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## Filaggrin deficiency promotes the dissemination of cutaneously inoculated vaccinia virus

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### Abstract

**Background**—Eczema Vaccinatum (EV) is a life threatening complication of smallpox vaccination in patients with atopic dermatitis (AD) characterized by dissemination of vaccinia virus (VV) in the skin and internal organs. Mutations in the filaggrin gene, the most common genetic risk factor for AD, confer a greater risk for eczema herpeticum in AD patients, suggesting that it impairs the response to cutaneous viral infections.

**Objective**—To determine the effects of filaggrin deficiency on the response of mice to cutaneous VV inoculation.

**Methods**—VV was inoculated by scarification of unsensitized skin, or skin topically sensitized with ovalbumin (OVA), in filaggrin-deficient, flaky tail (*fl/fl*) mice or wild-type (WT) controls. The size of primary and satellite skin lesions were measured and H&E staining was performed. VV genome copy number and cytokine mRNA levels were measured by quantitative PCR.

**Results**—VV inoculation in unsensitized skin of *fl/fl* mice, independently of the matted hair mutation, resulted in larger primary lesions, more abundant satellite lesions, heavier viral loads in internal organs, greater epidermal thickness, dermal cellular infiltration, and higher local *Il17a*, *Il4*, *Il13*, and *Ifng* mRNA levels than in WT controls. VV inoculation at sites of topical OVA application amplified all of these features in *fl/fl* mice, but had no detectable effect in WT controls. The number of satellite lesions and the viral loads in internal organs following cutaneous VV inoculation were significantly reduced in both unsensitized and topically sensitized *fl/fl;Il17a<sup>-/-</sup>* mice.

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**Conclusion**—Filaggrin deficiency predisposes to EV. This is mediated primarily through the production of IL-17A.

**Clinical Implications**—IL-17A blockade may prevent the development of EV.

**Capsule summary**—Filaggrin deficiency and allergic skin inflammation synergize to promote susceptibility to vaccinia virus dissemination. This susceptibility is mediated primarily by IL-17A, which is highly expressed in the skin of filaggrin deficient mice.

### Keywords

Vaccinia virus; filaggrin; IL-17A

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## Introduction

Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disorder that affects more than 15% of children in the USA <sup>1</sup>. AD is characterized by a defect in skin barrier function that results in dry itchy skin, and is aggravated by skin injury inflicted by scratching <sup>2</sup>. Patients with AD are prone to Th2-dominated immune responses as well as bacterial and viral infections of the skin <sup>3-5</sup>. In particular, they are susceptible to dissemination of viruses such as vaccinia (VV) and herpes (HSV) viruses <sup>6,7</sup>, resulting in eczema vaccinatum (EV) and eczema herpeticum (EH), respectively. In EV patients, VV spreads through skin, resulting in large primary lesions surrounded by satellite lesions, and infects internal organs. Although smallpox was declared eradicated in 1979, recent fears that variola virus might be used as a biological weapon, along with concerns regarding the EV susceptibility of unimmunized atopic populations, have prompted the development of new safer vaccines. Predicting the efficacy of such vaccines in the absence of human smallpox depends on understanding the mechanisms of the disease. The reasons why patients with AD are at risk for EV are not known.

Strong genetic associations exist between mutations in the filaggrin (*FLG*) gene and AD <sup>8-10</sup>. The *FLG* gene encodes for profilaggrin, the major component of keratohyalin granules. Patients with AD who carry *FLG* mutations have more persistent disease, a higher incidence of skin infections with herpes virus that results in EH <sup>11,12</sup>, and a greater risk of multiple allergies including asthma, allergic rhinitis, and peanut allergy <sup>13,14</sup>. Whether filaggrin deficiency predisposes to EV has not been studied.

Flaky tail (*ft/ft*) mice carry a 5303delA frame shift mutation in the *FLG* gene, which is in linkage disequilibrium with a second mutation at the matted (*ma*) locus that confers hair matting <sup>15, 16</sup>. *ft/ft* mice have skin barrier dysfunction <sup>17</sup>. We, and others, have previously reported that the homozygous *ft* mutation on an outbred genetic background is associated with spontaneous development of eczematous skin lesions, increased mRNA expression in skin of *Il17a*, *Il4*, *Il13*, and *Ifng*, and elevated serum IgE levels <sup>17,18</sup>. Following topical cutaneous exposure to ovalbumin (OVA), *ft/ft* mice on a mixed background develop allergic skin inflammation evidenced by epidermal thickening, dermal CD4<sup>+</sup> cell infiltration, and exaggerated local and systemic Th17 and Th2 responses <sup>18</sup>. In contrast, topical application of OVA elicited no detectable local or systemic immune responses in wild-type (WT)

BALB/c and C57BL/6 mice<sup>18</sup>. In WT mice, tape stripping, a surrogate for human scratching, is a prerequisite for induction of allergic skin inflammation and systemic immune responses to EC application of antigen. Genetic and environmental factors favoring the induction of a cutaneous Th2 response lead to impaired effector immune responses and ineffective elimination of VV and HSV in mice resulting in phenotypes analogous to human EV and EH<sup>19–22</sup>. Animal studies support the hypothesis that skin barrier dysfunction caused by *FLG* mutations or by mechanical injury secondary to scratching plays a key role in antigen sensitization that leads to the development of AD.

