Japanese general asthma cohort, there was a statistically significant association between the eight *FLG* mutations and asthma with AE. There was no statistically significant association between the *FLG* mutations and overall asthma patients, nor between *FLG* mutations and asthma without AE. This Japanese cohort has a completely different *FLG* mutation spectrum from those in the European and the North American populations. However, our results clearly confirm the strong association of *FLG* mutations with our Japanese cohort of AE patients with asthma complications, and the association of *FLG* mutations and asthma patients with AE complications.[50]

The mechanism of the asthma risk associated with *FLG* null alleles is not yet fully understood. *FLG* is expressed in the skin and in the outer layers of the oral and nasal mucosae, but not in the respiratory epithelium of the nose or the lower airways.[51,52] Therefore it has been suggested that *FLG*-associated asthma is mediated by percutaneous priming[53] and/or secondary, possibly systemic, immunologic mechanisms stimulated through the impaired skin barrier. Recent studies hypothesized that skin barrier defects caused by *FLG* mutations allow allergens to penetrate the epidermis and to interact with antigen-presenting cells (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.[53,54]

Filaggrin mutations and allergic rhinitis

Three case-control studies investigated the association between filaggrin gene defects and the risk of developing allergic rhinitis in people without AD.[42,55,56] Recent meta-analysis study showed that *FLG* mutations are significantly associated with allergic rhinitis without AD (OR: 1.78; 95%; CI: 1.16 - 2.73). In addition, the *FLG* mutations are significantly associated with allergic rhinitis with AD (OR: 2.84; 95%; CI: 2.08 - 3.88).[8] Filaggrin is expressed in the anterior vestibulum of the nose, but not in transitional and respiratory nasal epithelia.[56] Thus, it seems unlikely that *FLG* mutations exert organ-specific and localized effects in the upper airways. The mechanisms through which *FLG* mutations contribute to airway disease are not understood yet. Percutaneous priming and secondary immunologic effects from the induction of Th2 cytokines in epithelia are interesting hypotheses that need further investigation.

Novel treatment for AD based on recent *FLG* mutation studies

The epidermal barrier dysfunction caused by *FLG* mutations has been recognized as a major contributor to the pathogenesis of AD over the past few years. The skin barrier defect is the primary event that initiates disease pathogenesis, allowing the entrance of numerous antigens into the epidermis in patients with AD. Thus, the restoration of skin barrier function seems a feasible and promising strategy for prophylactic treatment of AD patients with *FLG* mutation. There have been efficient clinical methods to restore skin barrier function, including the application of general moisturizers and specific lipid replacement therapy.[57] When used under nursing supervision, moisturizers have been shown to reduce topical steroid

usage.[58] In addition, the topical application of ceramide-dominant lipid replacement therapy was proved effective in alleviating skin barrier defects and reducing AD severity significantly in childhood AD patients.[59]

Regarding the association between filaggrin deficiency and sensitization to specific antigens, allergen exposure during early life may increase the risk of AE, but the protective effect of reduction in allergen exposure remains uncertain. According to a population-based, longitudinal birth cohort study by Henderson et al., eczema associated with *FLG* mutations presents in early life and is persistent.[60] In addition, a strong association was identified between *FLG* mutations and sensitisation to grass, house dust, mites, and cat dander. Our study revealed that AD disease severity and specific IgE for house dust, mite allergen, and cat dander were significantly correlated in *FLG* mutation-related patients with AD.[61] In light of this, 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 AD development. Further studies are required to clarify the preventive effect of early intervention against AD in high-risk, filaggrin-deficient children.

B).

Figure legends

Fig. 1. Skin barrier function and allergic risk. (a) Normal skin: In the granular layer, keratohyaline granules composed of profilaggrin predominate. Upon terminal differentiation of keratinocytes, the products of degradation, filaggrins, aggregate keratin filaments and flatten the keratinocytes to form an effective barrier against external allergens. (b) In IV and AD with *FLG* mutation, there is a reduction or complete absence of filaggrin. The defective skin barrier allows external antigens to penetrate the epidermis, where they interact with antigen-presenting cells (Langerhans cells and dermal dendritic cells), which might further initiate the Th2 immune response and lead to the development of atopic disorders. (Modified from[3].)

