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Original

Ovariectomy-Induced Dry Skin Increases IL-31-Induced Scratching Behavior in Mice

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Summary

Dry skin and type 2 cytokines, IL-4/IL-13 and IL-31, have been reported to be involved in pruritus in skin diseases such as atopic dermatitis. IL-31 directly induces pruritus, but IL-4/IL-13 themselves have little or no potential to induce pruritus, whereas IL-4/IL-13 potentiate nerve activation by subthreshold doses of pruritogens such as histamine and IL-31 in vitro, and are involved in alloknesis in dry skin mouse models induced by ovariectomy and artificial barrier disruption. In this study, we investigated whether ovariectomy-induced dry skin with permeability barrier dysfunction enhances IL-31-induced pruritus in mice. Levels of spontaneous scratching behavior were higher than in ovariectomized mice than in sham-operated mice probably due to barrier disruption that could be accelerated by hair shaving. Levels of scratching behavior induced by intradermal administration of IL-31, regardless of single or repeated administration, were increased in ovariectomized mice compared to in sham-operated mice. Pre-intradermal administration of neutralizing antibodies against IL-4/IL-13 did not attenuate the level of IL-31-induced scratching behavior in ovariectomized mice. Our results show that the dry skin induced by ovariectomy is an itchy condition prone to pruritus, and this is the first report showing that the dry skin enhances the potential of IL-31 to induce pruritus in vivo.

Key Words: dry skin, IL-13, IL-31, IL-4, pruritus

Introduction

Dry skin is a well-known symptom that leads to itching. Patients with atopic dermatitis experience severe pruritus and dry skin with barrier dysfunction. Pruritus and dry skin are also peri- and post-menopausal problem in women[1].

Artificial barrier disruption, a model of dry skin, induces pruritus and alloknesis, an abnormal itch sensation induced by innocuous mechanical stimuli in mice[2]. Recently, we reported that ovariectomized (OVX-) mice exhibited dry skin and permeability barrier dysfunction that also led to alloknesis, which was attenuated by neutralizing antibodies to IL-4 and IL-13[3].

Type 2 cytokines, IL-4/IL-13 and IL-31, have been reported to be involved in pruritus in several skin diseases such as atopic dermatitis. IL-31 directly induces pruritus, but IL-4 and IL-13 themselves have little or no potential to induce pruritus[4,5], whereas IL-4 potentiates nerve activation by subthreshold doses of prurito-

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gens such as histamine and IL-31 in vitro[4], in addition to being involved in alloknesis in the dry skin mouse models[3]. In addition, IL-4/IL-13 and IL-31 have been reported to impair the permeability barrier function[6].

This study was designed to elucidate the effect of ovariectomy-induced dry skin on IL-31-induced pruritus in mice.

Materials and Methods

Ethical statement

The experimental protocol and procedures used were ethically reviewed and approved by the Animal Care and Use Committee of Dokkyo Medical University (approval number 1384, approval Date, June 28, 2021), and conducted according to the guidelines of the Animal Care and Use Committee of Dokkyo Medical University.

Mice and ovariectomy

Seven-week-old female C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). Mice were randomly assigned to either a sham surgery (sham) or bilateral ovariectomy (OVX) at eight weeks of age, as previously reported[7]. Details are provided in the Supplementary file.

Reagents and treatment

Mice were injected intradermally with 10 μL of phosphate-buffered saline (PBS) containing IL-31 (100 ng, and 300 ng) at the shaved nape 4 weeks after OVX or sham surgery. A 10 μL of PBS containing 1 μg anti-IL-4 antibody and 5 μg anti-IL-13 antibody or isotype IgG, was injected intradermally into the shaved nape 4-5 hours before IL-31 administration. Details are provided in the Supplementary file.

Scoring of scratching behavior

The frequency of scratching behavior was recorded using the SCLABA®-NEXT: real-time scratch counting system (Noveltec Inc., Kobe, Japan), and was evaluated using three types of scores as follows. The number of scratching sessions was visually counted in the video. Long-lasting scratching (LLS) was defined as the number of movements lasting from ≥ 1.5 seconds to ≤ 3 seconds each, which has been reported to be the most reliable score for assessing IL-31-induced pruritus[8]. The total number of behaviors measured was the number of all movements of the mice, including all behaviors such as scratching and jumping. Details are provided in the Supplementary file.