To understand whether filaggrin deficient mice are susceptible to developing EV, we backcrossed the mutant *FLG* allele of the *ft/ft* strain onto the Th2-prone BALB/c background. This inbred strain has been well characterized with respect to models of allergic skin inflammation and EV<sup>19,23–28</sup>. Transferring the *ft/ft* genotype into this background permits rigorous analyses of *ft/ft* phenotypes, and a comparison of the responses of the mutant and WT BALB/c controls. In this study we demonstrate that *ft/ft* mice inoculated with VV in the skin develop more severe skin lesions, greater viral dissemination, and more intense cutaneous inflammation than WT controls, indicating that filaggrin deficiency impairs VV containment. All these features were exaggerated in *ft/ft* mice inoculated with VV in skin topically sensitized with OVA, indicating that filaggrin deficiency and allergic inflammation synergize to impair VV containment. IL-17A was shown to mediate the susceptibility of *ft/ft* mice to VV, as EV features were markedly attenuated in *ft/ft.II17a<sup>-/-</sup>* mice.

## Methods

### Mice

Flaky tail (*ft/ft*) mice from Jackson laboratory were backcrossed onto the BALB/c mice from Charles River Laboratories. *ft/ft* mice on BALB/c background were subsequently crossed with *II17a<sup>-/-</sup>* BALB/c mice to generate *ft/ft.II17a<sup>-/-</sup>* mice. Later in the course of the study, we were able to remove by extensive backcrossing the matted hair mutation (*ma*) present in the original mixed strain. The mice with the removed *ma* mutation were designated *ft/ft.ma<sup>wt/wt</sup>*. The sample size in each group was n = 5. All mice were bred in our animal facility, and kept in a specific pathogen-free environment and fed an OVA-free diet. All procedures performed on the mice were in accordance with the Animal Care and Use Committee of the Boston Children's Hospital.

### Topical skin sensitization and VV inoculation

The dorsal skin of anesthetized six to eight-week-old female mice was shaved and rested for two days for recovery from any injury by shaving, then 100 µg OVA (Grade V; Sigma) in 100 µl of normal saline, or placebo (100 µl of normal saline), were placed on a patch of sterile gauze (1×1 cm), which was secured to dorsal skin with a transparent bio-occlusive dressing (Tegaderm, Westnet Inc.). Each mouse had a total of three one-week exposures to the patch separated by two-week intervals.

Shaved dorsal skin of unsensitized mice or the sensitized site of topically sensitized mice was inoculated with VV Western Reserve strain (VR-1354, ATCC) by means of skin scarification by using  $10^7$  plaque forming unit (pfu/mouse). Skin was examined 7 days after inoculation (see Fig. S1). Lesion sizes were analyzed with the National Institutes of Health's Image software Image J (National Institutes of Health, Bethesda, Md). No mortality was observed following VV inoculation.

### Histological analysis

Multiple 4  $\mu$ m sections of skin were stained with hematoxylin and eosin (H&E). Neutrophils were counted in a blinded fashion in 10–15 high-power fields (HPFs) at a magnification of 400 $\times$ . Epidermal thickness of 10 different randomly chosen sites was measured in each skin section from each mouse at a magnification of 400 $\times$ .

### Quantitative PCR analysis of VV genomes

Tissue samples were immediately frozen and stored at  $-80^{\circ}\text{C}$ . Quantification of VV genomes was performed as previously described<sup>20</sup>. Cytokine mRNA expression was shown as fold induction relative to uninfected WT skin unless otherwise specified. Viral genome measurements by PCR correlated with viral titer measurements by plaque assay.

### Quantitative PCR analysis of cytokines

Total RNA was extracted from homogenized skin tissue or from cultured cells with RNAqueous extraction kit (Ambion Inc) following the manufacturer's instructions. cDNA was generated with iScript cDNA synthesis kit (Bio-rad Laboratory). Quantitative real-time PCR was done using Taqman Gene Expression Assay, universal PCR master mix and the ABI Prism 7300 sequence detection system with commercial primers and probes, all from Applied Biosystems. Fold induction of target gene expression was calculated using the comparative method for relative quantitation by normalization to the internal control  $\beta$ 2-microglobulin, as described previously<sup>29</sup>.

### *In vitro* cytokine production and VV specific IgG2a antibody ELISA

These assays were performed as described previously<sup>30</sup>.

### Statistical analysis

A Mann-Whitney's U test was used to compare the distribution of each outcome between two groups. A Bonferroni corrected significance level of 0.05 was used to account for pairwise comparisons among three or more groups. All analyses were performed using the Graphpad Prism version 5.0 (Graphpad Software).

## Results

### *ft/ft* mice are susceptible to cutaneous VV inoculation

We tested the hypothesis that *ft/ft* mice are predisposed to the development of EV. Seven days after VV inoculation in the back skin, *ft/ft* mice developed significantly larger primary lesions and significantly higher numbers of satellite lesions than WT BALB/c controls (Fig.

1A–C). Furthermore, they exhibited significantly higher viral loads in internal organs than WT controls (Fig. 1D). These results suggest that *ft/ft* mice are more susceptible to developing features of EV following inoculation with VV.

Inoculation of *ft/ft* mice with VV resulted in a significant increase in epidermal thickness, cellular infiltration of the dermis by mononuclear cells, as well as skin *Il4*, *Il13*, and *Ifng* mRNA, as compared to uninfected *ft/ft* mice or VV inoculated WT controls (Fig. 1E,F,H). Inoculation of WT controls with VV resulted in a slight but significant increase in epidermal thickness, skin *Il4*, *Il13*, and *Ifng* mRNA as compared to uninfected WT controls (Fig. 1E,F,H). However, no increase in skin infiltrating neutrophils was observed in any of the experimental groups (Fig. 1E,G). Epidermal thickness and skin *Il17a* mRNA expression were increased in the skin of uninfected *ft/ft* mice compared to uninfected WT controls (Fig. 1F,H), as previously reported in *ft/ft* mice on mixed background<sup>18</sup>. The high basal levels of skin *Il17a* mRNA in *ft/ft* mice were significantly decreased following VV inoculation, but remained higher than the *Il17a* mRNA levels in the skin of VV inoculated WT mice (Fig. 1H). VV inoculation had no detectable effect on *Il17a* mRNA in the skin of WT controls. Splenocytes from *ft/ft* mice and WT controls inoculated with VV secreted comparable amounts of IL-4, IL-13, IFN- $\gamma$ , and IL-17A in response to *in vitro* stimulation with VV (Fig. S2A). Serum levels of VV specific IgG2a were higher in VV inoculated *ft/ft* mice than WT controls (Fig. S2B). These results demonstrate that *ft/ft* mice mount exaggerated cutaneous Th2 and Th1 responses to VV infection.

