

Altered mRNA and protein expression of filaggrin in the skin of a canine animal model for atopic dermatitis

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Background – Filaggrin is a structural protein that has attracted increasing interest over the past decade for its role in the pathogenesis of human atopic dermatitis (AD). Null mutations in its sequence are considered risk factors in the development of AD.

Hypothesis/Objectives – To investigate canine filaggrin mRNA and protein expression in the skin of atopic beagles with experimentally induced AD compared with breed-matched healthy control dogs.

Methods – All dogs were environmentally challenged for 3 days consecutively with allergens to which the atopic dogs had been sensitized. Skin biopsy specimens were taken from six healthy and seven atopic beagles before and after allergen challenge. Canine filaggrin mRNA was measured using quantitative real-time PCR. Indirect immunofluorescence was used to localize the filaggrin protein in canine skin. Analysis of variance with Tukey's multiple comparison test (over-time effect) and unpaired Student's *t*-test (treatment effect) were used. Values of $P \leq 0.05$ were considered significant.

Results – Analysis of variance showed a significantly higher expression of filaggrin mRNA in atopic dogs compared with healthy control dogs ($P = 0.004$ on day 3 and $P = 0.01$ on day 10) and a decreased mRNA expression on day 3 in healthy control dogs (effect of time, $P = 0.006$). On blinded evaluation, filaggrin immunofluorescence was distributed homogeneously in the stratum granulosum and the stratum corneum in healthy dogs. Atopic dogs showed a patchy immunofluorescence pattern, which was exacerbated after environmental challenge.

Conclusions and clinical importance – Altered epidermal filaggrin mRNA expression and protein distribution was detected in this experimental model.

Introduction

Atopic dermatitis (AD) is one of the most common allergic skin diseases in both humans and dogs.^{1,2} The pathogenesis of human AD is very complex and not completely elucidated. The most widely accepted theory (outside–inside–outside) hypothesizes a primary skin defect as a major predisposing factor in the development of AD along with an aberrant immunological response to common allergens.³ This theory is supported by multiple studies demonstrating an impairment of the skin barrier in atopic human patients compared with healthy individuals and increased risk for sensitization when the skin barrier is defective.^{4,5}

In humans, one of the most important predisposing factors for the disruption of the skin barrier of atopic patients includes an alteration of the transcription/translation of the filament-aggregating protein, filaggrin.^{6,7} Filaggrin is one of the most significant proteins involved in the development of the cornified envelope and, after its degradation,

participates in the formation of natural moisturizing factors.⁸ Many genetic studies, analysing different human populations (e.g. European, Chinese and Afro-American) have revealed up to 40 different semi-dominant, homozygous or compound heterozygous mutations present in patients affected by AD.^{9,10} In particular, two null loss-of-function mutations (R501X and 2282del4) have been identified, with 2282del4 being the most common.¹¹ The R501X and 2282del4 mutations are more common in patients affected by the extrinsic subtype of AD, in which allergen-specific IgE levels are increased, confirming that a barrier defect may lead to higher allergen penetration, causing a more severe allergic sensitization.^{12,13} However, filaggrin is not the only predisposing factor in AD development; mutations of the filaggrin gene are present in only 15% of human AD patients and are mainly associated with the most severe form of AD.^{14,15} One explanation for these differences may be the different immunological environment present in extrinsic versus intrinsic AD¹³; intrinsic AD is not characterized by a T helper 2 (Th2)-predominant environment like extrinsic AD.¹³ This hypothesis has been supported by a study in which inhibition of filaggrin production has been observed in normal keratinocyte cell culture after exposure to Th2, indicating that a local Th2 environment is able to decrease the expression of filaggrin, leading to a disruption of the

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skin barrier even in patients without filaggrin gene mutations.¹⁶

Animal models represent a valuable tool to help increase the knowledge of AD.¹⁷ Experimentally induced murine models of AD have been used to investigate immunological and structural alterations in atopic skin. However, the murine model has several limitations, including clinical differences and the fact that mice and human beings do not share the same environment, leading to different external stimuli. These differences between the two species lead to the necessity for animal models similar to human atopic disease. Recently, the dog has been shown to be a better model for human AD because of the development of clinical and immunological alterations similar to the human disease.^{18,19} In addition, dogs are phylogenetically closer to humans than mice and share the same environment and nutrition.^{18,19} A model for canine AD has been identified in a colony of high-IgE, experimentally sensitized atopic beagles able to develop lesions clinically, histologically and immunohistochemically similar to naturally occurring canine AD after environmental challenge with house dust mite (HDM).^{20,21}

In dogs, the disruption of the skin barrier, as a predisposing factor of AD, has only been hypothesized recently. Three studies showed a decreased immunohistochemical staining^{22,23} and mRNA expression²⁴ along with a nonhomogeneous distribution of filaggrin protein in canine skin, suggesting a potential involvement of filaggrin in the pathogenesis of canine AD. Chervet *et al.*²² specifically demonstrated that approximately 20% of dogs with naturally occurring AD showed a decreased expression of filaggrin when an antibody directed against the C-terminal portion of the molecule was used, supporting the usefulness of dogs as a model for the human disease. Recently, filaggrin single-nucleotide polymorphisms (SNPs) in specific canine breeds in both the UK and Thailand have also been demonstrated.^{25,26}

