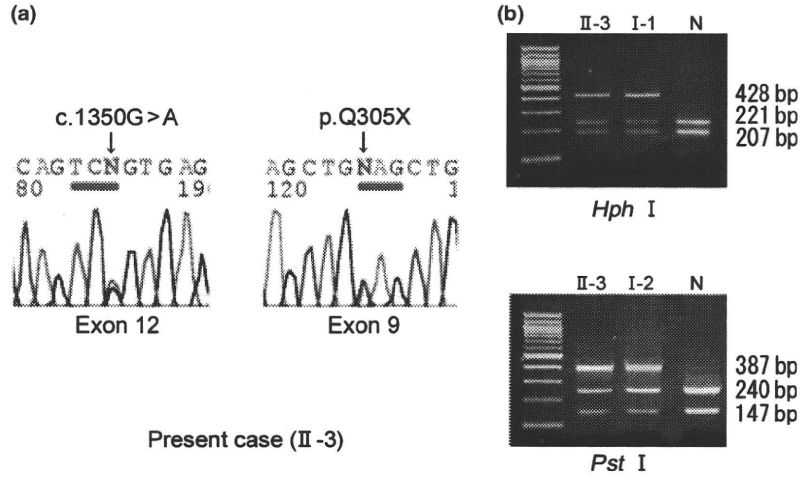

Figure 2 Analysis of the plectin gene mutations in genomic DNA from amniocytes of a fetus at risk. (a) Mutation analysis of genomic DNA from amniocytes shows both the c.1350G>A mutation in exon 12 and p.Q305X mutations in exon 9. (b) The presence of the mutations was verified by restriction enzyme digestion. The paternal mutation abolished a recognition site for the *Hph*I restriction enzyme. In the case of the normal allele, the 428-bp fragment was digested to 221 bp and 207 bp (lane N), whereas in the case of the mutant allele, a 428-bp fragment resisted digestion in the PCR product (father: lane I-1; present fetus: lane II-3). The maternal mutation also abolished a recognition site for the *Pst*I restriction enzyme. In the case of the normal allele, the 387-bp fragment was digested to 240 bp and 147 bp (lane N), whereas in the case of the mutant allele, a 387-bp fragment resisted digestion in the PCR product (mother: lane I-2; present fetus: lane II-3)

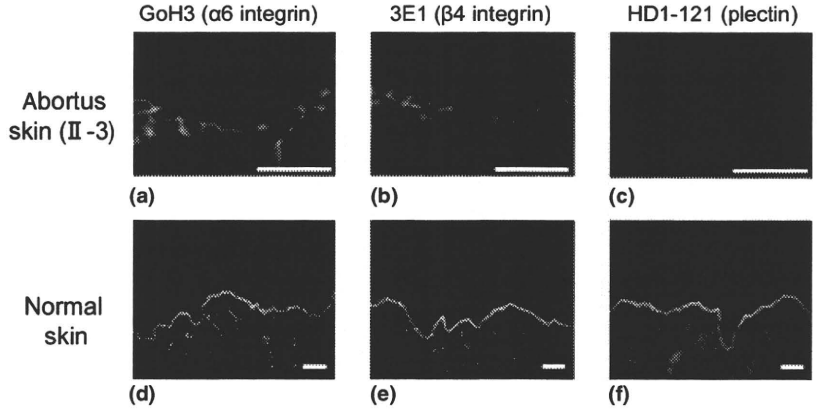

Figure 3 Absence of plectin expression in the abortus. α6 integrin (mAb GoH3) and β4 integrin (mAb 3E1) are expressed in the abortus skin (a, b) and the control skin (d, e). Staining with monoclonal antibody for plectin (mAb HD1–121) shows positive in the control skin (f) but negative in the skin of the abortus (c: blue frame). Note that the skin tissue from the abortus was subject to degeneration before skin sampling. Thus, protein localization cannot be evaluated in the degenerated tissue. Scale bar: 50 μm

was a compound-heterozygote and affected by JEB-PA. The parents elected for the fetus to be terminated at 20 weeks gestation.

Immunofluorescence analysis showed that immunoreactivity using the mAbs HD1–121 (plectin), GoH3 (α6 integrin), and 3E1 (β4 integrin) was positive in the normal control skin (Fig. 3d–f). The skin sample obtained from the abortus tested positive for α6 integrin and β4 integrin (Fig. 3a,b) but negative for plectin (Fig. 3c).

Discussion

This is the first successful PND of plectin-deficient EBS-PA, and the correct diagnosis was reconfirmed in the skin of the abortus. Given the universal mortality of EBS-PA due to *PLEC* mutations, there might be unreported PND cases for this form of EB. The prognosis of plectin-deficient EBS-PA is poor, and most patients commonly die within the first year of life,[13] as happened in the first- and

second-born progeny in the present family. Fetuses at risk of this condition are frequently terminated during pregnancy, and DNA-based PND plays an important role in prohibiting unnecessary termination of healthy fetuses at risk. Due to the recent elucidation of the causative genetic defects for genetic skin disorders, it has become possible to make DNA-based PND for severe genodermatoses by sampling of the chorionic villus or amniotic fluid in the earlier stages of pregnancy with a lower risk to fetal health and with a reduced burden on the mothers.

Plectin, a component of the hemidesmosome inner plaque, is involved in the attachment and crosslinking of the cytoskeleton and intermediate filaments to specific membrane complexes.[10] It has been described that EBS associated with muscular dystrophy (EBS-MD) results from *PLEC* mutations.[14,15] Mutations in the rod domain of *PLEC* are known to cause EBS-MD.[9,14,15] In addition, recent reports have confirmed that some *PLEC* mutations also lead to EBS-PA.[7–9,13] One alternative splice *PLEC* mRNA transcript that lacks exon 31 encoding the central core rod domain was identified in rat tissues.[16] By plectin-domain-specific reverse transcriptase-PCR, expression of this rodless alternative spliced form was confirmed in human keratinocytes.[17] Recently, our group demonstrated that loss of the full-length plectin with maintenance of the rodless plectin leads to EBS-MD, whereas complete loss or marked attenuation of full-length and rodless plectin expression underlies the EBS-PA phenotype.[12] The present family further supports the hypothesis that homozygotes or compound-heterozygotes for mutations that cause plectin truncation outside the rod domain show the EBS-PA phenotype.