Recently, it has become evident that filaggrin null (*Flg*<sup>-/-</sup>) mice differ from *ft/ft* mice in that they do not develop spontaneous dermatitis and do not have an abnormal transepidermal water loss<sup>31</sup>. However, *Flg*<sup>-/-</sup> mice, like *ft/ft* mice mount a Th2 response to topical sensitization of antigen<sup>31</sup>. The differences between *ft/ft* mice and *Flg*<sup>-/-</sup> mice raised the possibility that the homozygous *ma* mutation linked to the *ft* mutation might contribute to the susceptibility of *ft/ft* mice to cutaneous inoculation with VV. To address this issue, we backcrossed our *ft/ft* mice on BALB/c background to generate *ft/ft* mice that lack the *ma* mutation, as evidence by the lack of matted hairs and by genomic sequencing for the *ma* mutation<sup>32</sup>(data not shown). These mice were indistinguishable from *ft/ft* mice that harbor the *ma* mutation in their susceptibility to VV infection, as measured by the size of the primary lesion, the number of satellite lesions, viral loads in internal organs, epidermal thickening, and local cytokine expression profile (Fig. S3). The differences in aforementioned changes between *ft/ft* or *ft/ft.ma*<sup>wt/wt</sup> mice and WT controls, but not between *ft/ft* and *ft/ft.ma*<sup>wt/wt</sup> mice, were statistically significant. These results indicate that the *ma* mutation does not play an important role in the susceptibility of *ft/ft* mice to VV inoculation.

### VV inoculation of topically sensitized skin aggravates the features of EV in *ft/ft* mice

Topical application of OVA to the shaved *unstripped* back skin elicited local skin inflammation and a systemic immune response in *ft/ft* mice on BALB/c background (Fig. S4A–D and data not shown), as previously shown in *ft/ft* mice on mixed background<sup>18</sup>. In contrast, topical application of OVA to the shaved *unstripped* back skin did not result in the development of local skin inflammation or a systemic response in WT controls (Fig. S4A–D and data not shown). We tested the hypothesis that VV inoculation of *ft/ft* mice at sites of

topical OVA application would result in the development of more severe features of EV. Mice were topically sensitized with OVA or saline over a 7-week period, then were inoculated with VV at the site of sensitization and examined 7 days later (Fig. S4E). *ft/ft* mice inoculated with VV at OVA exposed sites developed significantly larger primary lesions, exhibited significantly higher number of satellite lesions, and had significantly greater VV loads in the ovary and kidney compared to *ft/ft* mice inoculated with VV at saline exposed sites or WT controls inoculated with VV at OVA exposed site (Fig. 2A–D). They also had significantly greater epidermal thickness, significantly denser dermal infiltration with neutrophils, and significantly higher expression of *Il17a*, *Il4*, *Il13*, and *Ifng* mRNA (Fig. 2E–H). The size of the primary lesion, epidermal thickness and expression of *Il17a*, *Il4*, *Il13*, and *Ifng* mRNA were comparable in WT mice inoculated with VV in OVA or saline exposed skin, and significantly reduced compared to those in *ft/ft* mice inoculated with VV at saline exposed sites (Fig. 2A–C,H). WT mice inoculated with VV in OVA or saline exposed skin had no satellite lesions, no detectable viral loads in the internal organs, and very few neutrophils in the primary lesion (Fig. 2C–E,G). Splenocyte cytokine secretion in response to VV stimulation was similar in *ft/ft* and WT mice (data not shown). VV specific serum IgG2a levels were higher in *ft/ft* mice than in WT mice, regardless of whether the skin was sensitized with OVA or saline (Fig. S5A). These results suggest that cutaneous sensitization to topically encountered antigens in *ft/ft* mice aggravates their susceptibility to EV.

### IL-17A contributes to the increased susceptibility of *ft/ft* mice to cutaneous VV inoculation

We have previously reported that treatment with anti-IL-17A attenuates EV features in WT mice inoculated with VV in tape stripped OVA sensitized skin<sup>30</sup>. Here we tested the hypothesis that increased IL-17A expression in the skin of *ft/ft* mice contributes to their increased susceptibility to VV inoculation. We bred the *ft/ft* genotype onto the IL-17A null background and examined responses to VV inoculation.

