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Letter to the Editor

Atopic dermatitis-like dermatitis emerges unevenly on different sites in flaky tail mice

Keywords:

Atopic dermatitis; Flaky tail mice

To the Editor,

Flaky tail mice (FTM; *ma/ma*, *Flg^{fl/fl}*) have two pathogenic genetic abnormalities related to cutaneous permeability barrier homeostasis, namely, gene mutations in filaggrin (*Flg^{fl}*) and matted (*ma*), and are known to exhibit atopic dermatitis (AD)-like dermatitis “spontaneously” and the spontaneous emergence is mainly attributed to the matted mutation [1–4]. Although FTM has been used as one of the murine AD models, whether such spontaneous emergence is consistent with the pathogenesis of human AD, in which genetic abnormalities are not the sole determinant but the combination of genetic abnormalities and environmental factors is the most important characteristic, remains questionable. In the present study, we determined whether site-dependent emergence of skin manifestations, which is one of clinical characteristics of human AD, is seen in FTM.

The severity of dermatitis and age of onset in FTM differ among laboratories [1,2,5,6]. Such inconsistency might be related to the presence or absence of the *matted* mutation and/or to variations in the genetic background of individual strains and in environmental factors [2,7]. In our laboratory, young FTM (7–8 weeks old) did not have any clinical or histological symptoms of dermatitis [8]. FTM aged over 40 weeks (old FTM) developed marked dermatitis on the ears, eyelids, cheeks and neck accompanied by elevation of serum IgE levels (Fig. 1, Supplementary Figs. S1–S3). The dermatitis on the neck was associated with increased TEWL, SC pH, and epidermal thickness compared with those of C57BL/6. In contrast, in old FTM, only slightly rough skin (but no apparent dermatitis) was observed on the dorsal flank and abdomen, accompanied by mild epidermal thickness on the abdomen (Fig. 1, Supplementary Figs. S1 and S3). No difference was observed between old FTM and C57BL/6 in TEWL of the skin in the dorsal flank and abdomen (Fig. 1). SC hydration in old FTM was decreased on both the dorsal flank and abdomen compared with that in C57BL/6 (Supplementary Fig. S2). Accordingly to the elevation of SC pH, serine protease activity and TSLP expression in the epidermis were also elevated on the face and neck compared with those in the epidermis of the dorsal flank skin in old FTM (Fig. 2). An ELISA examining the levels of TSLP in whole skin also revealed the extremely elevated levels

of TSLP in neck of old FTM (Supplementary Fig. S4). FTM frequently scratched their face and neck, but it seemed hard for FTM to extend their extremities to scratch the dorsal flank (Supplementary Fig. S1).

In the present study, by comparing several physiological and pathological parameters among different sites, we clearly demonstrated that spontaneous AD-like dermatitis did not emerge on the whole body evenly, but was site-dependent, especially affecting the face and neck. These observations suggest that the genetic abnormalities in FTM confer susceptibility to exogenous stimuli and are not the sole determinant of the disease. Accordingly, susceptibility to the development of dermatitis after allergen exposure [1,2], hapten treatment [5] and SC pH neutralization [8] in FTM has been demonstrated. In fact, the neck and face, where significant dermatitis was observed, are frequently affected by mechanical stimuli, including scratching, foods, and friction with wood tips. Meanwhile, the precise mechanism by which spontaneous dermatitis emerges with site-dependency in FTM still remains unclear. Possibility of uneven involvement of the gene mutation in different sites of the body also might have to be considered.