Fig. 2. The *FLG* gene, which is located within the epidermal differentiation complex on chromosome 1q21, comprises three exons and two introns. Exon 1 (15 bp) consists only of a 5' untranslated (UTR) sequence, exon 2 (159 bp) contains the translation initiation codon, and exon 3 contains a S100 calcium-binding domain, a B-domain and two imperfect filaggrin-repeat domains flanking 10 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 8th and/or 10th filaggrin repeat(s).

Fig. 3. Clinicopathological features of IV. (**a**) Marked, adherent scales are clearly visible on the pretibial region of this IV patient. (**b**) Marked plantar hyperlinearity is seen in this IV patient. (**c, e**) Hematoxylin and eosin staining. Normal control skin (**c**) shows abundant keratohyalin granules in the granular layers. In contrast, the IV patient who is heterozygous for S2554X (**e**) shows a lack of granular layers in the epidermis, where basophilic substances that resemble keratohyalin are present in only small amounts and only intermittently. (**d, f**) In immunohistochemical staining for filaggrin, normal control skin (**d**) stains strongly. The IV patient (**f**) shows a marked reduction in staining for filaggrin. Bar: 50 μ m.

Fig. 4. Reported *FLG* mutations in a diagram of the profilaggrin peptide. Several of the mutations are rare, but a number of recurrent mutations have been identified (bold). Note that *FLG* mutations in the European and the Asian populations appear to be unique to each population. Only two mutations (R501X and E2422X) were reported in both European and Asian populations. The *FLG* mutations among Asian populations are shown (red = Japanese, blue = Chinese, brown = Taiwanese, black = Korean). Mutations are distributed widely in the profilaggrin sequence and the mutation K4022X is the most distal mutation in the C-terminal incomplete filaggrin repeat. The duplications of the 8th and 10th filaggrin repeats are represented as 8^{-1}, 8^{-2}, 10^{-1} and 10^{-2}.

References

1. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005; 6:328-40.

2. Sybert VP, Dale BA, Holbrook KA. Ichthyosis vulgaris: Identification of a defect in synthesis of filaggrin correlated with an absence of keratohyaline granules. J Invest Dermatol 1985; 84(3), 191-194.

3. McGrath JA, Uitto J. The filaggrin story: Novel insights into skin-barrier function and disease. Trends Mol Med 2008; 14(1), 20-27.

4. McGrath JA. Filaggrin and the great epidermal barrier grief. Australas J Dermatol 2008; 49(2), 67-73; quiz 73-64 .

5. Sandilands A, Terron-Kwiatkowski A, Hull PR et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis and atopic eczema. Nat Genet 2007; 39,650-4.

6. Akiyama M. FLG mutations in ichthyosis vulgaris and atopic eczema:Spectrum of mutations and population genetics. Br J Dermatol 2010; 162,472-477.

7. Irvine Ad, McLean WH. Breaking the (un)sound barrier: Filaggrin is a major gene for atopic dermatitis. J Invest Dermatol 2006; 126(6), 1200-1202.

8. Van den Oord RA, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitisation and allergic disorders: Systematic review and meta-analysis. BMJ 2009; 339, b2433.

9. Akiyama M, Shimizu H. An update on molecular aspects of the non-syndromic ichthyoses. Exp Dermatol 2008; 17(5), 373-382.

10. Steinert PM, Cantieri JS, Teller DC, Lonsdale-Eccles JD, Dale BA. Characterization of a class of cationic proteins that specifically interact with intermediate filaments. Proc Natl Acad Sci U S A 1981; 78(7), 4097-4101 .

11. Dale BA, Resing KA, Lonsdale-Eccles JD. Filaggrin: A keratin filament associated protein. Ann NY Acad Sci 1985; 455, 330-342.

12. Listwan P, Rothnagel JA. Keratin bundling proteins. Methods Cell Biol 2004; 78, 817-827.

13. Sandilands A, Sutherland C, Irvine AD, McLean WH. Filaggrin in the frontline: Role in skin barrier function and disease. J Cell Sci 2009; 122 (Pt 9), 1285-1294.

14. Sandilands A, Terron-Kwiatkowski A, Hull PR et al. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. Nat Genet 2007; 39(5), 650-654.