Canine models able to mimic the clinical, immunological and structural alteration of the skin barrier in human AD may provide a useful tool to investigate the pathomechanisms of AD in people further.^{21,27,28} Thus, we performed the present study, using a canine model, to gain more insight into the role of filaggrin in canine AD. In order to do so, we analysed the mRNA expression of canine filaggrin in healthy and atopic beagles (lesional and nonlesional skin) using a quantitative reverse transcriptase PCR (qRT-PCR). In addition, we evaluated its protein distribution in the epidermis using indirect immunofluorescence (IIF). The use of such a model allowed us to show increased mRNA expression of filaggrin in lesional and nonlesional atopic skin compared with healthy control dogs. In addition, a nonhomogeneous distribution of filaggrin protein was seen in atopic skin compared with healthy control skin. These results confirmed that, as in human AD, this colony of atopic beagles have an impairment of the skin barrier function associated with alterations in filaggrin production and distribution.

Methods

The study protocol was approved by the Institutional Animal Care and Use Committee.

Animals

Atopic dogs

Experimentally sensitized atopic beagle dogs belonging to a colony that has been validated as a model for canine AD were used.^{17,20,21} These dogs were sensitized to HDM and show low-grade AD when not exposed to HDM, but react strongly when under allergenic challenge. These dogs were also used as control population in previously published studies on filaggrin expression and AD.^{29,30}

Normal dogs

Healthy, unrelated beagle dogs with no history of skin disease and pruritus were included in this study as breed-matched controls.

Housing conditions

The atopic dogs were housed indoors in individual temperature- and humidity-controlled cement runs (22–24°C; relative humidity of 68–72%). The runs were washed daily using a high-temperature and high-pressure wash (water and bleach). Air filters were changed routinely, and stuffed toys, carpets, soft bedding or anything that could trap dust was not allowed in the runs. MITE-T-Fast™ Allergen Detection System (Aveho Biosciences, Tullahoma, TN, USA) was used monthly, and between challenges, on walls and filters to ensure the absence of HDM in the environment. The dogs were fed a commercial maintenance diet (Hill's Science Diet Adult Maintenance kibble formulation; Hill's Pet Nutrition Inc., Topeka, KS, USA).

Likewise, the healthy control beagles were housed indoors, in individual cement runs, in a temperature- and humidity-controlled facility (22–24°C; relative humidity of 68–72%). The runs were cleaned daily using a high-temperature and high-pressure wash (water and bleach). The dogs were fed a commercial maintenance diet (Hill's Science Diet Adult Maintenance kibble formulation; Hill's Pet Nutrition Inc., Topeka, KS, USA).

Acute environmental challenge with HDM

All dogs (AD and control) were challenged for HDM as previously described.¹⁷ Briefly, the animals' intact skin surface was exposed to 50 mg of *Dermatophagoides farinae* (Greer®, Lenoir, NC, USA) placed on the bottom of their kennels. All dogs were allowed to stay in the kennels for 3 h/day, 3 days in a row.

Clinical evaluation

Severity of clinical signs was scored using the canine AD extent and severity index scoring system (CADESI).³¹ The CADESI used in the present study is a modified version of the validated CADESI-03.³² The sites evaluated are the same as for the CADESI-03, but papules are added as a clinical sign in this modified version. The other difference between the scoring system used in this study and the CADESI-03 is that the scores for each sign range from 0 to 3 rather than from 0 to 5, as done in the CADESI-03. Briefly, the dog's body was divided into small sections, each of which received a score based on the clinical signs evaluated. The total score was calculated by adding the scores of all clinical signs and body sites. Clinical signs evaluated included diffuse erythema, erythematous macules, papules, excoriations and alopecia. The scoring system ranged from 0 (absent) to 3 (severe) for each site and each sign. Total score was used in the statistical analysis. Dogs were scored at baseline [day 0; before allergen exposure (nonlesional skin)], 3 days after the beginning of the challenge [day 3; acute lesional skin (only atopic dogs)] and 7 days after discontinuation of allergen exposure [day 10; postchallenged skin with resolution of the cutaneous lesions (only atopic dogs)].

Skin sample collection

One 8 mm skin biopsy punch from either the inguinal or the ventral thorax area from nonlesional (day 0) and lesional areas (day 3; erythematous maculo-papular dermatitis) was collected following sub-

cutaneous (s.c.) injection of 1 mL of lidocaine (Lidocaine HCl 2%; Hospira Inc., Lake Forest, IL, USA). The skin biopsy specimen was immediately divided into two halves. One half was fixed in 10% neutral buffered formalin for IIF evaluation, while the other half was divided into two quarters, placed in 1.5 mL microfuge tubes and quickly frozen in liquid nitrogen. These latter samples were stored at -80°C until being processed for qRT-PCR.