In summary, this is the first report of DNA-based PND of EBS-PA. EBS-PA has now been added to the list of severe genodermatosis for which DNA-based PND is feasible.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan to H. Nakamura (Kiban C 1959129107) and H. Shimizu (Kiban A 21249063).

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BJD
British Journal of Dermatology

Childhood subepidermal blistering disease with autoantibodies to type VII collagen and laminin-332

DOI: 10.1111/j.1365-2133.2010.10065.x

MADAM, Autoimmune subepidermal blistering diseases include bullous pemphigoid, pemphigoid gestationis, linear IgA bullous dermatosis, mucous membrane pemphigoid (MMP), anti-p200 pemphigoid, epidermolysis bullosa acquisita (EBA) and bullous systemic lupus erythematosus.[1] Patients with EBA have IgG autoantibodies to type VII collagen while some patients with MMP have autoantibodies to laminin-332.[2,3] We describe a juvenile case of subepidermal blistering disease with autoantibodies to both type VII collagen and laminin-332. The present case is unique because of its childhood onset and successful remission following only topical steroid therapy.

A 12-year-old Japanese girl presented with pruritic eruptions on her scalp. A few weeks later, widespread pruritic vesicles gradually developed over her whole body. The vesicles were seen both on erythematous and normal skin (Fig. 1a, b). Blisters and erosions also appeared in her oral mucosa, but there was no involvement of genital or ocular mucous membranes (Fig. 1c). Neither nail changes nor alopecia were observed. She had no family history of any blistering disorders or autoimmune disease. There was no preceding illness or history of medication/vaccination that might have triggered her disease.

General laboratory examinations revealed no apparent abnormalities except for an increased serum IgE level (668·8 IU mL^{-1}; normal < 100 for age 7–14 years). A skin biopsy was taken from the edge of one blister on her right forearm. Light microscopy showed a subepidermal blister with an inflammatory cell infiltrate consisting of mainly neutrophils in the upper dermis (Fig. 2a). Direct immunofluorescence of the patient's lesional skin showed *in vivo* linear deposits of IgG and C3 at the epidermal basement membrane zone (Fig. 2b). On the blistered area, deposition of IgG and C3 was demonstrated on the dermal side of the separated skin (arrows, Fig. 2b). Indirect immunofluorescence with the patient's serum on 1 mol L^{-1} NaCl-split normal human skin showed IgG antibodies bound to the dermal side of the blister (Fig. 2c). Immunoblot analysis revealed that the patient's serum reacted with a 290-kDa protein in dermal extracts, and further with purified laminin-332 α3 protein (145, 165 kDa) (Fig. 2d, e). Laminin-332 was obtained from human keratinocytes and was purified using an antilaminin-332 affinity column as

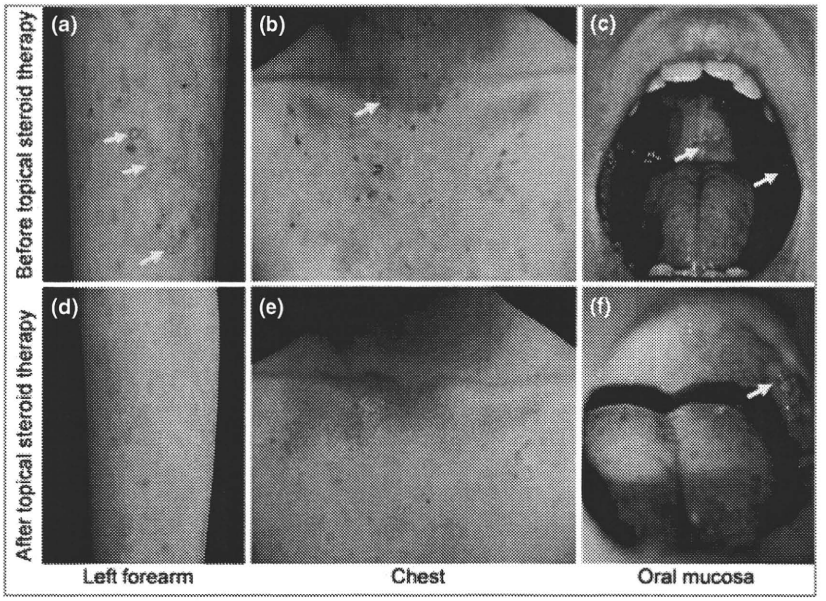

Fig 1. Clinical manifestations of the skin and oral mucosa. (a–c) Before topical steroid therapy. Erythema and tense vesicles on the left forearm and chest (a and b, arrows). Blisters and erosions over the oral mucosa (c, arrows). (d–f) After topical steroid therapy. Skin lesions healed within 9 days of the beginning of treatment, leaving residual pigmentation, scars and milia (d and e). Blisters and erosions on the oral mucosa subsided (f, arrow).