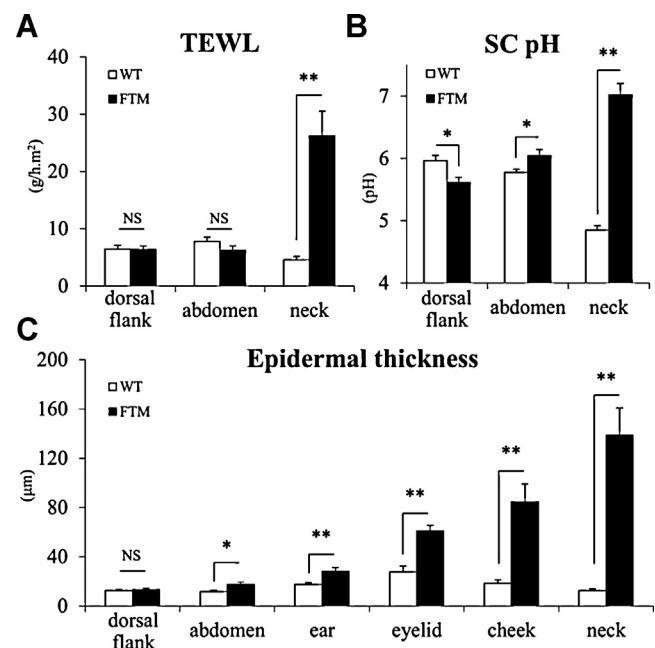


Fig. 1. Physiological and histological findings. Transepidermal water loss (TEWL, A), stratum corneum (SC) pH (B), and epidermal thickness (C) were compared at each site between old C57BL/6 (WT) and flaky tail mice (FTM) (40- to 90-week-old), as described in the supplementary file. *N* = 7–9 in A, *N* = 5–8 in B, and *N* = 6–10 in C. NS, not significant, * *P* < 0.05, ** *P* < 0.01.

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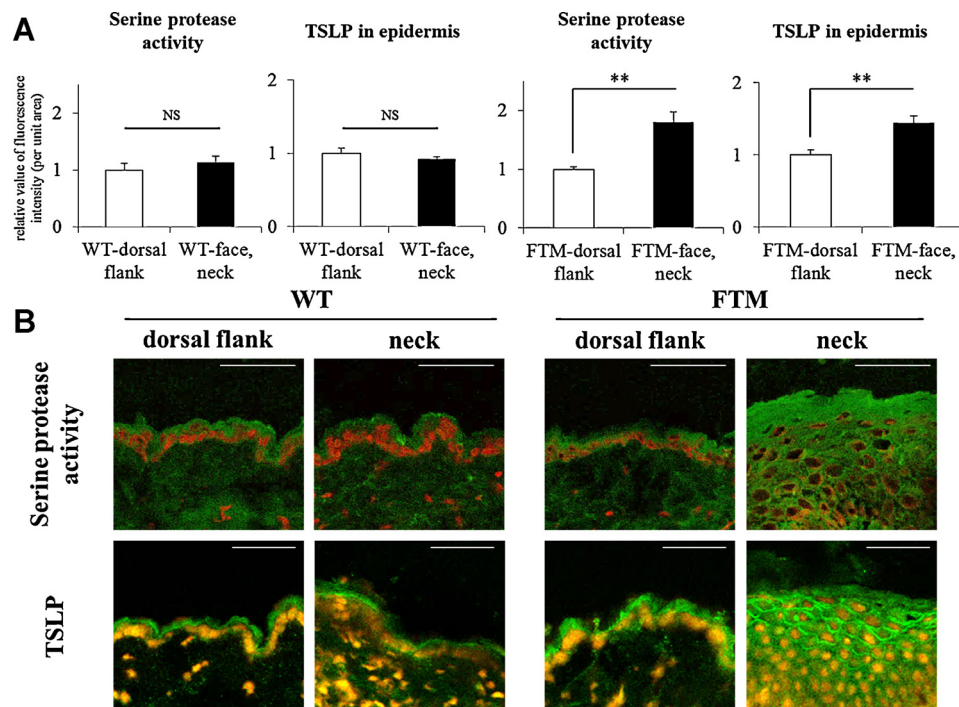


Fig. 2. Serine protease activity and expression of thymic stromal lymphopoietin (TSLP). *In situ* zymography for serine protease activity and immunohistochemistry for TSLP were performed as described in the supplementary file. Serine protease activity and expression of TSLP were enhanced in face and neck, compared to dorsal flank in flaky tail mice (FTM) (A). Representative images were shown in (B). Green staining represents activity of serine protease or expression of TSLP and red counterstaining represents nuclear staining with propidium iodide. WT, wild-type mice, NS, not significant, ** $P < 0.01$. Scale bar = 50 μm .

FTM is well known as a murine model that exhibits spontaneous AD-like dermatitis due to genetic abnormalities [2–4]. These studies showed that AD-like dermatitis occurred on the neck and face, but did not fully describe the skin condition at other sites. The present study, for the first time, focused on the site-dependent emergence of AD-like dermatitis in FTM, and indicated that the dermatitis of FTM is similar to that of human AD, in which genetic predisposition and environmental factors cooperatively contribute to the emergence of dermatitis. In addition, the present study showing the site-dependency of skin manifestations should be informative for researchers who are planning to use FTM as an AD model, although whether the site-dependent emergence in FTM is common among laboratories remains unclear.