Quantitative real-time PCR

One quarter of the 8 mm skin biopsy specimen was homogenized using a PowerGen 125 (Fisher Scientific, Pittsburgh, PA, USA) and then processed into RNA using the AllPrep[®] RNA/protein kit (Qiagen, Valencia, CA, USA) reagents, according to the manufacturer's protocol. A 260 nm ultraviolet spectrophotometry and an Agilent 2100 Bioanalyzer (Agilent Biotechnologies Inc., Santa Clara, CA, USA) were used to determine the total RNA concentrations and its integrity and quality. After DNase treatment using a Turbo DNA-free[™] kit (Invitrogen, Carlsbad, CA, USA), total RNA (0.5 μg) was converted to complementary DNA (cDNA) by reverse transcription of mRNA using the SuperScript First-Strand Synthesis System (Invitrogen). Forward and reverse primers for filaggrin (Table 1) were generated using Primer Designer software (Scientific and Educational Software Inc., Palo Alto, CA, USA). Each primer was designed to cross an exon-exon boundary, maximizing the amplification of the mRNA transcript target and minimizing amplification of any residual contaminating genomic DNA. The primers were generated from the previously published genomic sequence [ENSCAFT00000023034 (now renamed *FLG2* with code ENSCAFT00000049887)]. All primer sequences were subjected to Basic Local Alignment Search Tool (BLAST; blast.ncbi.nlm.nih.gov/) comparison to the canine genome build 2.0 for unintended homologies. The relative mRNA expression levels were quantified using a SYBR[®] Green assay (Qiagen) and ABI (Applied Biosystems Inc., Foster City, CA, USA) qRT-PCR methodology. All samples were performed in triplicate 50 μL reverse transcription reactions in an ABI 7500 Real Time PCR System (Applied Biosystems Inc.). The PCR amplifications were carried out as follows: 50°C for 2 min; 95°C for 10 min; and 40 cycles of 95°C for 15 s and 60°C for 60 s. Amplifications were followed by dissociation (melting) curves to ensure specificity of the primers. In initial tests, dilution curves were generated for each gene primer set to determine their amplification efficiencies (Table 1). The results were analysed using the comparative C_T (cycle threshold) method, and the relative mRNA expression of filaggrin was calculated using the $\Delta\Delta C_T$ method. When the efficiency of both the housekeeping gene and the target gene are similar and close to 100%, the formula to use is

$$2^{-[\Delta C_T \text{ experimental sample} - \Delta C_T \text{ controlsample}]},$$

where ΔC_T is the difference between the target gene C_T and normalizer gene C_T .³³ All samples were normalized against the *canine ribosomal protein L15 gene* (*RPL15*). This gene was chosen as member of the ribosomal proteins that showed high stability and consistent expression in canine skin.³⁴

Antibody preparation

Polyclonal anti-canine-filaggrin antibody was synthesized as previously described.²⁹ Briefly, the canine anti-filaggrin antibody was produced based on the genetic sequences previously identified [ENSCAFT00000023034 (now renamed *FLG2* with code ENSCAFT00000049887)]. The amino-acid sequence was subjected to BLAST comparison to the canine genome build 2.0 for unintended

homologies. Based on the aforementioned genetic and amino-acid sequences, the most immunogenic epitope (GRRESSVTESSTEND) on the C-terminus was chosen, and a synthetic peptide was created. Antibody production was carried out using one healthy, 3-month-old, female New Zealand rabbit, 3.4 kg in weight, purchased from Harlan Laboratories, Inc. (Indianapolis, IN, USA).

The peptide conjugates were mixed with an adjuvant and injected s.c. into the rabbit. Titermax (Sigma, St Louis, MO, USA) was used for the primary immunization and incomplete Freund's adjuvant for all subsequent immunizations. The rabbit was immunized four times, and its blood was tested for an immune response using an ELISA technique. The pre-immune serum was used as the negative control. When a satisfactory immune response (positive up to 1:51,200 dilution) was achieved, the rabbit was exsanguinated, and the crude serum was used. The specificity of the anti-canine-filaggrin antibody was tested by immune-absorption on tissue sections; the antibody was incubated with a high concentration (20 $\mu\text{g}/\mu\text{L}$) of the synthetic peptide overnight at 4°C before being applied to the tissue sections. In addition, a standard western blotting was also performed to verify the antibody specificity.

Western blotting

One quarter of the 8 mm skin biopsy specimen was weighed, and the protein was extracted using the AllPrep[®] RNA/protein kit (Qiagen) according to the manufacturer's protocol. The protein concentration was determined at 280 nm using the UV NanoDrop1000[®] spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Fifty and 80 μg of total skin proteins were loaded in well numbers 1 and 2 for gel electrophoresis; 80 μg of total skin proteins were loaded in well number 3 as a negative control, and then a standard western blot was performed. Briefly, 20 μL of total protein extract was loaded in a 10-well 10% Ready Gel Tris-glycine resolving gel (BioRad, Hercules, CA, USA) and run for 1 h at 100 mA. A polypeptide standard (Precision Plus Protein[™] Dual Color Standards; BioRad) was used as the marker. After electrophoresis, the gel was blotted to a 0.2 μm nitrocellulose transfer membrane (BioRad) using an electrophoretic transfer cell apparatus (Mini Trans-Blot[®]; BioRad) for 1 h at 100 mA. The membranes were blocked with a 3% dry milk in Tris buffer solution (TBS) for 1 h, then incubated for an additional 60 min with a 1:1000 dilution of anti-canine filaggrin followed by multiple washes with 0.05% Tween-TBS (BioRad). Then, the membranes were incubated for 1 h with a goat anti-rabbit IgG horseradish peroxidase conjugate (BioRad) as a secondary antibody at 1:3000 dilution according to the manufacturer's recommendation, followed by multiple washes with 0.05% Tween-TBS. A final wash was performed with TBS to remove the excessive secondary antibody not specifically bound to the membrane. The immunofluorescence signal was graded using a modified corrected total well fluorescence (CTWF) index previously described.³³ Briefly, CTWF was determined as follows: CTWF = integrated density – (area of selected well $\times$ mean fluorescence of background readings). The relative fluorescence was expressed in arbitrary units (a.u.).