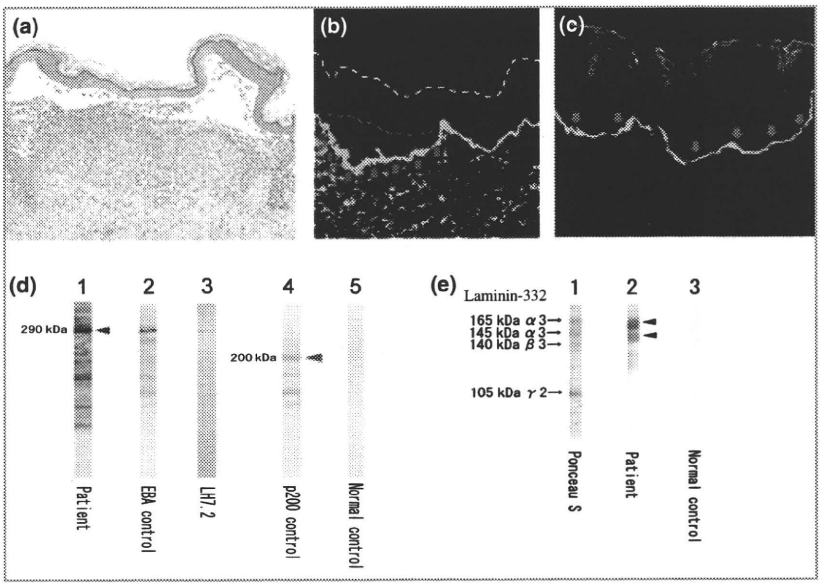

Fig 2. Histopathological findings, immunofluorescence staining and immunoblot analyses. (a) A subepidermal blister with an inflammatory cell infiltrate composed of mainly neutrophils in the upper dermis (haematoxylin and eosin; original magnification × 40). (b) Direct immunofluorescence showed *in vivo* linear deposits of IgG along the basement membrane zone. On the blister area, deposition of IgG was shown to be towards the dermal side of separated skin (arrows) (original magnification × 40; white dotted line is the skin surface and red dotted line is the roof side of separated skin). (c) Indirect immunofluorescence with the patient's serum on 1 mol L^{-1} NaCl-split normal human skin showed IgG antibodies bound to the dermal side (arrows) (original magnification × 40). (d) Immunoblot analysis revealed that the patient's serum (lane 1), like both serum from a reference patient with epidermolysis bullosa acquisita (EBA, lane 2) and monoclonal antibody LH7.2 to type VII collagen (lane 3), reacted with a 290-kDa protein in dermal extracts (arrowhead). Control anti-p200 serum did not react with the 290-kDa but with a 200-kDa protein (red arrowhead) (lane 4). Normal control serum (lane 5) showed reactivity with neither. (e) In immunoblotting of purified laminin-332, lane 1 shows Ponceau S stain (protein staining using amido black). Reactivity with 145-kDa and 165-kDa purified laminin-332 α3 protein (arrowheads) was indicated in the patient's serum (lane 2), but not in the normal control serum (lane 3).

previously described.[4,5] Purified laminin-332 was a generous gift from Dr S. Amano, Shiseido Life Science Research Centre, Yokohama, Japan. The patient was diagnosed as having an autoimmune subepidermal blistering disease with circulating autoantibodies to type VII collagen and laminin-332.

Treatment was initiated with 0·05% clobetasol propionate ointment 20 g daily to skin lesions, which healed within 9 days after the beginning of treatment, leaving residual pigmentation, scars and milia (Fig. 1d, e). Blisters and erosions on the oral mucosa subsided without any topical therapy (Fig. 1f). The dose of topical corticosteroids was progressively decreased, and no recurrence of skin lesions was observed. The titre of antibasement membrane zone antibodies in indirect immunofluorescence studies decreased from 1 : 320 to 1 : 40 over 2 months. We performed further immunoblot analyses on five serial serum samples obtained from the patient after her antibasement membrane zone antibodies decreased. All five samples showed similar reaction bands to both 290-kDa protein in dermal extracts and purified laminin-332 α3 protein (145, 165 kDa) (data not shown). Hence it is difficult to speculate the major target antigen in this patient from these results. No local or systemic side-effects of topical corticosteroids were noticed during the entire treatment duration.

EBA and MMP are distinct autoimmune bullous diseases that are both characterized by autoantibodies to dermoepidermal junction components.[1] Detection of autoantibodies to either type VII collagen or laminin-332 differentiates these two diseases.[1] Interestingly, besides antitype VII collagen antibodies, circulating antilaminin-332 α3 antibodies were also found in our patient's serum. According to our survey of the literature, three other previous cases of subepidermal blistering disease with circulating antibodies to both type VII collagen and antilaminin-332 have been reported (Table 1).[6–8] All of the reported cases are of adult onset, thus our report is the first juvenile case. Similar to our patient, these reported patients all presented with mucosal involvement.

Our case is unique in its course and prognosis as well as age at onset. All of the previously reported patients needed systemic corticosteroids or immunosuppressant agents for proper disease control. In the studies by Jonkman *et al.*[6] and Umemoto *et al.*,[7] the bullous lesions of the patients relapsed after systemic prednisolone was tapered. The skin lesions of the patient reported by Baican *et al.*[8] were refractory to systemic prednisolone, azathioprine and dapsone. However, our juvenile case was successfully treated with only topical steroids, and no recurrence was observed in the following 6 months. Our case suggests that the treatment outcome and prognosis of juvenile cases are better than those of adult-onset cases. Further accumulation of similar juvenile cases is needed to confirm this hypothesis. The differ-

Table 1 Comparison of four reported patients with circulating antitype VII collagen and antilaminin-332 antibodies