Statistical analysis

All experiments were repeated at least thrice. Statistical analyses were performed using the GraphPad Prism software 9 (GraphPad Software, San Diego, California, USA; www.graphpad.com). Data were analyzed using two-tailed unpaired *t*-tests and Mann-Whitney U test when sample size was 12 or larger and less than 12, respectively. Multiple t-test was used to monitor scratching behavior every hour for 24 h or 48 h. Error bars indicate mean ± standard deviation. Results with $P < 0.05$ were considered statistically significant.

Results

Effect of ovariectomy on pruritus

Spontaneous scratching behavior of OVX- and sham-mice operated 4 weeks prior was monitored for 30 min 3-5 days after shaving the neck hair. Except for LLS, the level of spontaneous scratching behavior observed on the video and all movements was significantly higher in OVX-mice than in sham-mice in the early experiments (Fig. 1A). On the other hand, there was no difference of spontaneous pruritus evaluated by the three scores in the later experiments performed by different experimenters with careful hair shaving resulting in normal appearance of the skin compared to the scaling of the skin in the early experiments (Fig. 1B).

Effect of ovariectomy on IL-31-induced pruritus

Initially, we used 100 ng IL-31, the threshold dose to induce pruritus in mice as reported previously[8], in the first set of experiments, and OVX-mice showed higher levels of scratching behavior observed in the video, LLS, and all movements than sham-mice (Fig. 2A). Repeated administration of IL-31 has been reported to increase pruritus. Thus, in the second set of experiments, a total of six doses of 100 ng IL-31 were administered over three days, and the levels of scratching behavior in the video after the second and fourth administrations of IL-31, and the levels of LLS after the second administration of IL-31 were higher in OVX-mice than in sham-mice with statistical significance