As for the mechanism of emergence of AD-like dermatitis, it should be noted that elevation of SC pH accompanied by augmentation of serine protease activity and TSLP expression occurred in parallel with the appearance of skin lesions, supporting recent evidence that points to the importance of aberrant SC pH in the pathogenesis of AD [8–10].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jdermsci.2015.01.008>.

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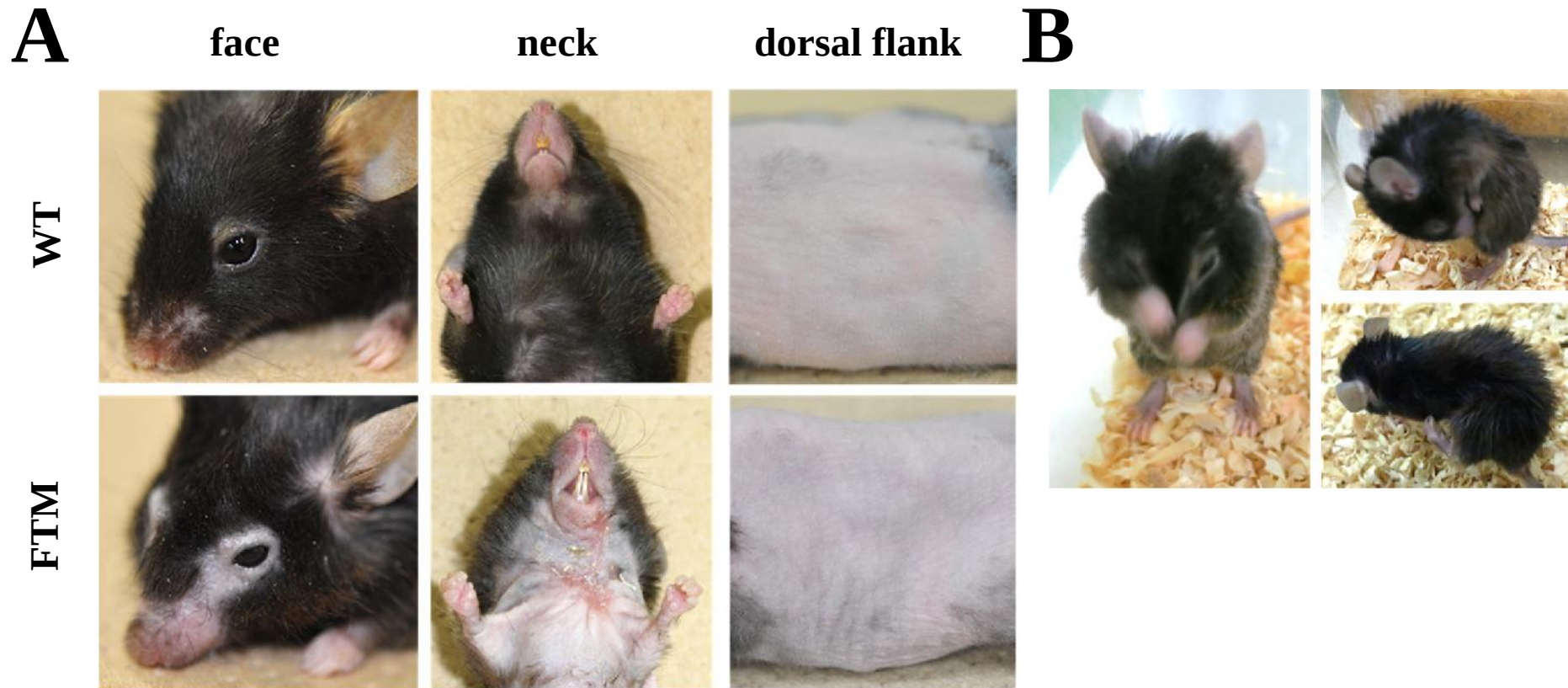


Figure S1: Clinical phenotype of old C57BL/6 (WT) and flaky tail mice (FTM) (40-90-week-old). Old FTM develop spontaneous dermatitis and alopecic lesion in face-neck area, but not in dorsal flank (A). Scratching behavior is often observed in FTM, but dorsal flank was not suffered from scratching (B).