Patient	Age (years)/sex	Skin lesion	Mucosal involvement	Treatment	Outcome	Immunoblot analysis	Reference
1	64/F	Blisters and erythema on the hands and feet	Oral/genital	Oral prednisolone 80 mg daily	Lesions resolved without scars/milia. Mild relapse occurred when tapering to oral prednisolone 5 mg daily	Type VII collagen, laminin-332 α3	Jonkman *et al.*[6]
2	46/M	Erythematous plaque, blisters, erosions and crusts on the trunk and extensor aspects of extremities	Oral	Oral colchicine 1·5 mg daily (refractory to prednisolone, azathioprine and dapsone)	Previous lesions healed with milia and scars. Free of new blisters but erythematous plaque persisted with erosions and crusts	Type VII collagen, laminin-332 α3, γ2	Baican *et al.*[8]
3	35/F	Vesicular lesions on the face, neck and upper back	Oral/genital	Oral prednisolone 40 mg daily	Lesions resolved without scars/milia. Mild relapse occurred when tapering to oral prednisolone 25 mg daily	Type VII collagen, laminin-332 α3, β3	Umemoto *et al.*[7]
4	12/F	Blisters, erosions and erythema on the face, trunk, hands and feet	Oral	Topical clobetasol propionate ointment 20 g daily	Lesions resolved with scars and milia. No recurrence was found	Type VII collagen, laminin-332 α3	Our patient

ences between childhood-onset and adult-onset cases seem to mirror those of EBA at different ages. Compared with adult cases, childhood EBA cases respond relatively better to treatment, and usually low-dose oral prednisolone and dapsone are effective and sufficient.[1]

In conclusion, we report the first juvenile case with autoantibodies to both type VII collagen and laminin-332, successfully treated with only topical steroid therapy. Our case suggests that juvenile cases have different characteristics from those of adult-onset cases in their course, including treatment outcome and prognosis. As topical steroid therapy has several advantages over systemic corticosteroids due to less severe complications, we consider topical steroids as preferable to systemic steroids for childhood-onset autoimmune subepidermal bullous disease.

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Funding sources: none.

Conflicts of interest: none declared.

thickness of the mucous layer of small intestines, resulting in the inhibition of small intestinal absorption.[4] In addition, PGE1 increases blood flow in the stomach and upregulates the digestion in the stomach. During the provocation test in our case, serum gliadin levels were not increased by administering misoprostol. However, sodium cromoglicate, a mast cell stabilizer commonly used to treat allergic rhinitis, allergic conjunctivitis, and asthma, could not affect serum gliadin levels in the provocation test, and therefore allowed the symptoms to occur. We consider that the effects of misoprostol on the alimentary tract are crucial for the prevention of FDEIA. Our observation indicates that the exacerbating effect of aspirin in FDEIA comes from the inhibitory effects of aspirin on PGE1 in the gastrointestinal milieu. Thus, misoprostol would be a promising prophylactic drug for FDEIA.

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Type XVII collagen ELISA indices significantly decreased after bullous pemphigoid remission

The major pathogenic epitope of bullous pemphigoid (BP) is known to be the noncollagenous extracellular domain (NC16A) of type XVII collagen (COL17).[1] Here we investigated indirect immunofluorescence (IIF) and COL17 NC16A domain enzyme-linked immunosorbent assay (ELISA)[2–5] data before treatment and after remission to evaluate the usefulness of ELISA analyses as indicators for BP disease activity.

We included ten consecutive BP patients [eight women and two men: between 33 and 80 years old (mean; 59 years old)] who showed typical clinical features before treatment and were successfully treated, resulting in complete or partial remission at our institute. The first day of each patient visit was within the last three years. In all patients, the diagnosis was confirmed by histopathological observation and immunofluorescence study, i.e. histopathological subepidermal blister formation was observed and direct and IIF studies revealed the presence of autoantibodies along the dermal–epidermal junction. All patients were successfully treated with oral prednisolone therapy of 30–50 mg/d with or without azathioprine or a combination therapy using tetracycline and nicotinamide. Treatment periods from initial diagnosis to remission ranged from four months to 35 months (mean; 14.6 ± 10.8 months). Serum samples were obtained for ELISA and IIF at least twice during the disease course for each patient.

Concentration of autoantibodies in the patients' sera directed against the NC16A domain of COL17 was measured using the COL17 NC16A ELISA kit following the kit's instructions.[6] IIF staining and evaluation were performed as previously described using normal human skin as a substrate.[7]

In all the cases, the ELISA indices showed a decrease during the successful treatment course. ELISA indices after remission were significantly reduced compared with those before treatment ($P < 0.0001$) (Fig. 1a). IIF titers also decreased after remission in six cases, but the titers were not apparently reduced in the other four cases, although a statistically significant reduction in combined IIF titer was observed after remission compared with those before treatment ($P < 0.05$) (Fig. 1b).

Positive correlation between ELISA indices and BP disease activity has been reported previously in the literature. Di Zenzo *et al.*[8] demonstrated that disease severity before treatment was well correlated with ELISA indices in BP patients. Izumi *et al.*[9] described ELISA indices and alteration of disease activity of five BP patients during various treatments. In this study, we compared the ELISA