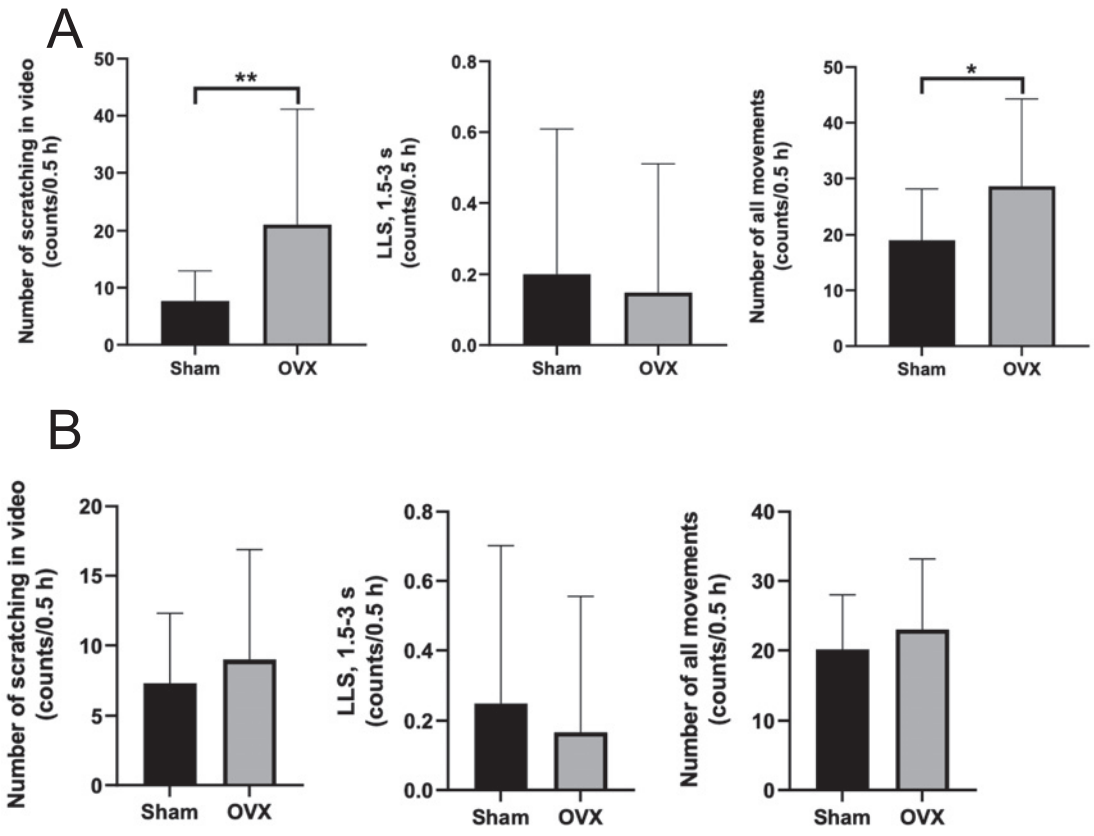

Figure 1 Spontaneous scratching behavior in ovariectomized and sham-operated mice

(A) Spontaneous scratching behavior in the early experiments using mice with the scaling of the skin after hair shaving. Scratching behavior of ovariectomized (OVX) and sham-operated (Sham) mice was evaluated for 30 min after 30 min of acclimatization with three types of scores, scratching behavior visually observed in the video, long-lasting scratching (LLS), and all movements, using the SCLABA®-NEXT: real-time scratch counting system. n = 27. **(B)** Spontaneous scratching behavior in the later experiments on mice with normal skin appearance after hair shaving. n = 18. Data are taken from at least two independent experiments. Results are expressed as mean ± standard deviation. Significant differences between groups are indicated by $^{*}P < 0.05$, $^{**}P < 0.005$.

(Fig. 2B). In the third set of experiments, OVX- and sham-mice received 300 ng IL-31 twice daily, and the levels of LLS were higher in OVX mice than in sham mice within 1 h or 2 h after each IL-31 administration, without statistical significance (Fig. 2C).

Effect of neutralizing antibodies against IL-4 and IL-13 on IL-31-induced increased scratching behavior in OVX-mice

Pre-intradermal administration of neutralizing antibodies against IL-4 and IL-13 had no effect on the level of IL-31-induced pruritus regardless of the dose of IL-31 (100 ng or 300 ng), single or repeated administration of IL-31 and the neutralizing antibodies, and observation period (30 min or 48 h) (Fig. 3A, 3B).

Discussion

In this study, we demonstrated increased levels of spontaneous scratching behavior in OVX-mice and also showed that IL-31-induced pruritus was higher in OVX-mice than in sham-mice, which was not attenuated by neutralizing antibodies against IL-4 and IL-13. This is the first report showing that the dry skin enhances the potential of IL-31 to induce pruritus in vivo.

OVX-mice may have higher levels of spontaneous pruritus than sham-mice, as evidenced by increased levels of scratching behavior in the video and all movements, but not in the LLS. This suggests that the LLS may be inappropriate for assessing spontaneous pruritus, where mild scratching is observed in the video, although the LLS has been reported to be the most reli-