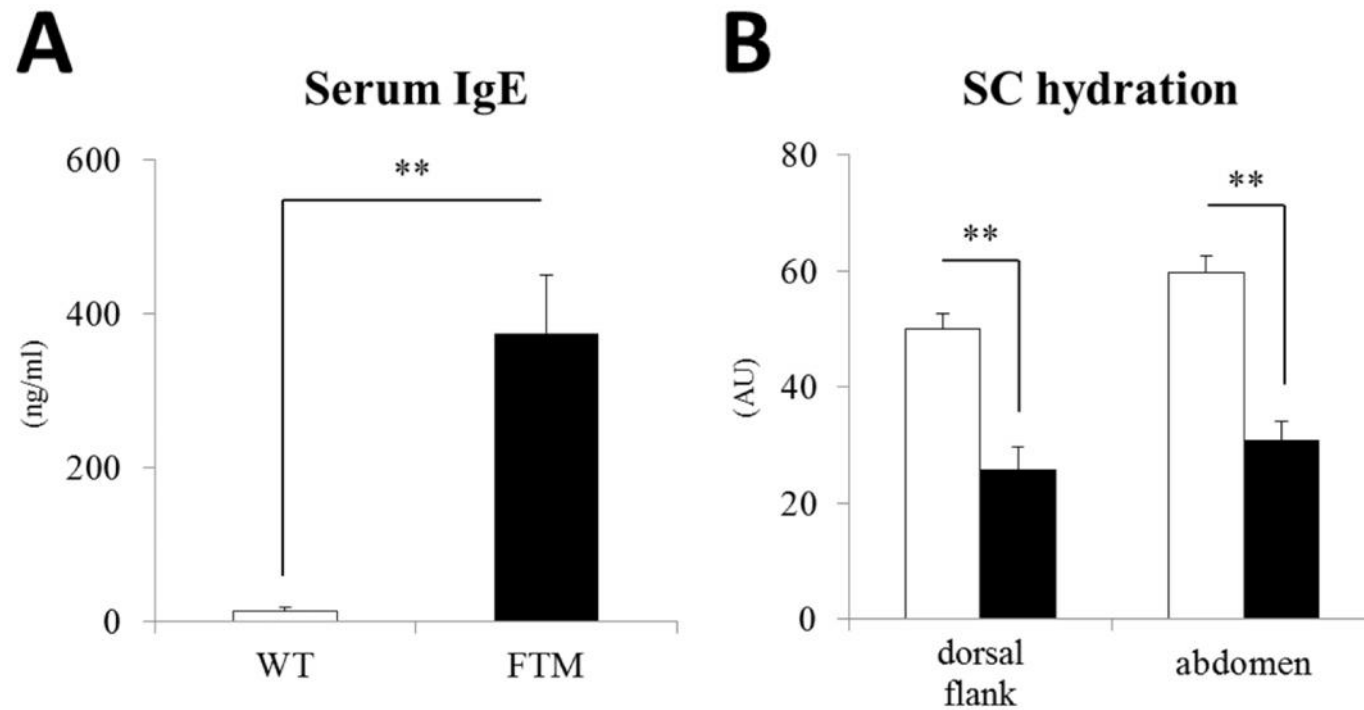


Figure S2: Serum levels of IgE and SC hydration. Serum levels of IgE in flaky tail mice (FTM) were higher than those in C57BL/6 (WT) (A). Stratum corneum (SC) hydration with FTM in both of dorsal flank and abdomen reduced, compared to WT (B). Serum levels of IgE were determined by ELISA with a mouse IgE quantitation kit (Bethyl Laboratories, Montgomery, TX, USA) according to each manufacturer's instructions. Measurement of SC hydration was performed as described in the supplementary file. $N=6$ in A, $N=7-9$ in B. ** $P < 0.01$. AU, arbitrary units.