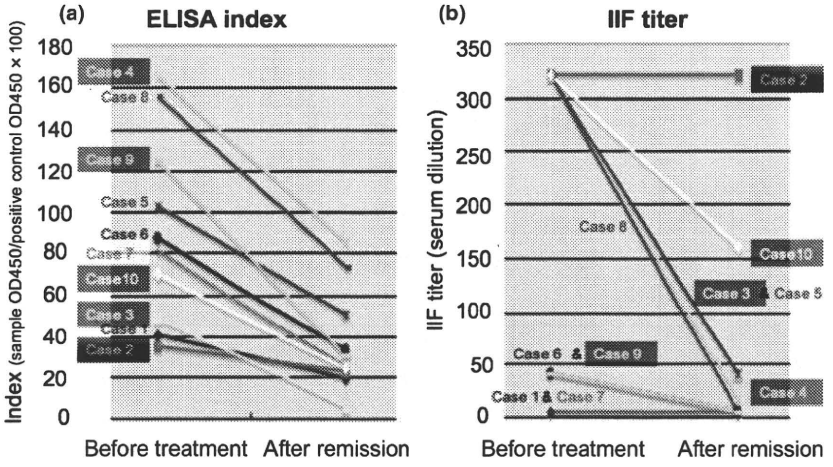

Figure 1 ELISA indices and indirect immunofluorescence (IIF) titers before treatment and after remission. (a) ELISA indices of successfully treated BP patients. Disease remission was defined as when erythema, bullae and erosions had completely healed (complete remission) or no more than three bullae or erythema were seen in a week (partial remission) and only a low dose of oral prednisolone (<5 mg/d) or no treatment was needed to maintain this condition. As ELISA indices after remission, we adopted ELISA indices at the time when each patient's disease activity was evaluated as being in "complete remission" or "partial remission" (as defined above) for the first time after treatment. Mean ELISA index of the 10 patients before treatment was 91.3 ± 45.7 (range: 35.6–165.6) and the mean index after remission was 37.4 ± 25.3 (range: 6.0–86.4). After complete or partial remission, the ELISA indices were significantly reduced ($P < 0.0001$). (b) IIF titers of the same patients. Apparent decreases in IIF titers after remission were seen only in six patients. Mean IIF titer of the 10 patients before treatment was 201 ± 154 (range: 5–320) and the mean titer after remission was 60.5 ± 102.8 (range: 5–320). A statistically, significant reduction was observed in combined IIF titers after remission compared with those before treatment ($P < 0.05$). Colors of the lines are specific for each patient in both figures (a) and (b)

indices before treatment and after remission in our BP patient cohort and clearly demonstrated that ELISA indices significantly decreased after remission. Feng *et al.*[10] reported similar results on correlation of ELISA indices with disease course in BP patients, although the time points for ELISA after treatment were just before the decrease in corticosteroid and when the dosage of corticosteroid was successfully decreased to half the initial dose in the report. In this study, we employed ELISA indices at the time when each patient's disease activity was evaluated as "complete remission" or "partial remission" for the first time after treatment. Thus, this study is unique in the point that we evaluated exact correlation between ELISA indices and disease remission.

In conclusion, the present results further support the idea that the COL17 NC16A ELISA indices demonstrate a correlation with the BP disease remission more accurately than IIF titers and are a useful tool to detect BP disease remission and to assess the efficacy of BP treatment.

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Conflict of interest: the authors state no conflict of interest.

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Allergology International. 2011;60:1-9
DOI: 10.2332/allergolint.10-RAI-0270

REVIEW ARTICLE

Filaggrin Gene Defects and the Risk of Developing Allergic Disorders

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ABSTRACT

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

allergic rhinitis, asthma, atopic dermatitis, atopic eczema, filaggrin, *FLG*, ichthyosis vulgaris

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 cross-linked 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 (>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.[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

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Received 22 September 2010.

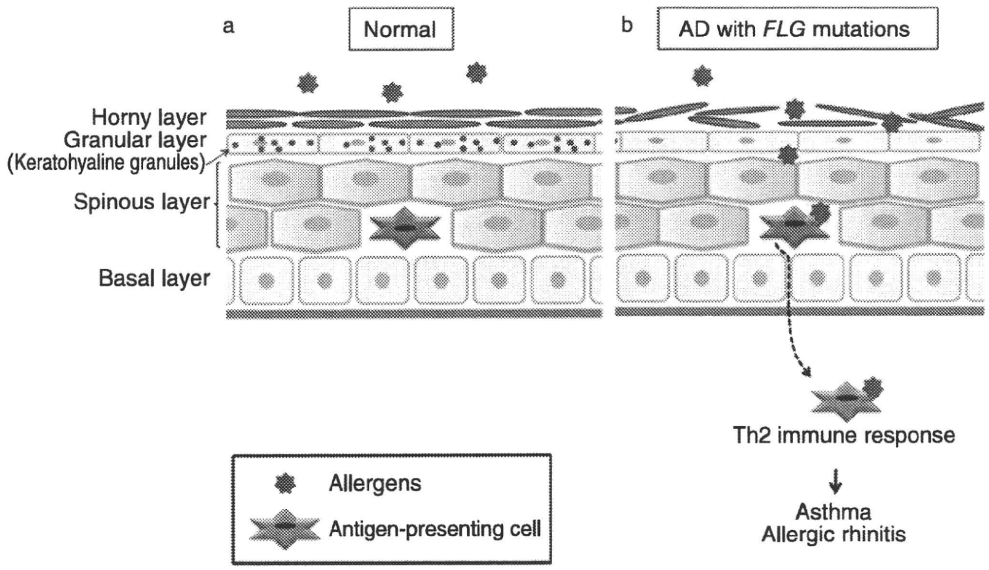

Fig. 1 Skin barrier function and allergic risk. (**a**) Normal skin: In the granular layer, keratohyaline granules composed of profilaggrin predominate. Upon terminal differentiation of keratinocytes, the products of degradation, filaggrins, aggregate keratin filaments and flatten the keratinocytes to 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].)

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 (>12 kb) 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

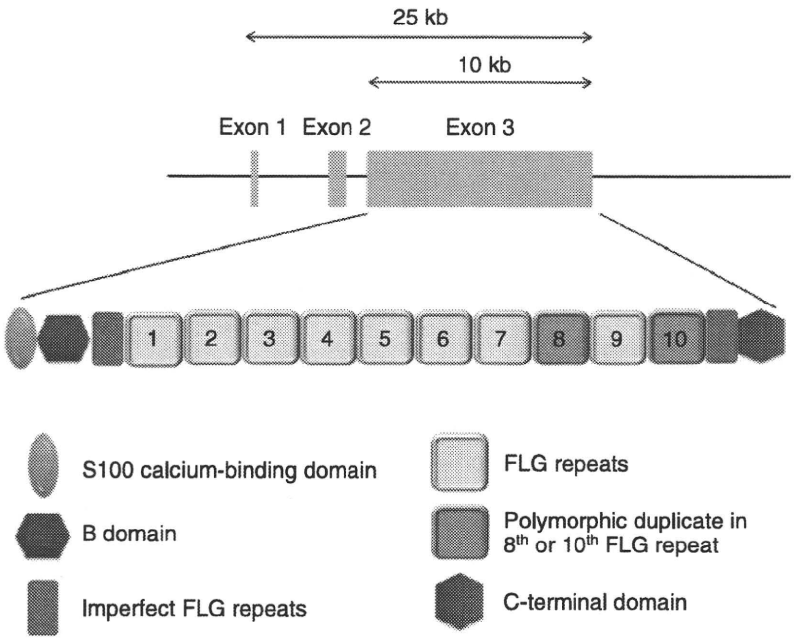

Fig. 2 The *FLG* gene, which is located within the epidermal differentiation complex on chromosome 1q21, comprises three exons and two introns. Exon 1 (15 bp) consists only of a 5' untranslated (UTR) sequence, exon 2 (159 bp) contains the translation initiation codon, and exon 3 contains a S100 calcium-binding 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).

