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Letter to the Editor

Filaggrin-deficient rats generated using zinc-finger nucleases spontaneously exhibit dry scaly skin

Dear Editor,

Filaggrin (FLG) is one of key proteins involved in the skin barrier function that is known to be decreased in the epidermis of atopic dermatitis (AD) patients.^{1,2} A previous study demonstrated that *Flg*-null mice exhibited dry scaly skin and decreased natural moisturizing factors (NMF) that are filaggrin degradation products; however transepidermal water loss (TEWL) was normal.³ On the other hand, homozygous *Flg*-deficient patients are known to exhibit an increase in TEWL,⁴ highlighting that *Flg* deficiency is not tolerated equally between mice and humans.

Rat skin is the most structurally similar to human skin among rodents, e.g. the skin permeability of drugs through rat skin correlates with human skin for drug permeation.⁵ In this study, we newly generated and characterized *Flg* knockout (*Flg*^{-/-}) rats using the Zinc-finger nucleases (ZFNs) technology.⁶

To generate *Flg*^{-/-} rats, we targeted the second exon of rat *Flg* gene using conventional ZFN reagents⁶ (Fig. 1A) and generated *Flg*^{-/-} rats confirmed by immunohistochemical staining (Fig. 1B). Neonatal *Flg*^{-/-} rats exhibited dry scaly skin with a shiny tone, constriction rings on the tail, and prominent areas of crista cutis surrounded by deep furrows on the back when compared with wild-type littermates (Fig. 1C).

It is known that profilaggrin is deposited on keratin fibers and exists as a major component of keratohyalin granules, and that F granules containing profilaggrin are keratohyalin granules.⁷ In this study, we have demonstrated that normal large F granules were observed in wild-type rats and *Flg*^{+/-} rats by electron microscopy (Fig. 1D).

Mild growth restriction was observed in *Flg*^{-/-} rats (Fig. 2A), but both *Flg*^{-/-} rats and wild-type rats exhibited normal hair growth, and the trunk skin phenotype of *Flg*^{-/-} rats became less obvious after hair outgrowth (data not shown). It is generally agreed that increased TEWL is indicative of stratum corneum (SC) barrier dysfunction. TEWL is thus used as a surrogate marker to assess skin dryness and SC barrier function in patients with AD.⁸ In contrast to the results of normal TEWL

in *Flg*^{-/-} mice,³ we found an increase in TEWL 3 days after birth in *Flg*^{-/-} rats compared to wild-type rats (Fig. 2B). In addition, *in vivo* confocal Raman microspectroscopic analysis revealed a significant decrease in NMF but not ceramide that is known to affect the skin barrier function and TEWL (Fig. 2C, D). However, as these rats grew, these findings became less apparent; there was no significant difference in TEWL between 8-week-old wild-type and *Flg*^{-/-} rats (Fig. 2E). Electron microscopic analysis revealed that both 8-week-old wild-type and *Flg*^{-/-} rats exhibited similar morphologies of keratohyaline granules and compact stratum corneum (Fig. 2F). Finally, we used AD-like dermatitis model induced by a vitamin D3 analogue MC903.⁹ There was no significant difference in clinical scores between wild-type and *Flg*^{-/-} rats (Fig. 2D). Hematoxylin and eosin staining revealed no significant difference in epidermal thickness (Fig. 2D).

We successfully generated *Flg*^{-/-} rats by using the ZFN technology. A previous report showed that neonatal *Flg*^{-/-} mice displayed unaltered TEWL values;³ however, *Flg*^{-/-} rats showed an increase in TEWL spontaneously in this study. Discrepancy in the results between *Flg*^{-/-} rats and mice may be derived from differences in animal species. In patients with ichthyosis vulgaris and AD that can be induced by *FLG* mutations, an increase in TEWL has been reported.^{4,8} In this regard, *Flg*^{-/-} rats can be useful for analyzing the barrier function of the skin. In addition, we consider that growth retardation in the immediate postnatal period is due to dehydration caused by barrier dysfunction.

In conclusion, the newly developed *Flg*^{-/-} rats revealed that FLG play an essential role in TEWL but not in the severity of MC903-induced AD-like dermatitis. It is thought that AD generally develops in infancy and childhood, and that the number of patients decreases with age. One of the possible reasons why the incidence of AD decreases with growth may be the restoration of the barrier function. *Flg*^{-/-} rats are expected to be a valuable resource in various fields and to serve as an important experimental model for the analysis of skin barrier function.