There was no detectable skin inflammation in 6–8 week old *ft/ftxIl17a<sup>-/-</sup>* mice or *ft/ft* mice. As expected, *Il17a* mRNA expression was undetectable in *ft/ftxIl17a<sup>-/-</sup>* mice. Following VV inoculation in unsensitized skin, *ft/ftxIl17a<sup>-/-</sup>* mice developed significantly less satellite lesions and significantly lower viral loads compared to *ft/ft* controls, but the size of the primary lesion was comparable between the two groups (Fig. 3A–D). VV inoculated *ft/ftxIl17a<sup>-/-</sup>* mice had significantly less epidermal thickening than VV inoculated *ft/ft* controls with no increase in dermal infiltration with neutrophils (Fig. 3E–G). However, VV inoculated *ft/ftxIl17a<sup>-/-</sup>* skin showed significantly higher levels of *Il4* mRNA expression than VV inoculated *ft/ft* skin. The levels of *Il13* and *Ifng* mRNA in VV inoculated skin were not significantly different between the two groups (Fig. 3H). VV inoculated *ft/ftxIl17a<sup>-/-</sup>* skin exhibited comparable epidermal thickness and dermal infiltration with neutrophils, but significantly higher levels of *Il4*, *Il13*, and *Ifng* mRNA as compared to uninfected *ft/ftxIl17a<sup>-/-</sup>* skin (Fig. 3E–H). Epidermal thickness, dermal infiltration with neutrophils, and skin *Il4*, *Il13*, and *Ifng* mRNA levels were comparable between uninfected *ft/ft* and *ft/ftxIl17a<sup>-/-</sup>* mice (Fig. 3E–H). Splenocyte secretion of IL-4, IL-13 and IFN- $\gamma$  in response to VV stimulation and VV specific serum IgG2a levels were comparable in *ft/ftxIl17a<sup>-/-</sup>* and *ft/ft* mice inoculated with VV (data not shown and Fig. S5B).

We next examined the response of *ft/ftxIl17a<sup>-/-</sup>* mice to VV inoculation at the site of cutaneous sensitization with OVA. Following VV inoculation in skin topically sensitized with OVA, *ft/ftxIl17a<sup>-/-</sup>* mice developed significantly smaller primary lesions, significantly less of satellite lesions, and significantly lower viral loads in internal organs compared to similarly sensitized *ft/ft* controls (Fig. 4A–D). They also developed significantly less epidermal thickening and significantly less neutrophil infiltration in the dermis; however, they expressed significantly more *Il4*, *Il13* and *Ifng* mRNA in VV infected skin than VV infected OVA-sensitized skin of *ft/ft* controls or saline exposed *ft/ftxIl17a<sup>-/-</sup>* skin (Fig. 4E–H). Splenocyte secretion of IL-4, IL-13 and IFN- $\gamma$  in response to VV stimulation and VV specific serum IgG2a levels were comparable in topically sensitized or saline exposed *ft/ftxIl17a<sup>-/-</sup>* and *ft/ft* mice inoculated with VV (data not shown and Fig. S5C). The size of primary lesions, number of satellite lesions, viral loads in internal organs, epidermal thickness, and dermal neutrophil infiltration were comparable between topically sensitized or saline exposed *ft/ftxIl17a<sup>-/-</sup>* mice inoculated with VV. These results suggest that increased expression of IL-17A in the skin of *ft/ft* mice contributes to their enhanced susceptibility to cutaneous infection with VV.

## Discussion

We demonstrate that filaggrin deficient *ft/ft* mice are more susceptible to developing features of EV following VV inoculation in the skin, and that filaggrin deficiency and allergic inflammation synergize to promote VV dissemination. The increased susceptibility of *ft/ft* mice to EV depends on IL-17A.

*ft/ft* mice, but not WT controls, developed features of EV following VV inoculation in unsensitized skin. These included larger primary lesions at the site of cutaneous VV inoculation, higher numbers of satellite skin lesions and greater viral dissemination to internal organs compared to WT controls. The increased susceptibility of *ft/ft* mice to VV dissemination, a feature of EV, is in line with the observation that filaggrin mutations predispose patients with AD to eczema herpeticum<sup>11</sup>. Taken together, these results suggest that filaggrin plays a critical role in containing viral dissemination following cutaneous infection.

The lesions in *ft/ft* mice were characterized by epidermal thickening, which was significantly more than in WT controls, and massive upregulation of Th1 and Th2 cytokine expression compared to WT controls. The Th1 cytokine IFN- $\gamma$  and IgG2a antibody play a protective role in limiting VV dissemination<sup>33</sup>. Splenocyte production of IFN- $\gamma$  in response to VV stimulation was comparable in *ft/ft* mice and WT controls, while VV specific IgG2a antibody levels were slightly increased in *ft/ft* mice, ruling out a role for a difference in these systemic immune responses in the increased susceptibility of *ft/ft* mice to VV. They rather suggest that disrupted barrier function underlies the propensity of *ft/ft* mice for VV dissemination. The increase in VV specific IgG2a antibody levels in *ft/ft* mice may be the consequence of their increased antigenic load.

*Il17a* mRNA expression is significantly elevated in the skin of *ft/ft* mice raised under specific pathogen free conditions<sup>18</sup>. The mechanisms by which filaggrin deficiency

predisposes to increased IL-17A skin expression may include increased absorption of microbes from the skin, which drives the production of IL-17A promoting cytokines that include IL-6 and IL-23<sup>18</sup>. This is supported by the observation that IL-17A expression in the skin is attenuated in *ft/ft* mice raised under germ free conditions (data not shown). Paradoxically, *Il17a* mRNA expression was attenuated following VV inoculation, possibly due to downregulation by the massive increase in *Il4* and *Ifng* expression<sup>34,35</sup>. The increased expression of Th1 and Th2 cytokines in VV inoculated skin of *ft/ft* mice was not simply a reflection of a stronger systemic antigen specific immune response in these mice. In fact, splenocytes from *ft/ft* mice and WT controls secreted comparable amounts of cytokines in response to VV stimulation. One possible explanation for the local increase in Th1 and Th2 cytokine production in VV inoculated skin of *ft/ft* mice could be increased cutaneous expression of molecules that promote cytokine production by infiltrating T cells. These could include thymic stromal lymphopoietin, which amplifies Th2 cytokine expression<sup>36</sup> and IL-12, which stimulates IFN- $\gamma$  expression by Th1 cells<sup>37</sup>. Increased dermal cellular infiltration in VV inoculated skin sites of *ft/ft* mice may also have contributed to the increased cytokine expression at these sites.

