学位論文の要旨

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学位論文名　Filaggrin-Gene Mutation Has Minimal Effect on the Disease Severity in the Lesions of Atopic Dermatitis

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論文内容の要旨

INTRODUCTION

Atopic dermatitis (AD) is a common chronic inflammatory dermatosis characterized by intractable itching and recurrent eczema caused by type 2 helper T lymphocyte (Th2)-dominant inflammation. The Pathogenesis of AD is thought to arise from the interplay between the genetic background and environmental factors. Filaggrin (FLG) plays an important role in epidermal differentiation, especially epithelial barrier formation in the skin. Loss-of-function mutations in the FLG gene (*FLG*) are the strongest known genetic risk factors for AD. Abnormal FLG proteins translated by loss-of-function mutations of the *FLG* result in an inability to maintain the cytoskeleton of keratinocytes and to produce natural moisturizing factors in the stratum corneum. It is still debatable how *FLG* mutations and the resulting abnormal amount of FLG protein contribute to skin barrier function and symptoms of AD. Aim of the present study was to clarify the effect of *FLG* mutations on the barrier condition or severity of dermatitis in the lesions of AD patients.

MATERIALS AND METHODS

We included Japanese patients with AD who visited the Department of Dermatology, Shimane University Hospital, between September 2014 and January 2015. The patients were 35 males and 20 females, with a mean age of 27.7 ± 11.9 years

(range, 3-48 years). Genomic DNA was isolated from the blood collected from the patients, and eight *FLG* mutations (p.R501*, c.3321delA, p.S1695*, p.Q1701*, p.S2554*, p.S2889*, p.S3296*, and p.K4022*) were screened. Skin scores were assessed visually for each of the three skin sites (extremities, neck, and trunk) to assess the severity of the symptoms using six index parameters (erythema, edema, lichenification, oozing/exudation, excoriation, and xerosis/dryness) for modified severity scoring of AD (mSCORAD). Skin water content (SWC) and trans-epidermal water loss (TEWL) were measured at each skin site. Tape-stripping methods were used to collect the stratum corneum. FLG content in the stratum corneum was defined as two independent methods, immunofluorescent staining with anti-FLG antibody (FLG immunofluorescence) and an ELISA kit specific for FLG from the stratum corneum (FLG protein content). The total amount of free amino acids was determined by the o-phthalaldehyde (OPA) method, and amino acids present in the stratum corneum were quantified by high-performance liquid chromatography (HPLC).

This study was approved by the ethics committee of Shimane University Faculty of Medicine (approval no. 1372).

RESULTS AND DISCUSSION

Eight patients had loss-of-function mutations in the *FLG* (seven had a mutation in one allele and one had a compound heterozygous of p.S2554* and p.S2889*). The background of the eight patients with *FLG* mutations and the 47 patients without *FLG* mutations did not differ concerning to age, sex, severity score of AD, and period of AD.

We found no significant difference between the mutation carriers and non-carriers in the FLG protein amount determined by the two different methods (FLG immunofluorescence and FLG protein content), except for FLG protein content on the trunk. In addition, there was no significant difference in the total amino acid content between the mutation carriers and non-carriers at the all cites.

In the mutation carriers, no significant correlation was observed between the FLG protein amount and the mSCORAD, except in the FLG immunofluorescence on the neck. No significant correlation was observed between the FLG protein amount and the SWC and TEWL. In the non-mutation carriers, no significant correlation was observed between FLG amount and mSCORAD, SWC and TEWL, except for the FLG immunofluorescence in the extremities and the FLG protein content in the neck.

A significant correlation was observed between total amino acid content and mSCORAD in the extremities of both mutation carriers and non-carriers, whereas no

correlation was observed in the neck and trunk of both mutation carriers and non-carriers. No significant correlation was observed between the total amino acid content and the SWC in both mutation carriers and non-carriers, except for the trunk in the non-carriers. A significant correlation was observed between total amino acid content and TEWL in the neck and trunk of the non-mutation carriers. However, no significant correlation was observed between the total amino acid content and TEWL in the non-carriers. No significant difference was observed between the mutation carriers and non-carriers in the amount of all 17 amino acids detected. When the amounts of 17 amino acids were compared between the patients with mSCORAD $\geqq$ 1 and those with mSCORAD = 0, serine, glycine, alanine, citrulline, threonine, and asparagine, which were supposed to derive from FLG, were significantly lower in the patients with mSCORAD $\geqq$ 1, suggesting a dominant role of inflammation on the FLG protein amount.

To date, there have been many reports showing that mutations in the *FLG* cause a decrease in the amount of *FLG* products and barrier function in the skin of patients with *FLG* mutations; however, most of these studies have been conducted in non-lesional areas of patients with AD. Conversely, there are many reports that activation of Th2-dominant inflammatory cells is involved in the suppression of FLG production. The results obtained from this study support these reports that the activation of Th2-dominant inflammatory cells, together with *FLG* abnormality, together with *FLG* abnormalities, plays a role in suppressing the production of FLG in skin lesions, because the relationship between the *FLG* mutation and the severity of clinical symptoms of AD was not confirmed when the condition of lesions was compared between the mutation carriers and non-carriers.

CONCLUSION

The involvement of *FLG* mutations in the condition of skin lesions, the severity of dermatitis, barrier function, and the amount of *FLG* product in the stratum corneum was not established in this study, supporting the idea that the amount of *FLG* products, especially amino acids derived from FLG, in the stratum corneum of AD lesional skin might be influenced by the development of dermatitis together with loss-of function mutations of *FLG*. A limitation of the study is that it included only eight *FLG* mutation carriers and 47 non-carriers, and the results should be confirmed in a large number of cases.