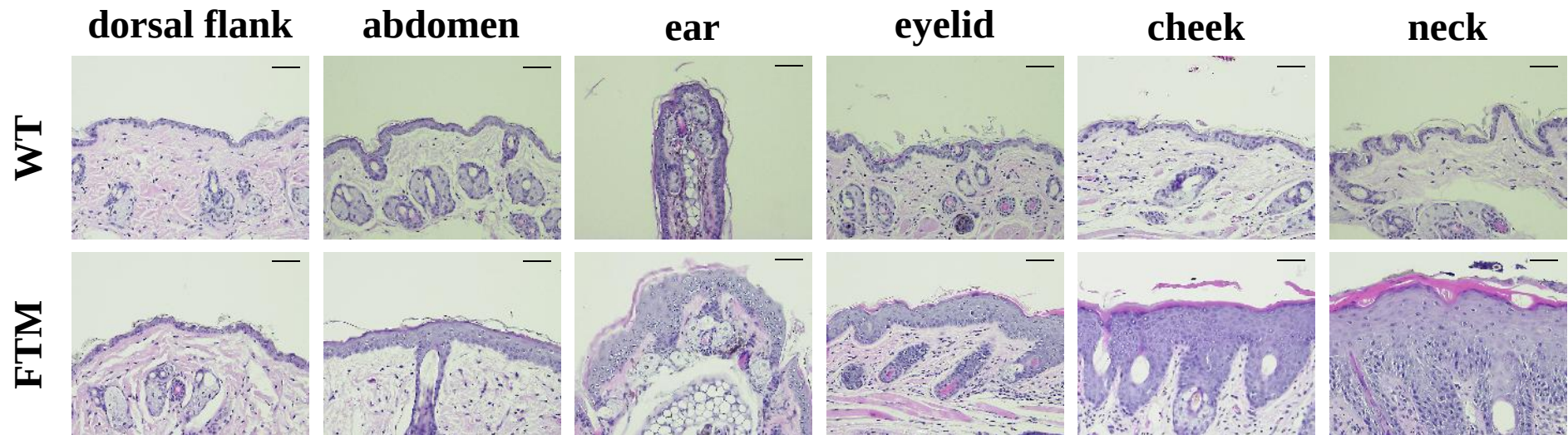


Figure S3: Representative histological images. Epidermis of areas around face, namely, ear, eyelid, cheek and neck in flaky tail mice (FTM) apparently thickened, while those of dorsal flank and abdomen were almost normal and slightly thickened, respectively, compared to C57BL/6 (WT). Scale bar = 50 μ m.