Cutaneous antigen sensitization in *ft/ft* mice mimics skin exposure to environmental antigens in patients with AD whose barrier dysfunction allows cutaneous sensitization upon exposure to antigen<sup>18,38</sup>. Topical sensitization with OVA elicited a vigorous Th2 response in the skin of *ft/ft* mice on BALB/c background as we had previously shown on mixed background<sup>18</sup>. VV inoculation of *ft/ft* mice in skin sites topically sensitized with OVA exaggerated the EV features that develop in these mice including primary lesion size, number of satellite lesions and viral loads in internal organs, suggesting that filaggrin deficiency and allergic inflammation synergize to promote VV dissemination. It also resulted in more severe skin inflammation characterized by intense dermal neutrophil infiltration and an increase in *Il17a* expression compared to VV inoculation in saline exposed or unsensitized skin from these mice. Consistent with our previous report<sup>18</sup>, topical application of OVA to shaved, unstripped WT skin did not result in the development of skin inflammation, and elicited minimal responses to VV inoculation. In contrast, EC sensitization of WT mice by application of OVA to tape stripped skin, which disrupts the skin barrier, elicits a vigorous Th2 response<sup>28</sup>. Moreover, VV inoculation in EC sensitized tape stripped skin of WT mice results in the development of EV features and in massive local neutrophil infiltration and *Il17a* expression<sup>30</sup>. These observations suggest that a preexisting cutaneous inflammatory response to an unrelated antigen promotes the development of EV features and enhances the local IL-17A response when VV is inoculated in the skin.

The increased susceptibility of *ft/ft* mice to develop satellite lesions and viral dissemination following VV inoculation in unsensitized skin was dependent on IL-17A. The number of satellite lesions and the viral loads in internal organs in *ft/ft;Il17a<sup>-/-</sup>* mice inoculated in unsensitized skin, or in skin topically sensitized with OVA, were reduced to the levels in WT controls. Th1 and Th2 cytokine secretion by VV stimulated splenocytes and serum levels of VV specific IgG2a antibody were comparable in *ft/ft;Il17a<sup>-/-</sup>* and *ft/ft* mice, indicating that IL-17A played no detectable role in the systemic Th1 and Th2 responses of *ft/ft* mice to cutaneous inoculation with VV. However, in the absence of IL-17A, epidermal



thickening was reduced and mRNA expression of *Il4* was increased in VV inoculated skin of *ft/ftxIl17a<sup>-/-</sup>* mice. These findings are consistent with previous observations that IL-17A activates keratinocytes<sup>39-41</sup> and downregulates Th2 cytokine expression<sup>35</sup>. Neutrophil infiltration in VV inoculated OVA sensitized skin, which was observed in *ft/ft mice*, was abolished in *ft/ftxIl17a<sup>-/-</sup>* mice. This finding is consistent with the role of IL-17A in driving neutrophil chemotaxis via the induction of CXCL2 expression in target cells<sup>42</sup>. It is also in agreement with our previous finding that administration of anti-IL-17A neutralizing antibody inhibits neutrophil accumulation in tape stripped EC sensitized skin of WT mice<sup>30</sup>. These findings suggest that IL-17A plays a critical role in the development of EV and in the local neutrophilic infiltration of VV inoculated skin in *ft/ft* mice.

The vast majority of AD patients with *FLG* mutations are heterozygous<sup>43</sup>. However, mice heterozygous for the *ft* mutation have no detectable skin abnormalities and do not mount an immune response to topical sensitization with antigen (our unpublished observations). Consistent with their lack of a cutaneous phenotype, *ft/+* heterozygous mice had a comparable response to VV inoculation as WT controls (data not shown). Altogether, these findings suggest that genetic and/or environmental factors operate in humans to modulate the effect of heterozygous *FLG* mutations in humans on skin biology, and thereby susceptibility of VV inoculation.

Patients with AD can develop EV even in the absence of active skin lesions<sup>44</sup>. The filaggrin deficient mice we have studied had no grossly visible dermatitis, yet they were susceptible to developing EV features following VV inoculation in the skin. This parallel with the human AD phenotype further supports the validity of using filaggrin deficient mice as a model for the study of EV. The protective effect of IL-17A deficiency in these mice suggests that neutralization of IL-17A might be an effective therapy for EV in filaggrin deficient patients with AD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

<b>AD</b>	atopic dermatitis
<b>EH</b>	eczema herpeticum
<b>EV</b>	eczema vaccinatum
<b>FLG</b>	filaggrin