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

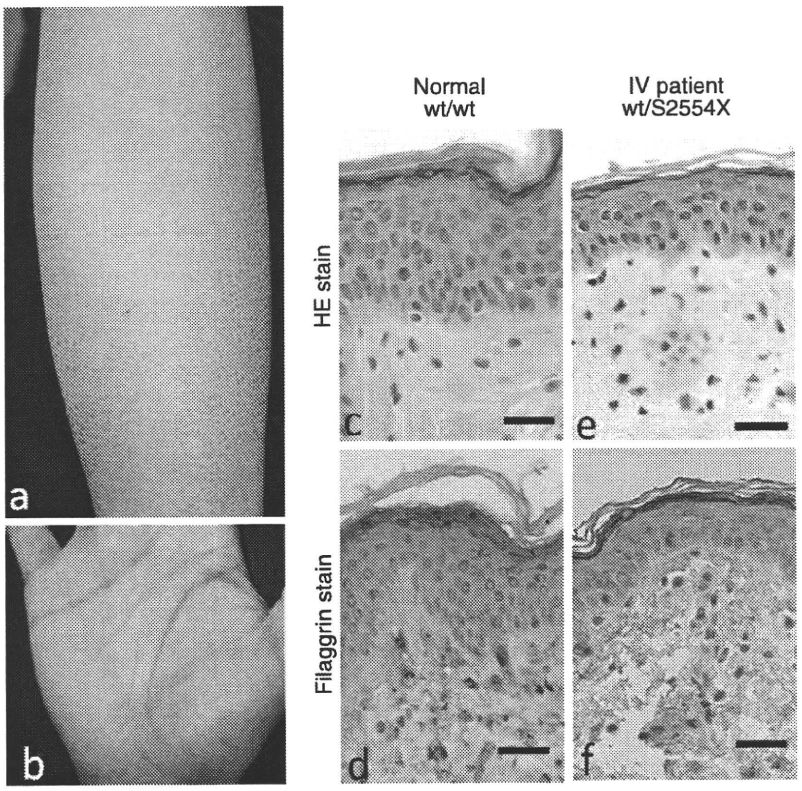

Fig. 3 Clinicopathological features of IV. (**a**) Marked, adherent scales are clearly visible on the pretibial region of this IV patient. (**b**) Marked plantar hyperlinearity is seen in this IV patient. (**c, e**) Hematoxylin and eosin staining. Normal control skin (**c**) shows abundant keratohyalin 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 μm.

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

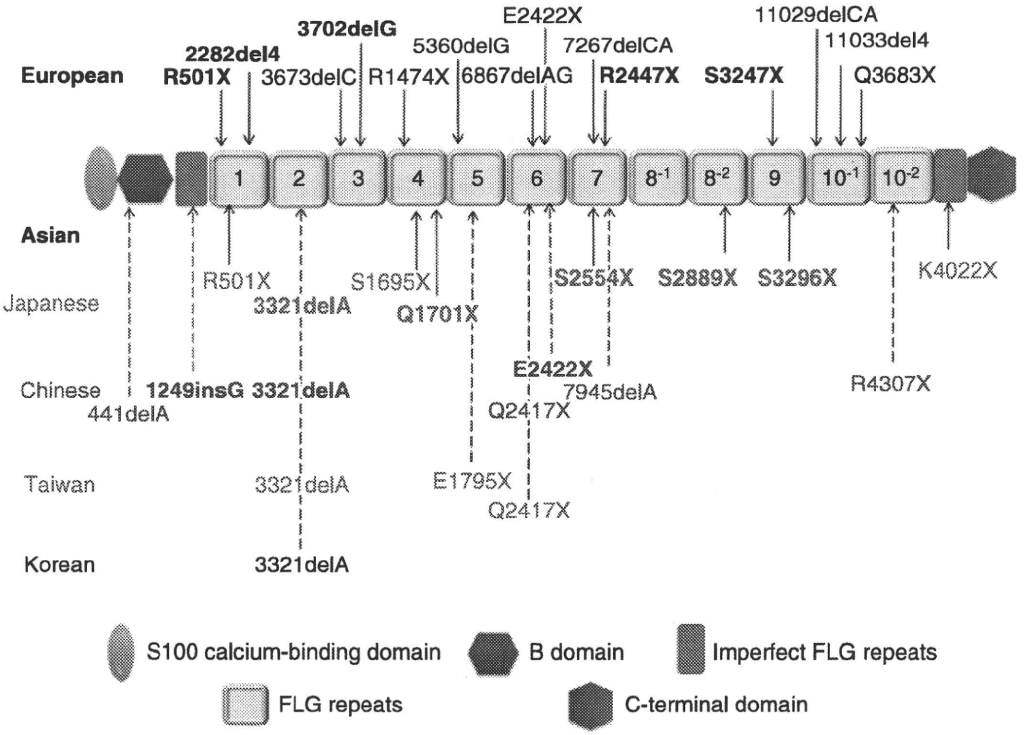

Fig. 4 Reported *FLG* mutations in a diagram of the profilaggrin peptide. Several of the mutations are rare, but a number of recurrent mutations have been identified (bold). Note that *FLG* mutations in the European and the Asian populations appear to be unique to each population. Only two mutations (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}.