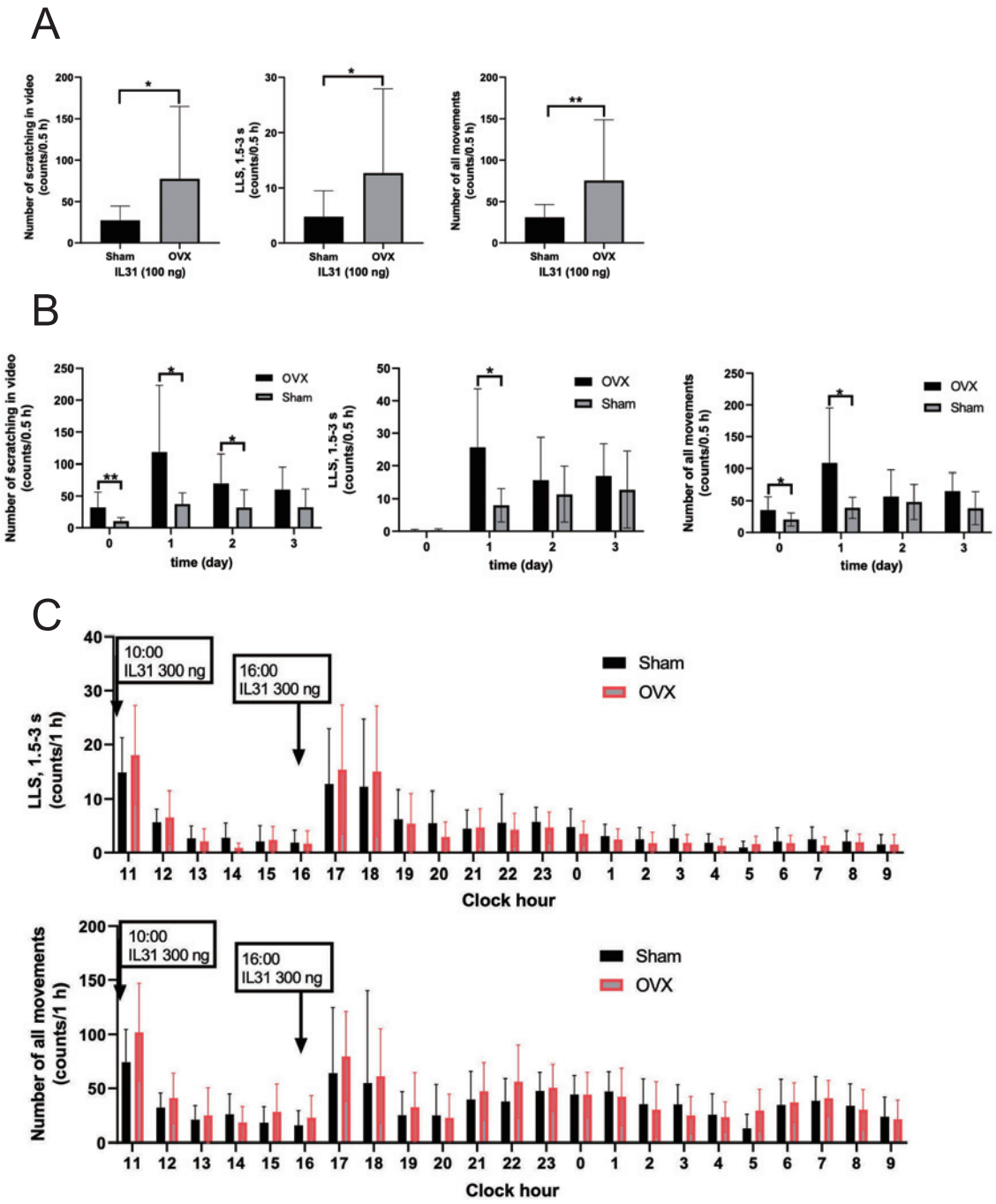

Figure 2 IL-31-induced scratching behavior in ovariectomized and sham-operated mice

(A) Scratching behavior induced by intradermal administration of 100 ng IL-31. Scratching behavior in ovariectomized (OVX) and sham-operated (Sham) mice was evaluated for 30 minutes after 30 minutes acclimatization following intradermal administration of 100 ng IL-31 with three types of scores, scratching behavior visually observed in the video, long-lasting scratching (LLS), and all movements, using the SCLABA®-NEXT: real-time scratch counting system. n = 27. **(B)** Scratching behavior induced by repeated administration of 100 ng IL-31. OVX- and Sham-mice received 100 ng IL-31 twice daily for 3 days, and scratching behavior was assessed for 30 minutes after a 30-minute acclimation period before the first IL-31 administration and after the second and forth IL-31 administration. n = 11. Day 0 indicates a state before IL-31 administration; day1-3 represent after the second, fourth, and sixth IL-31 administrations. **(C)** Scratching behavior induced by repeated administration of 300 ng IL-31 monitored for 24 h. OVX- and Sham-mice received 300 ng IL-31 twice daily, and LLS was assessed every one hour for 24 h. n = 18. Data are taken from at least two independent experiments. Results are expressed as mean ± standard deviation. Significant differences between groups are indicated by $^{*}P < 0.05$, $^{**}P < 0.005$.