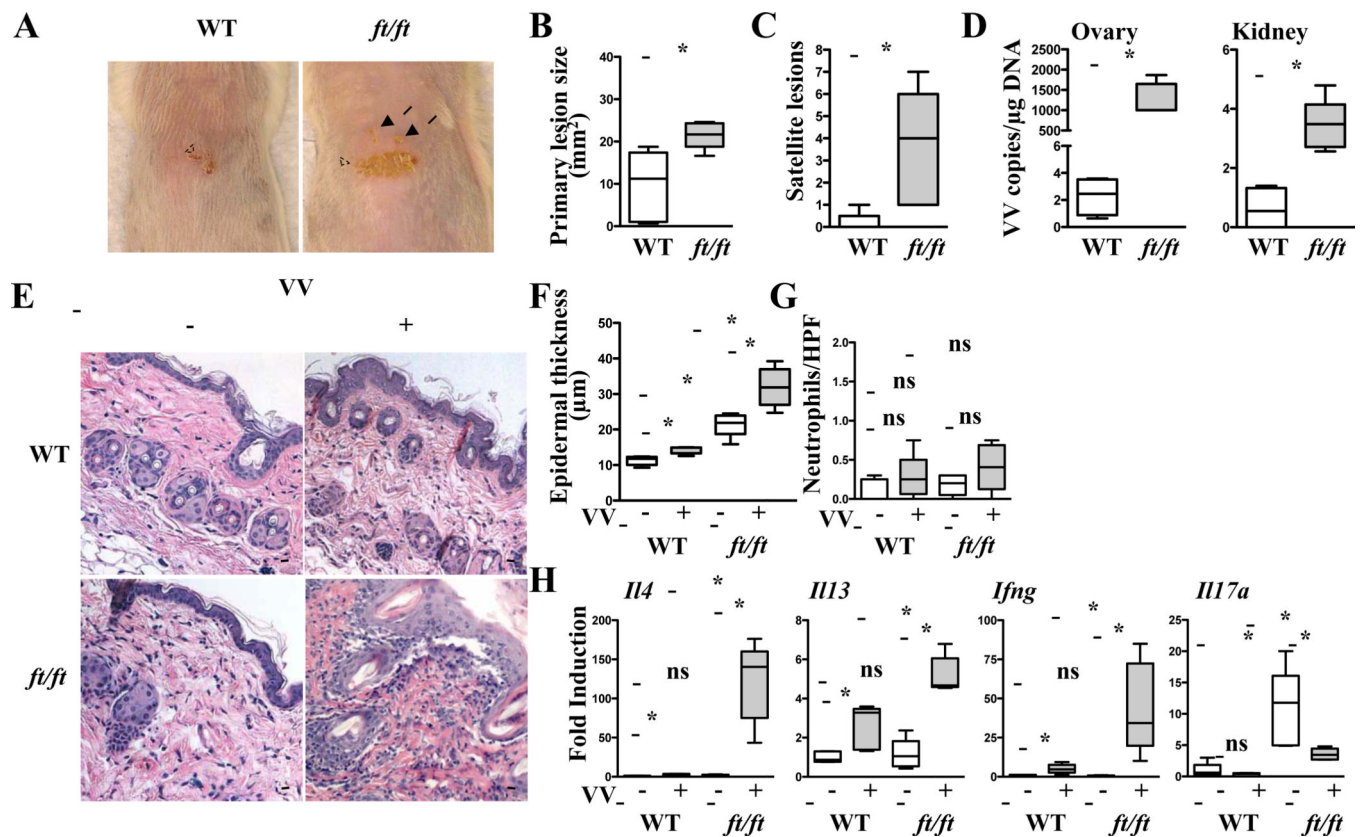
<b><i>ft/ft</i></b>	flaky tail
<b>H&amp;E</b>	hematoxylin and eosin
<b>HPF</b>	high power fields
<b>HSV</b>	herpes simplex virus
<b>IL</b>	interleukin
<b><i>ma</i></b>	matted
<b>OVA</b>	ovalbumin
<b>pfu</b>	plaque-forming unit
<b>WT</b>	wild-type
<b>VV</b>	vaccinia virus