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] Furthermore, 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 an 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 Q 2417X 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 2282 del4) 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.

ACKNOWLEDGEMENTS

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).

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THE JOURNAL OF DERMATOLOGY

doi: 10.1111/j.1346-8138.2011.01209.x

Journal of Dermatology 2011; **38**: 353–363

SPECIAL REPORT

Prevalence of dermatological disorders in Japan: A nationwide, cross-sectional, seasonal, multicenter, hospital-based study

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ABSTRACT

To clarify the prevalence of skin disorders among dermatology patients in Japan, a nationwide, cross-sectional, seasonal, multicenter study was conducted in 69 university hospitals, 45 district-based pivotal hospitals, and 56 private clinics (170 clinics in total). In each clinic, information was collected on the diagnosis, age, and gender of all outpatients and inpatients who visited the clinic on any one day of the second week in each of May, August, and November 2007 and February 2008. Among 67 448 cases, the top twenty skin disorders were, in descending order of incidence, miscellaneous eczema, atopic dermatitis, tinea pedis, urticaria/angioedema, tinea unguium, viral warts, psoriasis, contact dermatitis, acne, seborrheic dermatitis, hand eczema, miscellaneous benign skin tumors, alopecia areata, herpes zoster/postherpetic neuralgia, skin ulcers (nondiabetic), prurigo, epidermal cysts, vitiligo vulgaris, seborrheic keratosis, and drug eruption/toxicoderma. Atopic dermatitis, impetigo, molluscum, warts, acne, and miscellaneous eczema shared their top-ranking position in the pediatric population, whereas the most common disorders among the geriatric population were tinea pedis, tinea unguium, psoriasis, seborrheic dermatitis, and miscellaneous eczema. For some disorders, such as atopic dermatitis, contact dermatitis, urticaria/angioedema, prurigo, insect bites, and tinea pedis, the number of patients correlated with the average high and low monthly temperatures. Males showed a greater susceptibility to some diseases (psoriasis, erythroderma, diabetic dermatoses, *inter alia*), whereas females were more susceptible to others (erythema nodosum, collagen diseases, livedo reticularis/racemosa, hand eczema, *inter alia*). In conclusion, this hospital-based study highlights the present situation regarding dermatological patients in the early 21st century in Japan.

Key words: age, Japan, prevalence, sex, skin diseases.

INTRODUCTION

Skin forms the outermost part of the human body and it acts as a vital barrier to external and internal damage. Various external and internal stimuli, which can be either short- or long-term, can affect the homeostasis of the skin, leading to a variety of disorders. The development and perpetuation of skin disorders are multifactorial in nature, and can result from genetic, environmental, mechanical, meteorological and even cultural effects. Skin disorders therefore include a vast range of diseases.

Although it is difficult to know the exact prevalence or incidence of skin diseases, several hospital-based

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Received 1 September 2010; accepted 1 September 2010.

studies have shown that skin diseases are very common. Of a total of 11 191 patients seen by a general practitioner in the UK, 2386 (21%) presented dermatological complaints. Among these there was a preponderance of females (1604, 67%), and the most common skin diseases seen were viral warts, eczema and benign tumors.[1] In the Netherlands, 235–460/1000 person-years of children aged 0–17 years contacted general practitioners in 1987 and 2001,[2] and these contacts frequently involved bacterial, viral, fungal, eczematous or traumatic skin diseases.[2] Tamer *et al.* reported on 6300 pediatric cases aged 0–16 years who visited dermatological clinics in Turkey; this group showed a preponderance of bacterial, viral and eczematous skin diseases.[3] In the case of Japan, there is no authentic report in the published work on any investigation of the prevalence of skin diseases; therefore, the Japanese Dermatological Association conducted a nationwide, cross-sectional, seasonal, multicenter, hospital-based study.

METHODS

A total of 190 dermatology clinics at 76 university hospitals, 55 district-based pivotal hospitals and 59 private clinics participated in this study. At each clinic,

Table 1. Numbers of patients recruited in each season

	Number of patients				
	May 2007	August 2007	November 2007	February 2008	Total
University Hospitals $n = 69$	8558	7944	7782	7778	32 062 (47.54%)
District-based Hospitals $n = 45$	3505	3450	2890	2864	12 709 (18.84%)
Private clinics $n = 56$	5779	6709	5364	4825	22 677 (33.62%)
Total	17 842	18 103	16 036	15 467	67 448 (100%)

Table 2. Age distribution and sex difference of patients

Age distribution (years old)	Number of patients	Male patients	Female patients	Sex undescribed
0–5	4192 (6.22%)	2200 (7.12%)	1983 (5.49%)	9
6–10	2099 (3.11%)	1047 (3.39%)	1047 (2.9%)	5
11–15	1711 (2.54%)	815 (2.64%)	893 (2.47%)	3
16–20	2270 (3.37%)	995 (3.22%)	1266 (3.5%)	9
21–25	3219 (4.77%)	1245 (4.03%)	1960 (5.43%)	14
26–30	3516 (5.21%)	1378 (4.46%)	2126 (5.89%)	12
31–35	4050 (6%)	1546 (5%)	2483 (6.87%)	21
36–40	3807 (5.64%)	1604 (5.19%)	2180 (6.03%)	23
41–45	3298 (4.89%)	1387 (4.49%)	1879 (5.2%)	32
46–50	3201 (4.75%)	1326 (4.29%)	1848 (5.12%)	27
51–55	4062 (6.02%)	1763 (5.71%)	2279 (6.31%)	20
56–60	5543 (8.22%)	2503 (8.1%)	3012 (8.34%)	28
61–65	5413 (8.03%)	2533 (8.2%)	2846 (7.88%)	34
66–70	5629 (8.35%)	2775 (8.98%)	2824 (7.82%)	30
71–75	6157 (9.13%)	3195 (10.34%)	2923 (8.09%)	39
76–80	4777 (7.08%)	2487 (8.05%)	2259 (6.25%)	31
81–85	2636 (3.91%)	1297 (4.2%)	1318 (3.65%)	21
86–90	1098 (1.63%)	508 (1.64%)	583 (1.61%)	7
91–100	427 (0.63%)	166 (0.54%)	259 (0.72%)	2
≥101	16 (0.02%)	3 (0.01%)	2 (0.01%)	11
Age undescribed	327 (0.48%)	126 (0.41%)	155 (0.43%)	46
Total	67 448 (100%)	30 899 (100%)	36 125 (100%)	424