Indirect immunofluorescence

One half of the 8 mm skin biopsy specimen was fixed in 10% neutral buffered formalin solution for no more than 48 h, after which it was placed in phosphate buffer solution (PBS) until processed for IIF. Briefly, 3 μm skin tissue sections were processed using the immunohistochemical polymer procedure. The sections were blocked using a casein solution (Power Block[®]; BioGenex, San Ramon, CA, USA). A second blocking step was added using normal goat serum (BioGenex,

Table 1. Primers used

Canine gene	Primer sequences	Efficiency (%)	Amplicon length (bp)
<i>Canine filaggrin</i>	Forward: CTCAGCACAAAGGAAGACAGG	110	105
	Reverse: TTGTGTCTCTGGTGGCTTGTC		
<i>Canine ribosomal protein L15 gene</i>	Forward: TTGTGGCTGCTGCTCCTGTG	101	107
	Reverse: ATCCTCGTCCGATTCTCCG		

San Ramon, CA, USA). The sections were then stained for 1 h at room temperature using primary polyclonal rabbit antibodies specific for canine filaggrin. The primary antibodies were used at a dilution of 1:200. The pre-immune serum at 1:200 dilution was used as a negative control. The sections were washed with a blocking solution (Power Block[®]) and then incubated for 30 min at room temperature with a polyclonal goat anti-rabbit antibody bound with a green fluorochrome (Alexa Fluor[®] 488; Invitrogen) at a 1:1000 dilution, according to the manufacturer's recommendations. Finally, the sections were counterstained using 4',6-diamidino-2-phenylindole (DAPI; Invitrogen) for nuclear detection. Specimens were mounted on glass slides using Vectashield[®] Mounting Medium (Vector Laboratories, Burlingame, CA, USA).

The skin sections were examined using an inverted fluorescence microscope (Nikon Eclipse TE 2000-S[®]; Nikon Inc., Shelton, CT, USA). The images were analysed using MetaMorph[®] software (Version 63r1; Molecular Devices, Sunnyvale, CA, USA). Five representative fields from each tissue section were examined at $\times 400$ magnification and digital images collected. The intensity of the immunofluorescence signal was graded using a computer imaging program (ImageJ[®] 1.41; <http://rsb.info.nih.gov/ij/>). The stratum granulosum and stratum corneum were traced, and a modified corrected total cell fluorescence (CTCF) index was determined as previously described.³³ Corrected total cell fluorescence was determined as follows: CTCF = integrated density – (area of selected cells $\times$ mean fluorescence of background readings). The relative fluorescence was expressed in a.u.

Statistical analysis

The Kolmogorov–Smirnov test of normality was used ($\alpha = 0.05$). Mean values and 95% confidence intervals were calculated for all results. Differences between ΔC_T (C_T filaggrin – C_T RPLO) at each time point (between groups) were compared using Student's unpaired *t*-test. Differences between ΔC_T over time (within group) were compared using repeated measurement ANOVA. Tukey's multiple comparison test was used for *post hoc* testing. Values of $P \leq 0.05$ were considered significant. Statistical analysis was performed using SAS[®] statistical software version 9.2 (SAS[®] Institute Inc., Cary, NC, USA).

Results

Animals

Seven 4-year-old atopic beagle dogs (three males, four females) and six 4- to 6-year-old clinically healthy female beagle dogs were used.

Clinical scores

For the clinical score, the ANOVA showed a significant effect between the time points ($P = 0.0118$). In particular, the Tukey's multiple comparison test detected a difference between day 0 and day 3 ($P = 0.03$) and between day 3 and day 10 ($P = 0.007$); however, no difference was detected between day 0 and day 10 ($P = 0.7$; Figure 1).

Expression of mRNA

Quantitative real-time PCR was used to compare filaggrin expression between healthy dogs and dogs with AD. When the *FLG* mRNA expression measured within each group at various time points (day 0 versus day 3, day 0 versus day 10, and day 3 versus day 10) was evaluated, ANOVA showed a significant effect of time only in the control group, with a decreased mRNA expression on day 3 ($P = 0.006$) and on day 10 ($P = 0.02$) compared with day 0 (Figure 2b). When mRNA expression of filag-

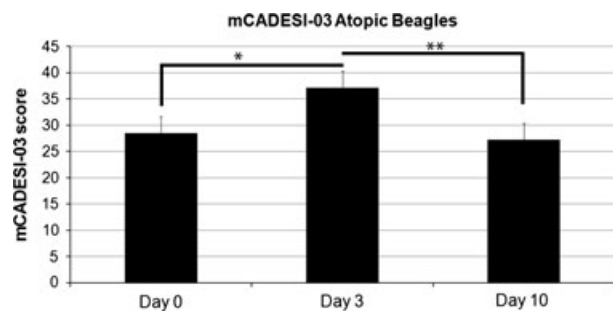


Figure 1. Average of the modified CADESI-03 (mCADESI-03) score before, during and after environmental challenge. (* $P \leq 0.05$; bars represent SEM). Day 0, before allergen exposure; day 3, acute lesional skin; and day 10, postchallenged skin with resolution of the cutaneous lesions.