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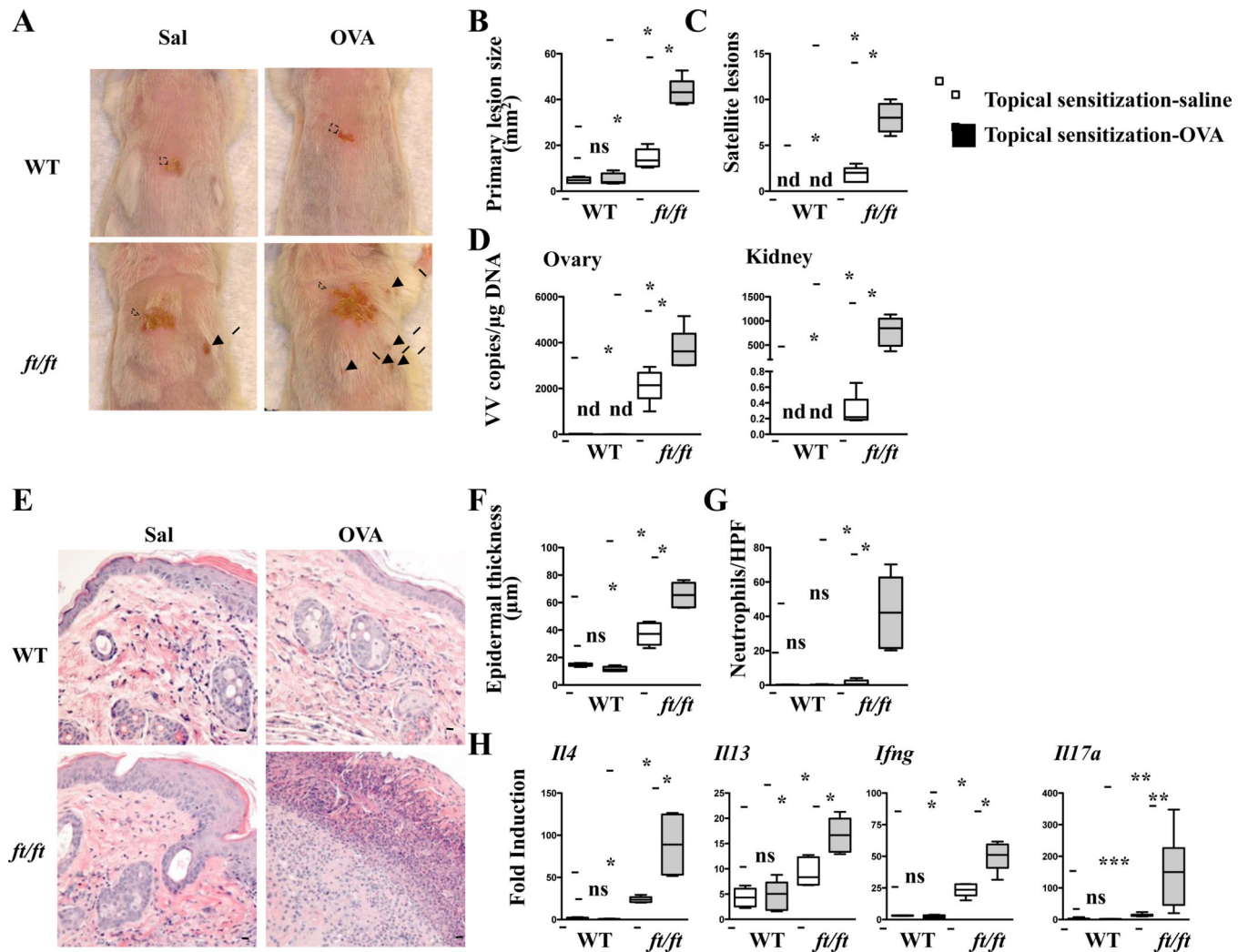
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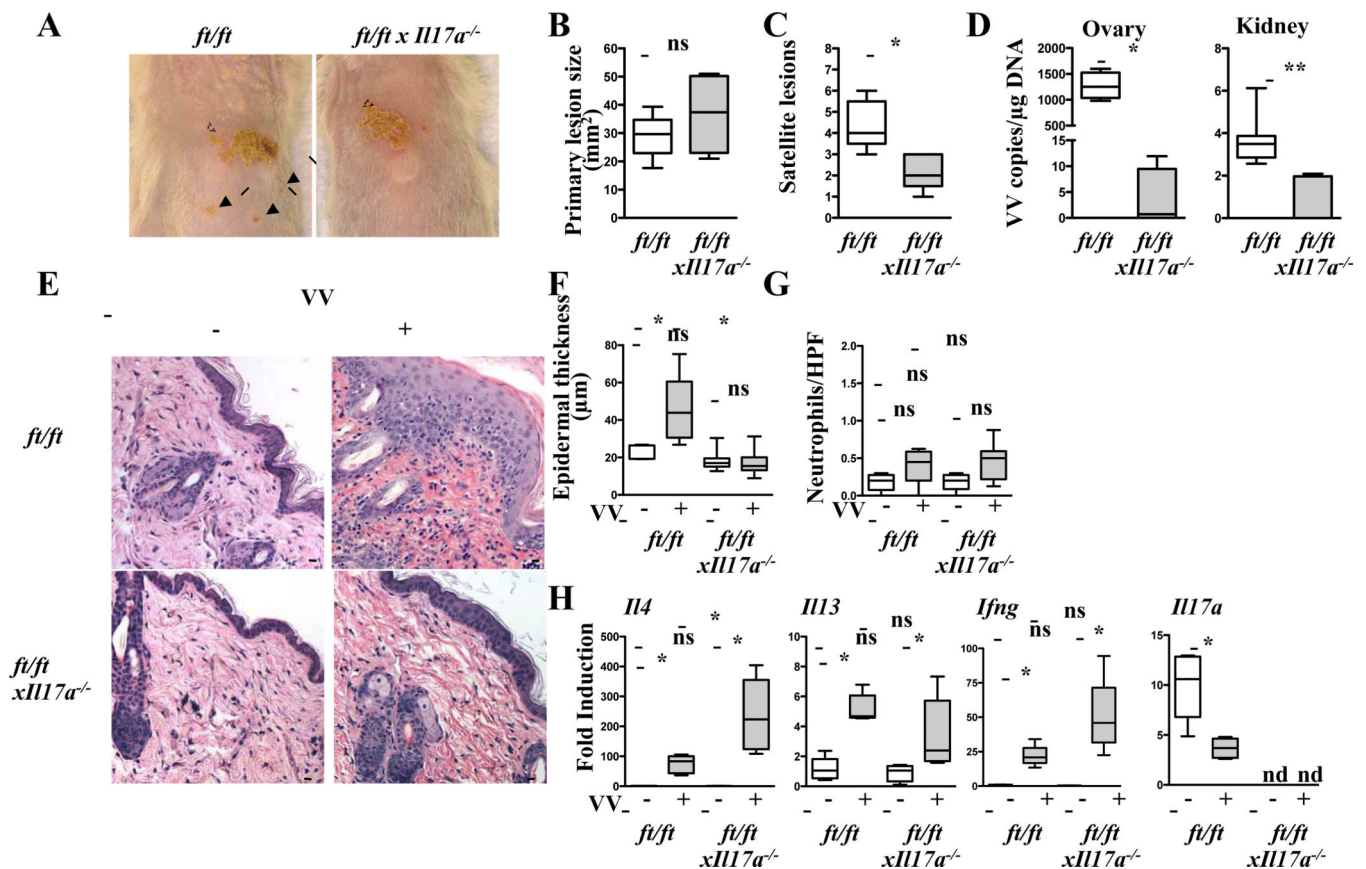


**Figure 1. *ft/ft* mice develop features of EV in response to cutaneous VV inoculation**

**A–C.** Gross appearance (A), area of primary lesions (B), and number of satellite lesions (C). Dashed circles indicate primary lesions. Arrows indicate satellite lesions. **D.** Viral load. **E–G.** H&E stained skin sections (200× magnification) (E), epidermal thickness (F), number of neutrophils (G), cytokine mRNA expression (H). Scale bars: 100 µm. The line in the box indicates median and the whiskers represent from the minimum to maximum (n = 5 per group). \*P < .05. ns, not significant.

