



Short Communication

Decreased expression of caspase-14 in an experimental model of canine atopic dermatitis

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ABSTRACT

Alterations in skin barrier function and filaggrin expression have been reported in atopic dermatitis (AD). Caspase-14, a protease important for filaggrin processing, is decreased in human AD. Atopic Beagle dogs with skin barrier alterations have been validated as model for AD. This study aimed to investigate caspase-14 in normal and atopic Beagle dogs. Skin biopsies from non-lesional and control skin were analyzed for caspase-14 by immunofluorescence. Six images/sections were blindly scored for intensity. Data were tested with unpaired Student's *t* test. A *P* value of <0.05 was considered significant. Caspase-14 was decreased in atopic compared to normal skin both quantitatively ($P < 0.001$) and qualitatively ($P = 0.006$; agreement = 0.93; consistency = 0.94). In conclusion, caspase-14 is decreased in this model similarly to reports in humans, highlighting the relevance of filaggrin metabolic defects in AD.

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Skin barrier dysfunction plays an important role in atopic dermatitis (AD), both in dogs and humans (Marsella et al., 2011; Czarnecki et al., 2014). Filaggrin, a key protein for skin barrier function, is decreased in both atopic dogs and humans. Caspase-14 is an important enzyme for the metabolism of filaggrin and the production of natural moisturizing factors (Hoste et al., 2011). Decreased caspase-14 expression has been reported in human patients with AD (Yamamoto et al., 2011). This study aimed to investigate caspase-14 expression in a canine model of AD which has been shown to have altered filaggrin expression (Marsella et al., 2009).

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida (number 200902424; modification number 3 approved on 26 March 2009). Eight atopic Beagle dogs (four females and four male; average age, 5 years old) and six healthy Beagle dogs (three males and three females; average age, 5 years old) were used as controls. The atopic Beagle dogs had been epicutaneously sensitized to house dust mites during puppyhood and were known to develop skin lesions and pruritus upon allergen exposure (Marsella et al., 2006). The controls were selected based on the lack of history of skin disease, lack of skin lesions on physical examination, and lack of reactivity after challenge with allergens (results not shown here). Both the AD and the healthy Beagle dogs were housed with the same environmental and nutritional conditions.

Two 8 mm biopsy skin samples were taken from the inguinal area under local anesthesia with 1% lidocaine. In the atopic dogs,

samples were taken from clinically non-lesional areas. Specimens were fixed in 10% neutral buffered formalin solution for no more than 48 h, after which they were placed in phosphate buffer solution until processed for indirect immunofluorescence using the immunohistochemical polymer procedure. Sections were stained for 1 h at room temperature using primary polyclonal goat antibodies (1:20) specific for the human caspase-14 p10 subunit (Santa Cruz). Sections were then incubated for 30 min with a polyclonal donkey anti-goat antibody bound with a red fluorochrome (Alexa Fluor 647; Life Technologies) at a 1:1000 dilution and mounted using Vectashield Mounting Medium with 4',6-diamidino-2-phenylindole for nuclear detection (Vector Laboratories). An identical procedure was used for negative controls except for the substitution of goat serum instead of primary antibody. Sections were examined using an upright fluorescence microscope (Leica). Six representative fields from each section were examined at a $\times 200$ magnification and digital images were collected. Images were analyzed using MagnaFire Software v 070121-01A (Opotronics). Caspase-14 expression was measured objectively and subjectively by investigators who were blinded to the source of the samples.

For objective evaluation, the intensity of the immunofluorescence signal was graded using a computer imaging program (ImageJ 1.41¹). The epidermis was traced, and a modified corrected total cell fluorescence (CTCF) index was determined as previously described (Santoro et al., 2013). Corrected total cell fluorescence was determined as follows: CTCF = (integrated density – [mean fluorescence of selected area $\times$ mean fluorescence of background readings])/area in

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¹ See: <http://rsb.info.nih.gov/ij/> (accessed 26 November 2015).

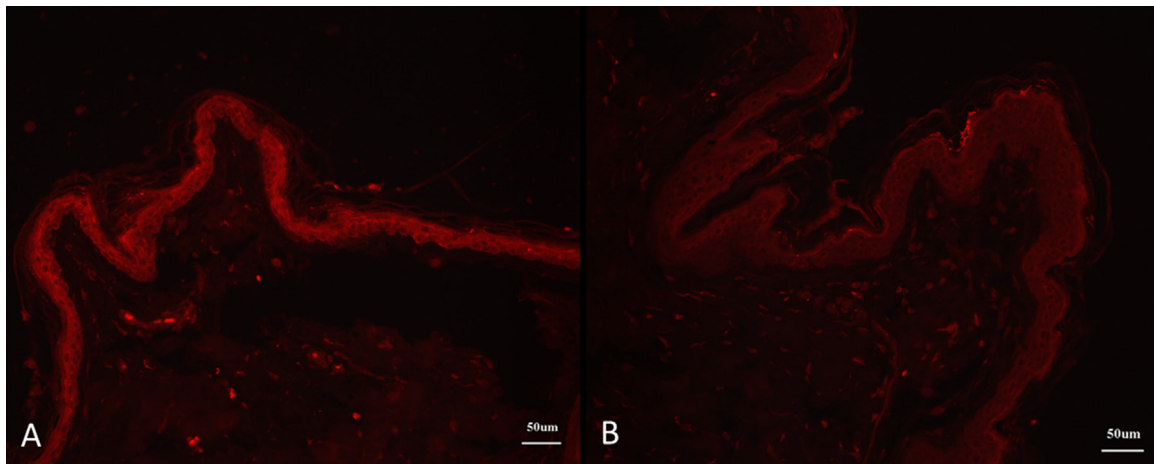


Fig. 1. Indirect immunofluorescence staining of sections of normal (A) and atopic (B) Beagles. Staining was evident in the stratum corneum in normal samples and markedly decreased to absent in atopic samples.