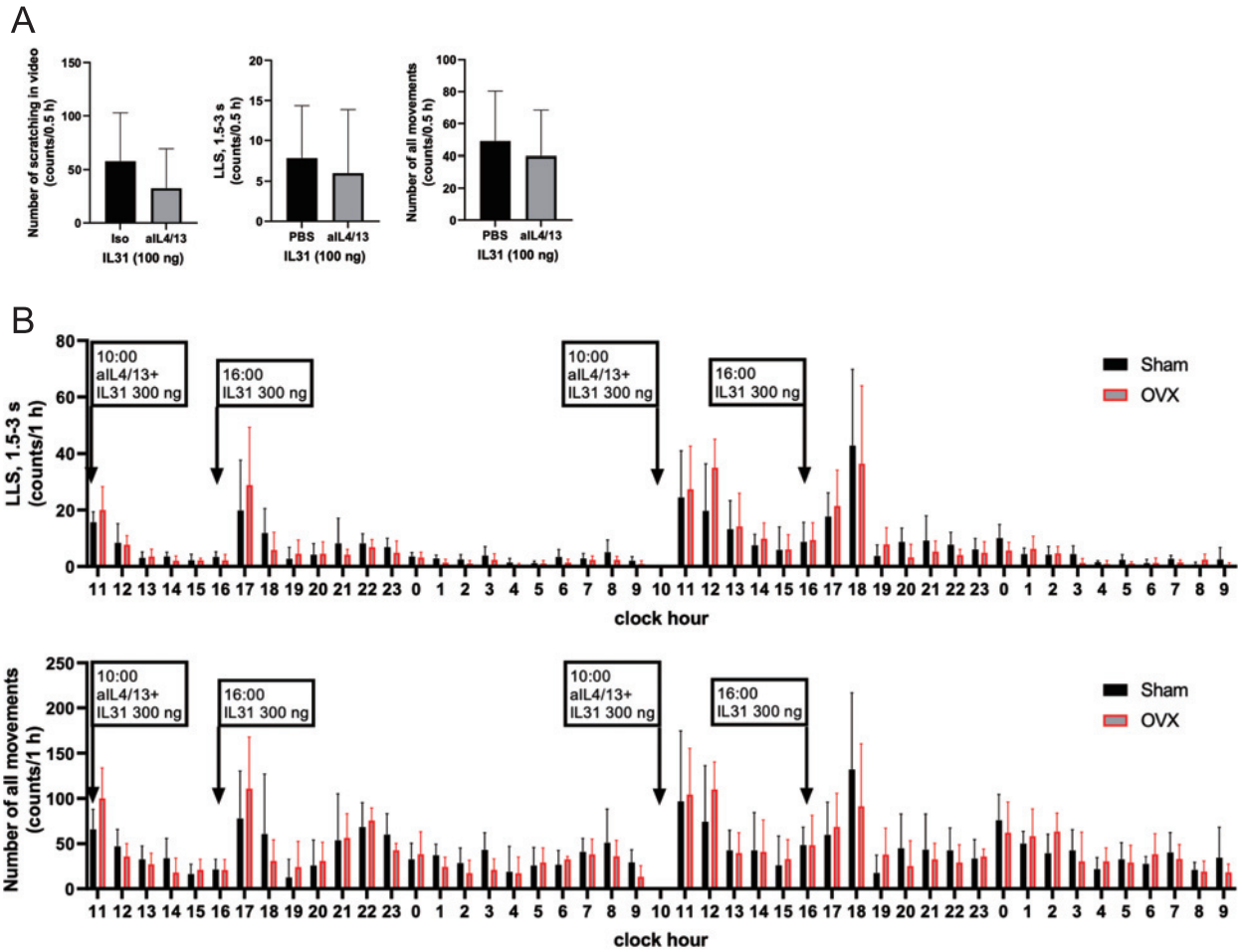

Figure 3 Effect of neutralizing antibodies against IL-4 and IL-13 on IL-31-induced increased scratching behavior in OVX-mice