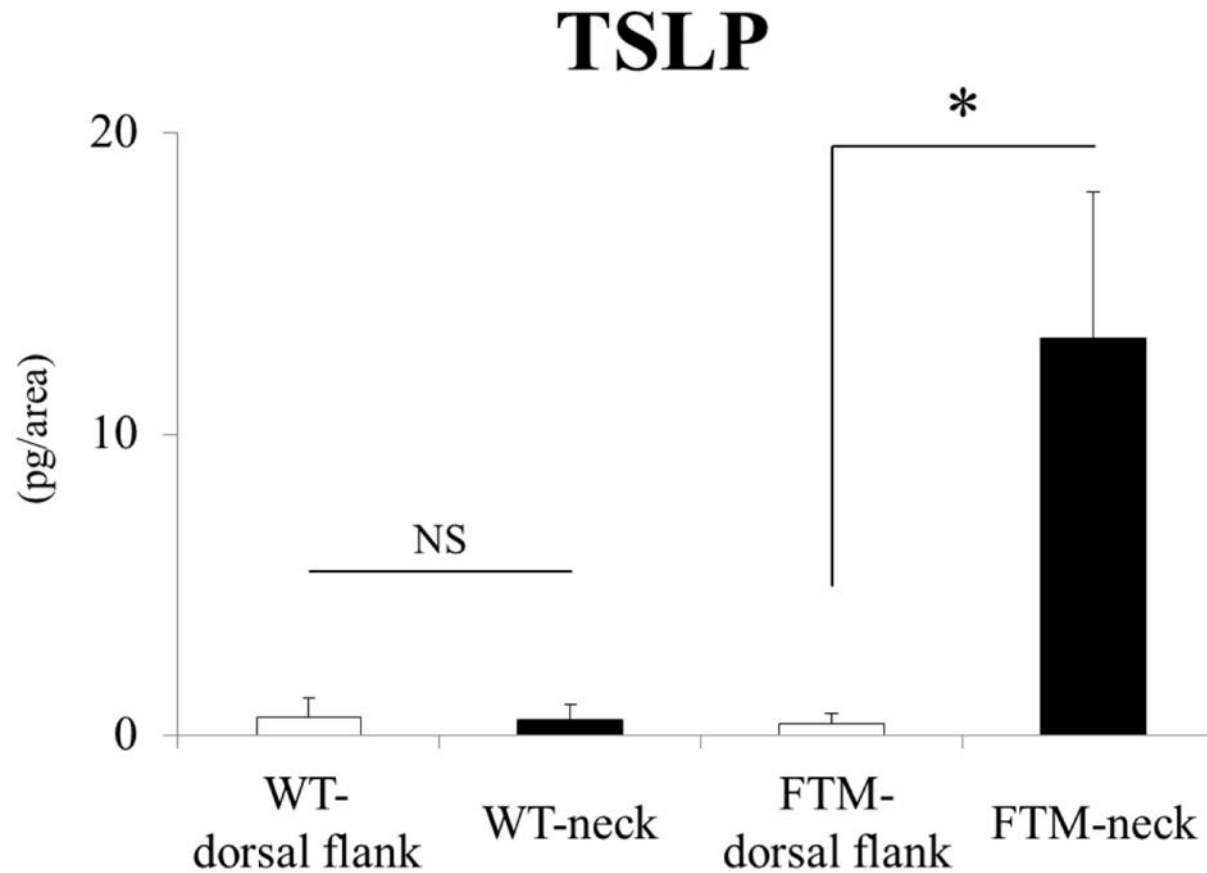


Figure S4: Protein levels of TSLP measured by ELISA. TSLP in whole skin measured by ELISA increased only in neck of FTM. Whole skin were collected with punch biopsy (5mm in diameter) and chopped in RIPA Buffer (Thermo Fisher Scientific Inc.) with Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific Inc.). After incubation at 4°C for 20 minutes, the samples were sonicated on ice and centrifuged at 14,000 rpm for 10 minutes. The equal amount of the supernatant fluids were applied to ELISA assay. Levels of TSLP in whole skin were determined by ELISA with Quantikine for TSLP (R&D Systems) according to each manufacturer's instructions. N=6,. NS, not significant, * $P < 0.01$.

Supplementary File

Title: Atopic dermatitis-like dermatitis emerges unevenly on different sites in flaky tail mice

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Materials and methods

Animals

Female C57BL/6 mice, which were used as wild-type mice (WT, Japan SLC Inc., Hamamatsu, Japan), and female flaky-tail mice (FTM, Jackson Laboratory, Bar Harbor, ME, USA) were used at 40-90 weeks old. All animals were housed under conventional conditions and had free access to a commercial diet and water. All experiments with mice were approved by the Ethics of Animal Experimentation Committee of Oita University.

Physiological assessments

After shaving hair of mice, transepidermal water loss (TEWL), stratum corneum (SC) pH, and SC hydration were measured with Tewameter (TM300; Courage & Khazaka, Cologne, Germany), skin pH meter (PH 905; Courage & Khazaka) and Corneometer (CM825; Courage & Khazaka), as described previously [1]. TEWL and SC pH in neck area were measured without shaving, because neck area is so narrow that shaving without injury is difficult.

Quantitative assessment of skin morphology

Skin samples from each indicated area were fixed in 10% buffered formalin and embedded in paraffin. Multiple 4- μ m sections were stained with hematoxylin and eosin. The thickness of the epidermis was measured with the scaled ocular lens of a light microscope [1].

Immunohistochemical staining

Immunohistochemical analysis of skin was performed with cryosections of 6- μ m thickness, as described previously [1]. Specimens were stained with primary polyclonal antibodies against thymic stromal lymphopoietin (TSLP; R&D Systems, Minneapolis, MN, USA) and Alexa Fluor® 488-conjugated donkey anti-goat secondary antibodies (Life Technologies, Carlsbad, CA, USA), and counterstained with propidium iodide. Staining was evaluated under a confocal microscope (LSM710 ZEN; Zeiss, Oberkochen, Germany).

Zymographic assessment of serine protease

The activity of serine protease was assessed by *in situ* zymography, as described previously [1-3]. 6- μ m frozen sections of skin samples were incubated with

BODIPY-FL-casein (EnzChek®Protease Assay Kit; Life Technologies) and counterstained with propidium iodide. The sections were examined under the confocal microscope (LSM710 ZEN).

Quantitative determination of the fluorescence intensities

For quantitative determination of the fluorescence intensities of immunohistochemical staining and *in situ* zymography, confocal images were analyzed, as described previously [1]. Exposure and acquisition setting were fixed and were such that no signal saturation occurred. The relative values of fluorescence intensity in epidermis of each compared specimen were calculated in one preparation, using LSM Software ZEN (Zeiss).

Statistical analysis

All experiments were analyzed by two-tailed Student's t-test or Welch's t-test. All results are presented as means $\pm$ SEMs of *N* experiments. A *P*-value of less than 0.05 was considered evidence of statistical significance.

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