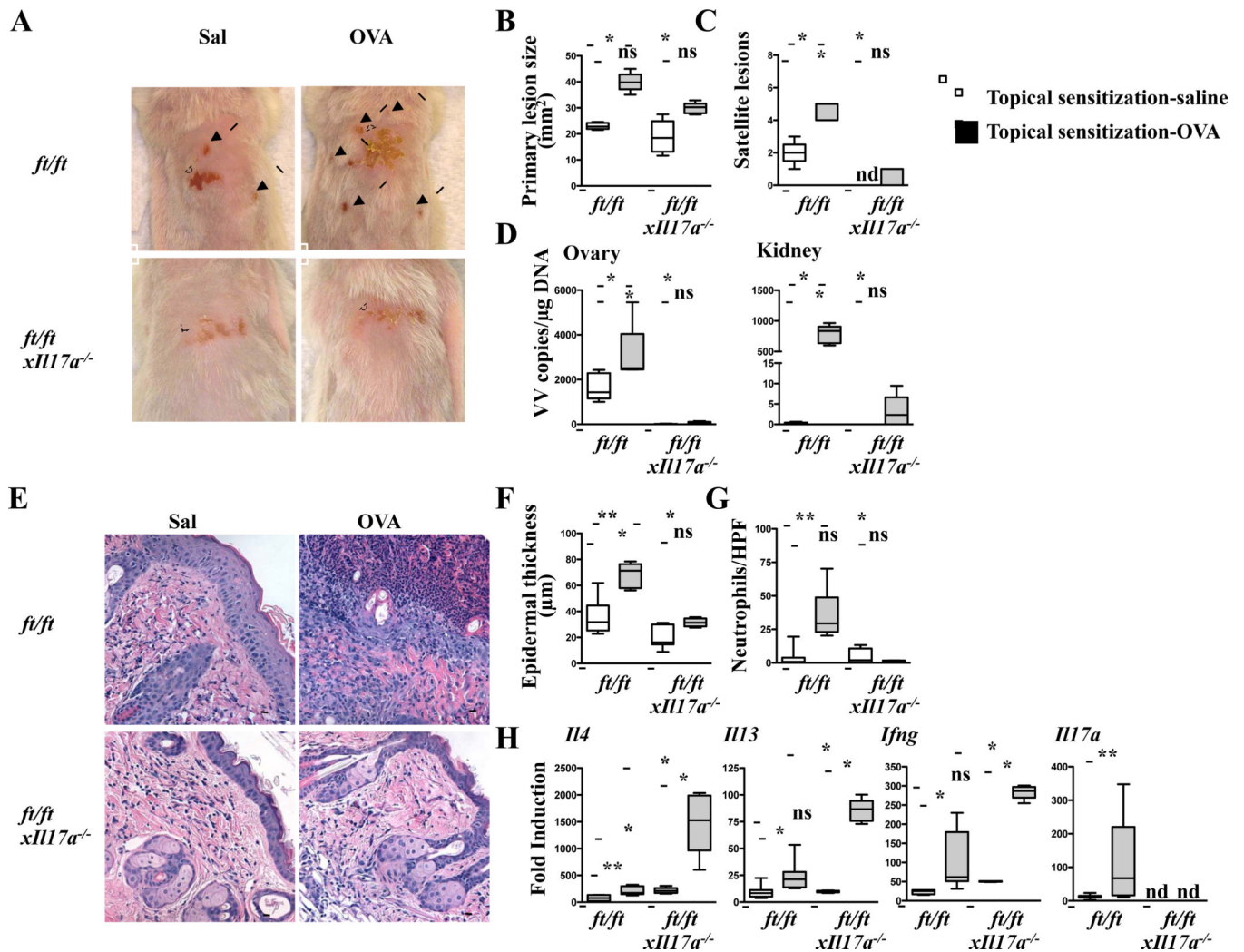


**Figure 2. Exaggerated EV features in *ft/ft* mice inoculated with VV in OVA sensitized skin**  
**A–C.** Gross appearance (A), area of primary lesions (B), and number of satellite lesions (C). Dashed circles indicate primary lesions. Arrows indicate satellite lesions. **D.** Viral load. **E–H.** H&E stained skin sections (200× magnification) (E), epidermal thickness (F), number of neutrophils (G), cytokine mRNA expression (H). Scale bars: 100 μm. The line in the box indicates median and the whiskers represent from the minimum to maximum (n = 5 per group). \*P < .05, \*\*P < .01 and \*\*\*P < .001. ns, not significant. nd, not detectable.



**Figure 3. Absence of IL-17A attenuates EV features caused by VV inoculation in the unsensitized skin of *ft/ft* mice**

**A–C.** Gross appearance (**A**), area of primary lesions (**B**), and number of satellite lesions (**C**). Dashed circles indicate primary lesions. Arrows indicate satellite lesions. **D.** Viral load. **E–H.** H&E stained skin sections (200× magnification) (**E**), epidermal thickness (**F**), number of neutrophils (**G**), cytokine mRNA expression (**H**). Scale bars: 100 μm. The line in the box indicates median and the whiskers represent from the minimum to maximum (n = 5 per group). \*P < .05 and \*\*P < .01. ns, not significant. nd, not detectable.



**Figure 4. Absence of IL-17A attenuates EV features caused by VV inoculation in the OVA sensitized skin of *ft/ft* mice**

**A–C.** Gross appearance (A), area of primary lesions (B), and number of satellite lesions (C). Dashed circles indicate primary lesions. Arrows indicate satellite lesions. **D.** Viral load. **E–H.** H&E stained skin sections (200× magnification) (E), epidermal thickness (F), number of neutrophils (G), cytokine mRNA expression (H). Scale bars: 100 μm. The line in the box indicates median and the whiskers represent from the minimum to maximum (n = 5 per group). \*P < .05 and \*\*P < .01. ns, not significant. nd, not detectable.