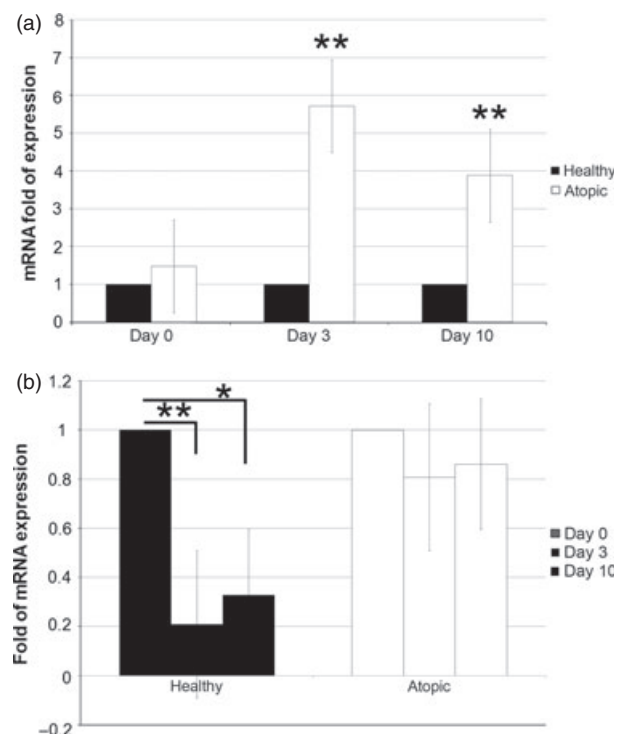


Figure 2. Relative mRNA expression between normal age- and breed-matched healthy and atopic beagles for *FLG* at each time point (a) and over time within each group (b). (* $P \leq 0.05$, ** $P \leq 0.01$; bars represent SEM). Day 0, before allergen exposure; day 3, acute lesional skin; and day 10, postchallenged skin with resolution of the cutaneous lesions.

grin was compared at each time point, between the two groups (healthy versus AD), a statistically significant higher expression of filaggrin was found in atopic dogs on day 3 and day 10 ($P = 0.004$ and $P = 0.01$, respectively), but not on day 0 ($P = 0.2$). In particular, filaggrin mRNA expression was 1.5-, 5.7- and 3.9-fold higher in atopic beagles than in healthy dogs on days 0, 3 and 10, respectively (Figure 2a).

Indirect immunofluorescence

Indirect immunofluorescence was used to analyse the distribution and to assess semi-quantitatively the amount of filaggrin in the skin of healthy and atopic beagles. In the subjective blinded assessment of the distribution pat-

tern, as expected, filaggrin immunofluorescence was detectable only in the stratum granulosum and deeper stratum corneum in all dogs. When the distribution of filaggrin in healthy beagles was compared with the distribution in atopic beagles, a different pattern of distribution was detected (Figure 3). More specifically, in healthy control dogs, a more continuous and laminar distribution of the fluorescence was detected at each time point, whereas in atopic dogs a more patchy distribution of filaggrin immunofluorescence was seen, with complete lack of the immunofluorescence in some areas. In acute lesional skin (day 3) of atopic dogs, the filaggrin immunofluorescence had the highest degree of discontinuity compared with nonlesional skin (day 0) or atopic skin after resolution of the skin lesions (day 10; Figure 3). When the fluorescence intensity was analysed objectively using ImageJ[®] 1.41, no significant difference in signal intensity was seen in atopic dogs compared with healthy control dogs (data not shown).

Western blotting

The western blotting revealed a single band with a size of ~54 kDa showing a high specificity of the anti-canine-filaggrin antibodies. Relative differences in filaggrin intensity were present between well numbers 1 (10 a.u.) and 2 (17 a.u.), as expected (Figure 4).