information on diagnosis, age and sex was collected from all outpatients and inpatients who visited the clinics or who were hospitalized on any single day of the second week in each of May, August and November 2007 and February 2008. Reports on the monthly average values of the high and low temperatures and humidities were collected from the Meteorological Agency. The information on 67 448 cases from 170 clinics (69 university hospitals, 45 district-based pivotal hospitals and 56 private clinics) that participated in all of the four seasonal examinations was analyzed. Statistical analyses were performed by using Spearman's rank correlation coefficient. A P-value of <0.05 was considered to be statistically significant. This study was approved by the internal ethical review boards of the Japanese Dermatological Association.

Table 3. Prevalence of skin diseases in 67 448 patients

Burn	899 (1.33%)	Syphilis	24 (0.04%)
Trauma	409 (0.61%)	Miscellaneous sexually transmitted diseases	41 (0.06%)
Skin ulcer (nondiabetic)	1334 (1.98%)		
Pressure ulcer	608 (0.9%)	Bullous pemphigoid	510 (0.76%)
Miscellaneous physico-chemical skin damage	681 (1.01%)	Pemphigus	424 (0.63%)
		Miscellaneous bullous diseases	141 (0.21%)
Diabetic dermatoses	436 (0.65%)	Systemic sclerosis	619 (0.92%)
Atopic dermatitis	6733 (9.98%)	Systemic lupus erythematosus	525 (0.78%)
Hand eczema	2024 (3%)	Dermatomyositis	304 (0.45%)
Contact dermatitis	2643 (3.92%)	Miscellaneous collagen diseases	915 (1.36%)
Seborrheic dermatitis	2213 (3.28%)	Anaphylactoid purpura	171 (0.25%)
Miscellaneous eczema	12590 (18.67%)	Reticular/racemous livedo	81 (0.12%)
Urticaria/angioedema	3369 (4.99%)	Miscellaneous vasculitis/purpura/ circulatory disturbance	632 (0.94%)
Prurigo	1229 (1.82%)		
Drug eruption/toxicoderma	1018 (1.51%)	Mycosis fungoides	427 (0.63%)
Psoriasis	2985 (4.43%)	Miscellaneous lymphomas	285 (0.42%)
Palmoplantar pustulosis	832 (1.23%)	Pigmented nevus	709 (1.05%)
Miscellaneous pustulosis	172 (0.26%)	Seborrheic keratosis	1095 (1.62%)
Lichen planus	200 (0.3%)	Soft fibroma/acrochordon	231 (0.34%)
Miscellaneous inflammatory keratotic disorders	241 (0.36%)	Epidermal cyst	1194 (1.77%)
		Lipoma	173 (0.26%)
Tylosis/clavus	917 (1.36%)	Dermatofibroma	111 (0.16%)
Ichthyosis	61 (0.09%)	Miscellaneous benign skin tumors	1666 (2.47%)
Miscellaneous keratinization disorders	502 (0.74%)	Actinic keratosis	261 (0.39%)
Ingrown nail	597 (0.89%)	Basal cell carcinoma	324 (0.48%)
Miscellaneous nail disorder	397 (0.59%)	Squamous cell carcinoma/Bowen's disease	455 (0.67%)
Alopecia areata	1653 (2.45%)		
Androgenic alopecia	210 (0.31%)	Paget's disease	224 (0.33%)
Miscellaneous skin appendage disorders	266 (0.39%)	Malignant melanoma	808 (1.2%)
Scabies	98 (0.15%)	Miscellaneous malignant skin tumors	534 (0.79%)
Insect bite	762 (1.13%)	Vitiligo vulgaris	1134 (1.68%)
Tinea pedis	4379 (6.49%)	Chloasma/senile freckle	336 (0.5%)
Tinea unguium	3231 (4.79%)	Miscellaneous pigmented disorders	154 (0.23%)
Miscellaneous tinea	610 (0.9%)	Erythema multiforme	197 (0.29%)
Candidiasis	408 (0.6%)	Erythema nodosum	111 (0.16%)
Miscellaneous mycosis	211 (0.31%)	Miscellaneous disorders with erythematous plaques	130 (0.19%)
Acne	2430 (3.6%)		
Impetigo contagiosum	507 (0.75%)	Nevus/phacomatosis (other than pigmented nevus)	267 (0.4%)
Folliculitis	755 (1.12%)		
Erysipelas	81 (0.12%)	Rosacea/rosacea-like dermatitis	150 (0.22%)
Cellulitis	594 (0.88%)	Granulomatous diseases	192 (0.28%)
Miscellaneous bacterial infection	914 (1.36%)	Keloid/hypertrophic scar	186 (0.28%)
Molluscum contagiosum	604 (0.9%)	Cheilitis/angular cheilitis/mucous membrane diseases	95 (0.14%)
Herpes simplex	691 (1.02%)		
Herpes zoster/zoster-associated pain	1609 (2.39%)	Erythroderma	63 (0.09%)
Viral wart	3028 (4.49%)	Other diseases	666 (0.99%)
Miscellaneous viral disorders	353 (0.52%)	Total	67 448 (100%)