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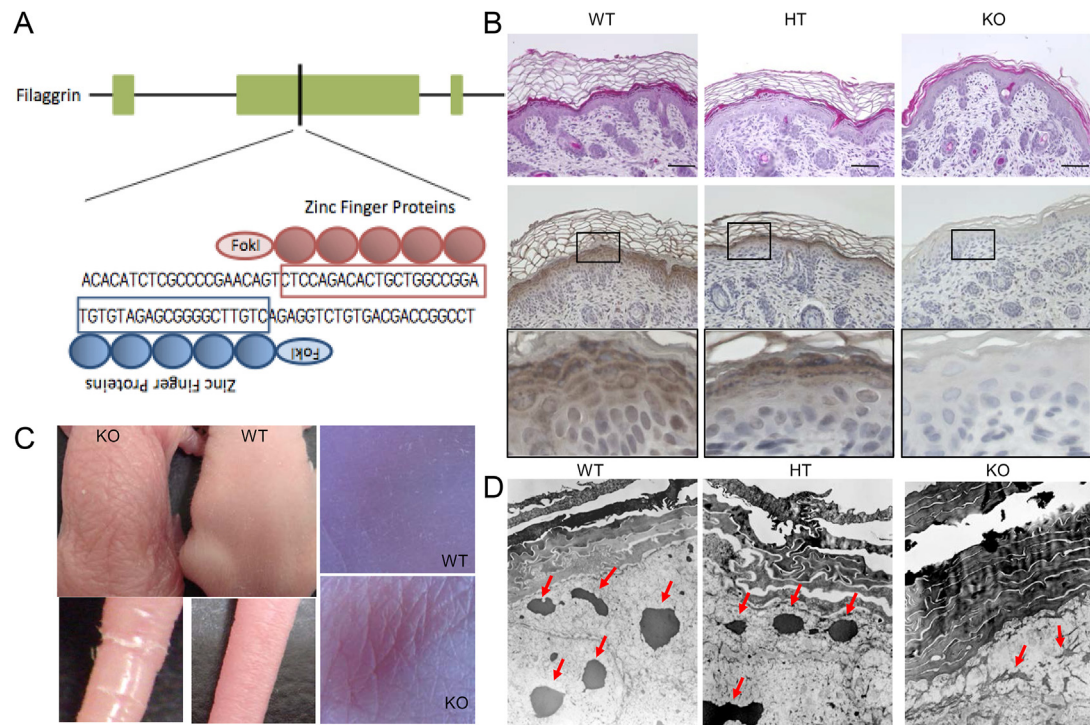


Fig. 1. Filaggrin-deficient rats generated with zinc-finger nucleases exhibit ichthyotic skin (A) Schematic representation of part of the rat *Flg* gene. The magnified views illustrate the binding sites for the ZFN pairs. The validated ZFN mRNA was microinjected into fertilized F344/Stm oocytes, which were then transferred into the oviducts of pseudopregnant Crlj:WI female rats (pseudopregnant foster mothers). Screening of eight newborn animals revealed that four of them (50%) carried mutations, comprising deletions from 7 bp to 2622 bp, which were frame-shift mutations resulting in the complete loss of Flg protein expression (B) Histologic (hematoxylin and eosin staining) images of wild-type (WT), *Flg*^{+/-} (HT), and *Flg*^{-/-} (KO) rats. Immunohistochemical staining of FLG was performed as below. Deparaffinized sections underwent antigen retrieval with 10 mM sodium citrate buffer and were immersed in 0.3% H₂O₂ to abolish endogenous peroxidase activity before being incubated with a monoclonal mouse anti-filaggrin (clone, AKH-1; Santa Cruz Biotechnology, Dallas, Texas) for 1 h at room temperature. The secondary antibody, ABC reagent, and DAB peroxidase substrate were added according to the manufacturer's protocol (VECTOR). The skin sections were counter-stained with hematoxylin (C) Macroscopic images of wild-type and *Flg*^{-/-} rats (D) Electronic microscopic images of wild-type, *Flg*^{+/-}, and *Flg*^{-/-} rats. Histological analysis was performed by using the samples of three days after birth (B and D) and macroscopic images were taken on the day four after birth (C).