Discussion

In this study, an increased mRNA expression of canine filaggrin in the skin of atopic beagle dogs when compared with healthy control dogs was found on days 3 and 10 but not on day 0 (Figure 2a). The results of our study are dif-

ferent from what is reported in humans, where a decreased mRNA and protein expression of filaggrin has been seen in AD patients.^{9,10,35} In contrast, the results of this study are in agreement with those of others who evaluated mRNA expression in dogs and reported an increased mRNA expression of filaggrin and some keratins (keratin-1, -2e, -5, -14 and -17) and involucrin in lesional and nonlesional skin of atopic dogs.³⁶

In addition, when we compared the filaggrin mRNA and protein expression, we noted that the significant increase in filaggrin mRNA level in atopic dogs is not accompanied by increase in filaggrin protein expression, suggesting an increase in protein degradation or a post-transcriptional alteration. Decreased^{22,23} or invariant protein expression of filaggrin in atopic dogs may be due to an increased degradation rather than a decreased synthesis. It has been reported that canine atopic skin has increased degradation of ceramides^{37–39} due to increased enzymatic activity. The same may be true for increased degradation of proteins, such as filaggrin, explaining why most studies in atopic dogs have failed to identify a genetic mutation in the filaggrin gene.^{24,25} It is also known that an increased cutaneous pH in atopic skin induces an increase in the activity of proteases,⁷ which may result in excessive degradation of proteins, including filaggrin. Another explanation could be the increased activity of anti-proteases, which does not allow the degradation of profilaggrin into filaggrin monomers, as recently reported in people with AD.⁴⁰ Unfortunately, the authors did not report the mRNA expression levels to confirm an increase in filaggrin mRNA expression as well.⁴⁰ A third possible explanation for an increased transcription of filaggrin without an increase in protein translation in the affected tissue could indicate a defect in the translation machinery (e.g. translational efficiency and/or a post-translational dysregulation) leading to increased protein instability followed by increased protein degradation. In this case, the increased mRNA expression could be due to a 'feedback mechanism', in which the keratinocyte would increase the transcription signal in an attempt to compensate for the lack of protein expression. Genetic mutations (SNPs) in the filaggrin gene and their association with canine AD are still controversial. Two studies^{25,26} have identified SNPs not confirmed by others.^{24,41,42} In the former studies,^{25,26} an association between SNPs, breed and geographical location has been suggested; three SNPs associated with canine AD have been identified in Labrador retrievers in the UK and small-breed dogs (toy poodles, shih tzus and pugs) in Thailand.^{25,26}

The experimental model for canine AD has many advantages. One of these is the potential to monitor filaggrin mRNA expression in lesional and nonlesional skin over time in AD. The present study showed a decrease in filaggrin mRNA expression in healthy and atopic beagles after exposure to HDM (day 3) followed by a slow trend towards a normalization of the filaggrin mRNA expression after resolution of cutaneous lesions (day 10); however, the decreased expression was significant only in control beagles. Healthy beagles were exposed to HDM in a similar manner, in order to reduce the variability due to the mite treatment; however, a greater decrease in filaggrin mRNA expression in healthy beagles, after exposure to

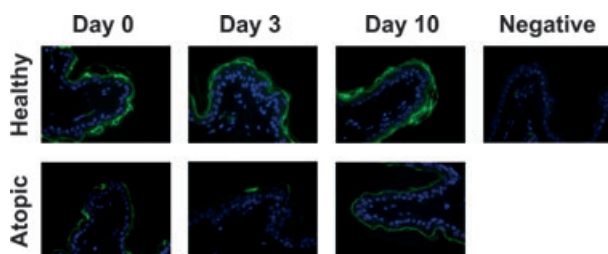


Figure 3. Photomicrographs of canine skin showing indirect immunofluorescence of canine filaggrin. The immunofluorescence signal is detectable only in the stratum granulosum and lower stratum corneum. A more continuous, laminar and homogeneous distribution of the fluorescence is seen in healthy dogs, compared with a patchy and discontinuous pattern seen in atopic dogs at each time point. The pictures are representative of all samples. Day 0, before allergen exposure; day 3, acute lesional skin; and day 10, postchallenged skin with resolution of the cutaneous lesions. Magnification, $\times 400$.

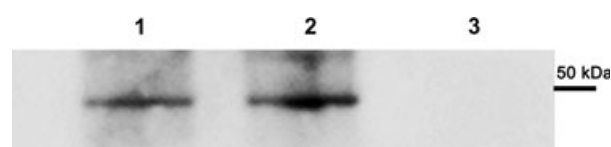


Figure 4. Western blotting analysis of the polyclonal anti-canine filaggrin antibody tested on nonatopic canine skin extract. Lane 1 contains 50 µg of protein extract; lane 2 contains 80 µg of protein extract; and lane 3 contains 80 µg of protein extract and was used as negative control (no primary antibody).

HDM, could lead to the question of whether the increased expression in atopic skin is real or due to the relative decrease in filaggrin mRNA expression in healthy skin. Unfortunately, due to the nature of the technique used in the present study (relative quantitative PCR) we cannot answer this question, and more studies, using an absolute quantitative technique, are needed.