pixel. For the subjective evaluation, five investigators separately scored the staining for intensity using a score ranging from 0 to 3 (0 = no staining, 1 = mild, 2 = moderate and 3 = severe). Mean scores were used for statistics.

Data were tested for normality and analyzed using the unpaired Student's *t* test. The Cohen's κ coefficient was used to evaluate the agreement (inter-rater) among the investigators, and the intra-class correlation coefficient (ICC) was used to measure the reliability/consistency of the ratings (intra-class). $P < 0.05$ was considered statistically significant. MedCalc 12.0 statistical software was used for the analysis (MedCalc Software).

Caspase-14 immunostaining was evident in all normal skin biopsies and concentrated in the stratum corneum (Fig. 1A). In atopic samples, the staining was almost completely absent or patchy in the stratum corneum (Fig. 1B). A significant decrease ($P < 0.001$) was found in atopic samples compared to controls both with the objective (Fig. 2A) and the subjective evaluation ($P = 0.006$; Fig. 2B). A very good total agreement ($\kappa = 0.93$) and consistency (ICC = 0.94) was evident among the investigators and the ratings.

Decreased expression of caspase-14 was found in atopic Beagle dogs compared to healthy controls in this study. This is the first report of filaggrin metabolism alteration in canine AD. The results of this study are similar to the results in humans (Yamamoto et al., 2011) where decreased expression of caspase-14 was found in patients with AD. It is known that caspase-14 levels are decreased by inflammation and a recent study has described how caspase-14 levels

inversely correlate with severity of disease and degree of inflammation in humans (Jung et al., 2014). In our study, skin biopsies in the atopic dogs were taken from non-lesional areas, although clinically non-lesional atopic skin does not equate to normal skin as low grade inflammation is detectable on hematoxylin and eosin staining (Marsella et al., 2006). Future studies will aim at investigating the role of allergen exposure and how a flare up of clinical lesions affects the expression of caspase-14. Future studies are also needed to see if the findings reported in this colony can be replicated in privately owned atopic dogs belonging to different breeds.

In conclusion, this preliminary study suggests that abnormality in filaggrin metabolism may exist in dogs as it does in humans. Although the number of samples used for this pilot study was small, the changes were significant and consistency was found between the objective and the subjective approaches, confirming the validity of these results. This raises the question of whether the changes in filaggrin expression found in dogs, both privately owned (Roque et al., 2011; Theerawatanasirikul et al., 2012) and research Beagle dogs (Marsella et al., 2009; Santoro et al., 2013), are primary or secondary to inflammation and abnormal metabolism. Further studies are needed to address this issue. As we learn more about the relevance of skin barrier dysfunction in this complex disease, it is important to investigate the metabolism of proteins, like filaggrin, playing a crucial role in the keratinization process in order to identify new treatments aimed at correcting these abnormalities and decrease allergen absorption through the skin.

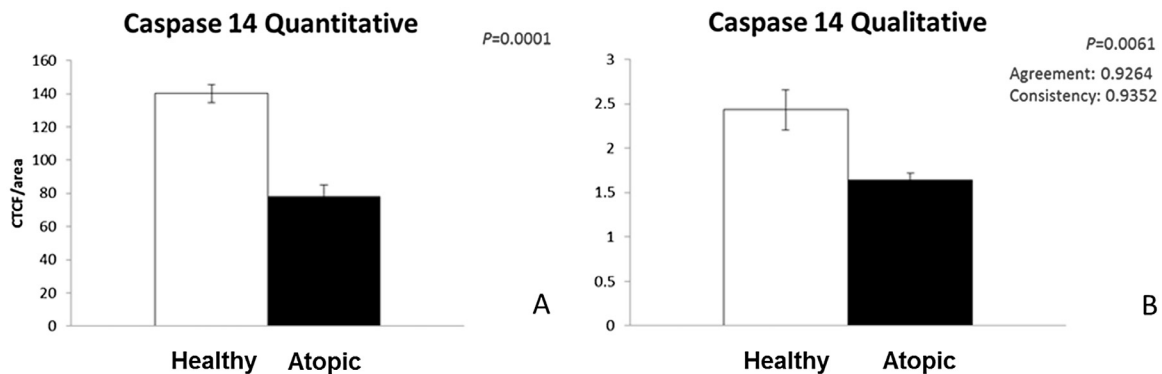


Fig. 2. Objective evaluation of caspase-14 immunostaining (A) and subjective evaluation of caspase-14 immunostaining (B). The figure shows the mean and standard error of the corrected total cell fluorescence measured using Image J. Significant decrease of staining was found in the atopic dogs when compared to the controls. Groups were compared using the unpaired Student's *t* test.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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