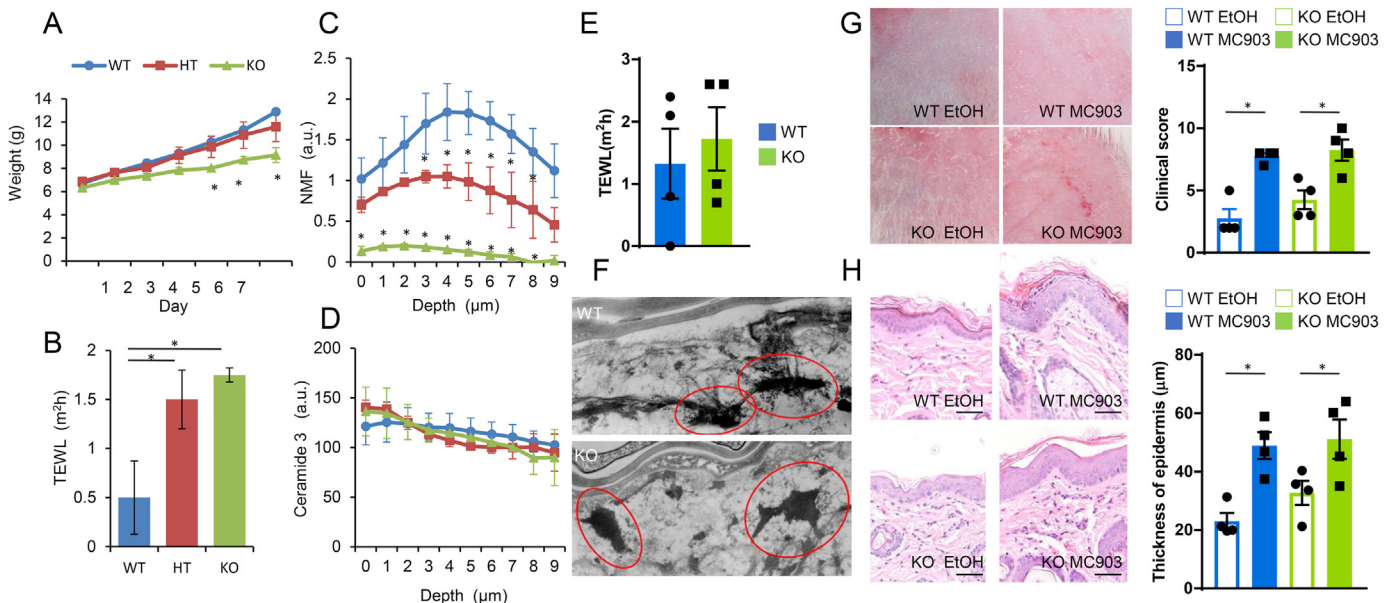


Fig. 2. Barrier functions in filaggrin-deficient rats (A) Body weight after birth (B) Comparison of TEWL among wild-type, *Flg*^{+/-}, and *Flg*^{-/-} rats. Measurements of TEWL were performed at room temperature (22–26 °C) and 40–60% humidity. TEWL was measured using a VAPOSCAN AS-VT100RS machine (Asahi Biomed). Dorsal skin NMF (C) and ceramide 3 (D) were measured *in vivo* using confocal Raman microspectroscopy (E) Comparison of TEWL among 8-week-old wild-type and *Flg*^{-/-} rats (F) Electronic microscopic images of 8-week-old wild-type and *Flg*^{-/-} rats. The area circled in red is a small keratohyaline granule (G) Clinical photographs after 7 days of daily application on the back of MC903 and the clinical score. The total clinical score for skin lesions was designated as the sum of individual scores, graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe), for the symptoms of erythema, edema, erosion, and scaling (H) Hematoxylin and eosin staining. Epidermal thickness was measured at three locations per field of view and averaged over five fields. Scale bar 50 μm. Each column represents the mean ± standard deviation (SD). **p* < 0.05, significantly different from the control group (*n* = 2–5 per group) using the Student's *t*-test.

Conflict of interest

The authors have no conflict of interest to declare.

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References

1. Otsuka A, Doi H, Egawa G, Maekawa A, Fujita T, Nakamizo S, et al. Possible new therapeutic strategy to regulate atopic dermatitis through upregulating filaggrin expression. *J Allergy Clin Immunol* 2014;**133**:139–46. e10.
2. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;**38**:441–6.
3. Kawasaki H, Nagao K, Kubo A, Hata T, Shimizu A, Mizuno H, et al. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. *J Allergy Clin Immunol* 2012;**129**:1538–46. e6.
4. Perusquia-Ortiz A, Oji V, Sauerland M, Tarinski T, Zareeva I, Seller N, et al. Complete filaggrin deficiency in ichthyosis vulgaris is associated with only moderate changes in epidermal permeability barrier function profile. *J Eur Acad Dermatol Venereol* 2013;**27**:1552–8.
5. Morimoto Y, Hatanaka T, Sugibayashi K, Omiya H. Prediction of skin permeability of drugs: comparison of human and hairless rat skin. *J Pharm Pharmacol* 1992;**44**:634–9.
6. Mashimo T, Takizawa A, Voigt B, Yoshimi K, Hiai H, Kuramoto T, et al. Generation of knockout rats with X-linked severe combined immunodeficiency (X-SCID) using zinc-finger nucleases. *PLoS One* 2010;**5**:e8870.
7. Ishida-Yamamoto A, Hohl D, Roop DR, Iizuka H, Eady RA. Loricrin immunoreactivity in human skin: localization to specific granules (L-granules) in acrosyringia. *Arch Dermatol Res* 1993;**285**:491–8.
8. Proksch E, Fölster-Holst R, Jensen J-M. Skin barrier function, epidermal proliferation and differentiation in eczema. *J Dermatol Sci* 2006;**43**:159–69.
9. Kim BS, Siracusa MC, Saenz SA, Noti M, Monticelli LA, Sonnenberg GF, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med* 2013;**5**:170ra16–ra16.

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