In terms of the protein expression, using IIF, a patchier distribution of the signal was detected in the lesional skin (day 3) of atopic dogs when compared with healthy control animals after HDM exposure (day 3). In addition, a patchier distribution of filaggrin expression was also seen in the atopic dogs when day 3 was compared with days 0 and 10. These results may suggest that the allergen exposure can alter filaggrin protein expression in both healthy and atopic beagles, as suggested in human patients.^{16,43,44} In particular, when the skin of healthy or atopic patients is disrupted, experimentally or naturally, an alteration of filaggrin mRNA and protein, as well as other proteins, occurs.^{16,43,44} This phenomenon is due not only to the mechanical disruption of the skin barrier resulting from increased activity of exogenous and endogenous proteases, including caspase 14, but also to the interaction between proteases and Th2 cytokines.^{16,43,44}

Furthermore, a decrease in filaggrin mRNA levels over time seen in healthy dogs is most probably due to a skin barrier disruption induced by exposure to HDM proteases. It has been reported recently that a marked decrease in filaggrin gene expression is present in healthy human skin after artificial barrier disruption using sodium lauryl sulfate, followed by an increase in mRNA levels 4 and 7 days postexposure.⁴⁵ However, once the stimulus was eliminated and resolution of clinical lesions achieved, a partial recovery was found of both filaggrin mRNA and protein expression. Filaggrin expression is affected by cytokines, as demonstrated by *in vitro* studies using normal human keratinocyte cell cultures and showing decreased filaggrin expression after exposure to Th2 cytokines, such as interleukin-4, interleukin-13 and interleukin-22^{40–42}; these cytokines are highly expressed in acute lesional skin of both human and canine atopic patients.^{3,15,46}

Examination of filaggrin protein distribution shows some distinct differences between humans and dogs. While only decreased signal intensity has been reported in atopic humans,⁴⁷ different patterns of distribution have been reported in canine skin. Chervet *et al.*²² analysed the immunofluorescence signal in the skin of 18 naturally affected atopic dogs (various breeds) and 16 healthy dogs (various breeds), using an anti-canine-filaggrin polyclonal antibody against the C-terminus and showed four different patterns of distribution. The filaggrin signal was similar to that of healthy control dogs (three of 18) or visibly decreased (seven of 18) or visibly decreased with a more granular and discontinuous distribution and large aberrant cytoplasmic vesicles (four of 18) or with a complete lack of immunofluorescence signal (four of 18).²² Compared with this study, our results showed a similarity with the third pattern; a discontinuous distribution of filaggrin staining. In addition, in our study a visible alteration of the filaggrin distribution associated with areas of complete loss of the immunofluorescence was mainly visible after

allergen exposure (day 3). The altered immunofluorescence signal was followed by an amelioration of the distribution after clinical resolution of the cutaneous lesions (day 10), suggesting an increase in protein stability.⁴⁸

Very recently, a new gene belonging to the filaggrin family, filaggrin-2, has been identified in human and murine skin.^{49,50} This gene shares many similarities in genomic sequence as well as protein distribution with filaggrin, and its involvement in skin barrier alterations has been hypothesized.⁵⁰ In dogs, an updated version of the canine database and its comparison with the human database suggested that the previously identified canine filaggrin gene, reported in this and in previous studies,^{22,24–26,36,41,42,51} is more similar to human filaggrin 2 than to the human filaggrin gene. However, given that little information on the human and murine filaggrin 2 genes has been published, and given that all the veterinary literature on filaggrin has been based on the same gene, we preferred to use the name 'filaggrin' rather than filaggrin 2 for the gene/protein studied here.

In summary, this study demonstrated that, as it is in people, filaggrin expression is altered in the skin of atopic dogs. Based on our results, the action of HDM may play a fundamental role in the skin barrier defect in canine AD. In fact, increased protein degradation may lead to an increase in filaggrin gene expression due to a feedback mechanism. More studies are necessary in order to understand better the possible transcriptional/translational alterations present in atopic dogs and to investigate the presence of germline filaggrin mutations and their relation with canine AD. Finally, we confirmed that canine filaggrin is characterized by a ~54 kDa protein identified by western blotting, confirming that canine filaggrin repeats are larger than its human counterpart.⁵¹

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References

1. Bieber T. Atopic dermatitis. *Ann Dermatol* 2010; 22: 125–137.
2. Hillier A, Griffin CE. The ACVD task force on canine atopic dermatitis (I): incidence and prevalence. *Vet Immunol Immunopathol* 2001; 81: 147–151.
3. Elias PM, Steinhoff M. "Outside-to-inside" (and now back to "outside") pathogenic mechanisms in atopic dermatitis. *J Invest Dermatol* 2008; 128: 1067–1070.
4. Proksch E, Folster-Holst R, Jensen JM. Skin barrier function, epidermal proliferation and differentiation in eczema. *J Dermatol Sci* 2006; 43: 159–169.
5. Pilgram GS, Vissers DC, van der Meulen H *et al.* Aberrant lipid organization in stratum corneum of patients with atopic dermatitis and lamellar ichthyosis. *J Invest Dermatol* 2001; 117: 710–717.
6. Wolf R, Wolf D. Abnormal epidermal barrier in the pathogenesis of atopic dermatitis. *Clin Dermatol* 2012; 30: 329–334.