(A) Neutralizing antibodies to IL-4 and IL-13 and isotype control IgG were given to OVX-mice 4 h before intradermal administration of 100 ng IL-31. Scratching behavior was assessed for 30 minutes after 30 minutes of acclimatization following IL-31 administration. n = 12. **(B)** OVX- and Sham-mice received 300 ng IL-31 twice daily with or without neutralizing antibodies against IL-4 and IL-13 as indicated in the figure, and long-lasting scratching (LLS) was assessed every hour for 48 h. n = 6. Results are expressed as mean ± standard deviation. There is no statistical difference between the two groups described in this figure.

able score for IL-31-induced pruritus[8], presumably severe pruritus, which was also confirmed in this study.

The mechanism of the pruritogenic effect in OVX-mice is unclear. An artificial dry skin model induced by treatment with acetone, diethyl ether, and water shows spontaneous pruritus in addition to epidermal innervation and barrier dysfunction[2] similar to OVX-mice[3]. Thus, these barrier dysfunction and related changes may be the mechanism of the pruritogenic effect in OVX-mice. We have previously reported that OVX HR mice, a hairless mouse without hair shaving, have reduced stratum corneum hydration, impaired permeability barrier recovery, and weakened stratified corneocyte integrity. Thus, OVX mice have abnormal skin barrier function even in the absence of shaving effects, which may increase pruritus. Furthermore, the barrier disrupting factor, such as shaving, may also increase the pruritogenic effect that is a characteristic of the dry skin in OVX mice as follows. The degree of barrier dysfunction induced by hair shaving could also be critical for pruritus, as reflected by the skin conditions with scaling or normal appearance. Mice used in Fig. 1A, 2A, and 2B showed scaling of the skin after shaving the hair on the neck where intradermal administration of IL-31 was given. On the other hand, mice used in Fig. 1B, 2C, 3A and 3B showed normal-

appearance skin after careful hair shaving performed by another experimenter. The difference in the skin conditions after hair shaving seems to depend on the shaving technique, rough or careful, and is related to the degree of permeability barrier disruption due to impaired permeability barrier recovery function in OVX mice as previously reported[7]. Thus, the former may be associated with more severe permeability barrier disruption leading to increased levels of spontaneous scratching and IL-31-induced pruritus in OVX mice than the latter. We regret that we did not evaluate the permeability barrier function after hair shaving in each experiment.

From a different perspective, considering the effect of OVX on the effect of IL-31 on itching in this experiment, it is possible that the dose of IL-31 may have influenced the determination of the effect of OVX. Pruritus was induced by 300 ng of IL-31 in the later experiments (Fig. 2C and 3B) versus 100 ng in the earlier experiments (Fig. 2A, 2B and 3A), and the higher dose of IL-31 might attenuate the pruritus-promoting effects of the dry skin. In order to evaluate the effect of OVX induced dry skin on IL-31-induced pruritus, we should use 100 ng IL-31 in combination with careful hair shaving. In addition, a small amount of IL-31, a threshold dose to induce pruritus, should be used for tests, although we unfortunately used 100 ng and 300 ng of IL-31 to induce pruritus. Taken together, it may be that it is not OVX itself, but OVX-induced dry skin that is vulnerable to damaging factors, including hair shaving, that is an itchy condition prone to pruritus. We have previously reported that alloknesis in OVX-mice was inhibited by neutralizing antibodies to IL-4 and IL-13[3], which had no effect on IL-31-induced pruritus. Other pruritogens and cytokines associated with dry skin, particularly keratinocyte-derived factors such as TSLP and IL-33[9], protease-activated receptors (PARs)[10,11], and their combination with IL-4, IL-13 and IL-31 may play a role in the IL-31-induced pruritus in OVX mice.

Our study results may explain the association between dry skin with permeability barrier dysfunction and pruritus. However, our study results are based on mouse models and have inherent limitations in their application to humans. Therefore, further studies are needed to validate the role of these cytokines and other pruritogens in chronic pruritic models including dry skin, in future studies.

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Author Contributions

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors state no conflict of interest.

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