7. Kubo A, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. *J Clin Invest* 2012; 122: 440–447.
8. Nemes Z, Steinert PM. Bricks and mortar of the epidermal barrier. *Exp Mol Med* 1999; 31: 5–19.
9. Chen H, Common JE, Haines RL *et al.* Wide spectrum of filaggrin-null mutations in atopic dermatitis highlights differences between Singapore Chinese and European populations. *Br J Dermatol* 2011; 165: 106–114.
10. Kawasaki H, Kubo A, Sasaki T *et al.* Loss-of-function mutations within the filaggrin gene and atopic dermatitis. *Curr Probl Dermatol* 2011; 41: 35–46.
11. Lesiak A, Kuna P, Zakrzewski M *et al.* Combined occurrence of filaggrin mutations and IL-10 or IL-13 polymorphisms predisposes to atopic dermatitis. *Exp Dermatol* 2011; 20: 491–495.
12. Weidinger S, Rodríguez E, Stahl C *et al.* Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. *J Invest Dermatol* 2007; 127: 724–726.
13. Tokura Y. Extrinsic and intrinsic types of atopic dermatitis. *J Dermatol Sci* 2010; 58: 1–7.
14. Henderson J, Northstone K, Lee SP *et al.* The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. *J Allergy Clin Immunol* 2008; 121: 872–877.e9.
15. Bieber T, Novak N. Pathogenesis of atopic dermatitis: new developments. *Curr Allergy Asthma Rep* 2009; 9: 291–294.
16. Howell MD, Kim BE, Gao P *et al.* Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007; 120: 150–155.
17. Marsella R, Olivry T. Animal models of atopic dermatitis. *Clin Dermatol* 2003; 21: 122–133.
18. Willemse T. Comparative aspects of canine and human atopic dermatitis. *Semin Vet Med Surg (Small Animal)* 1988; 3: 255.
19. Helton Rhodes K, Kerdel F, Soter NA. Comparative aspects of canine and human atopic dermatitis. *Sem Vet Med Surg (Small Animal)* 1987; 2: 166–172.
20. Marsella R, Olivry T, Nicklin C *et al.* Pilot investigation of a model for canine atopic dermatitis: environmental house dust mite challenge of high-IgE-producing beagles, mite hypersensitive dogs with atopic dermatitis and normal dogs. *Vet Dermatol* 2006; 17: 24–35.
21. Marsella R, Girolomoni G. Canine models of atopic dermatitis: a useful tool with untapped potential. *J Invest Dermatol* 2009; 129: 2351–2357.
22. Chervet L, Galichet A, McLean WH *et al.* Missing C-terminal filaggrin expression, NFκB activation and hyperproliferation identify the dog as a putative model to study epidermal dysfunction in atopic dermatitis. *Exp Dermatol* 2010; 19: e343–e346.
23. Marsella R, Samuelson D, Harrington L. Immunohistochemical evaluation of filaggrin polyclonal antibody in atopic and normal beagles. *Vet Dermatol* 2009; 20: 547–554.
24. Roque JB, O'Leary CA, Kyaw-Tanner M *et al.* Real-time PCR quantification of the canine filaggrin orthologue in the skin of atopic and non-atopic dogs: a pilot study. *BMC Res Notes* 2011; 4: 554.
25. Wood SH, Ollier WE, Nuttall T *et al.* Despite identifying some shared gene associations with human atopic dermatitis the use of multiple dog breeds from various locations limits detection of gene associations in canine atopic dermatitis. *Vet Immunol Immunopathol* 2010; 138: 193–197.
26. Suriyaphol G, Suriyaphol P, Sarikaputit M *et al.* Association of filaggrin (FLG) gene polymorphism with canine atopic dermatitis in small breed dogs. *Thai J Vet Med* 2011; 41: 509–517.
27. Olivry T. What can dogs bring to atopic dermatitis research? *Chem Immunol Allergy* 2012; 96: 61–72.
28. Marsella R, Olivry T, Carlotti DN; International Task Force on Canine Atopic Dermatitis. Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. *Vet Dermatol* 2011; 22: 239–248.
29. Marsella R, Santoro D, Ahrens K *et al.* Investigation of the effect of probiotic exposure on filaggrin expression in an experimental model of canine atopic dermatitis. *Vet Dermatol* 2013; 24: 260–e57.
30. Marsella R. Does filaggrin expression correlate with severity of clinical signs in dogs with atopic dermatitis? *Vet Dermatol* 2013; 24: 266–e59.
31. Marsella R, Messinger L, Zabel S *et al.* A randomized, double-blind, placebo-controlled study to evaluate the effect of EFF1001, an *Actinidia arguta* (hardy kiwi) preparation, on CADESI score and pruritus in dogs with mild to moderate atopic dermatitis. *Vet Dermatol* 2010; 21: 50–57.
32. Olivry T, Marsella R, Iwasaki T *et al.*; International Task Force On Canine Atopic Dermatitis. Validation of CADESI-03, a severity scale for clinical trials enrolling dogs with atopic dermatitis. *Vet Dermatol* 2007; 18: 78–86.
33. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001; 29: 2002–2007.
34. Santoro D, Bunick D, Graves TK *et al.* Expression and distribution of antimicrobial peptides in the skin of healthy beagles. *Vet Dermatol* 2011; 22: 61–67.
35. Osawa R, Akiyama M, Shimizu H. Filaggrin gene defects and the risk of developing allergic disorders. *Allergol Int* 2011; 60: 1–9.
36. Theerawatanasirikul S, Sailasuta A, Thanawongnuwech R *et al.* Alterations of keratins, involucrin and filaggrin gene expression in canine atopic dermatitis. *Res Vet Sci* 2012; 93: 1287–1292.
37. Yoon JS, Nishifuji K, Sasaki A *et al.* Alteration of stratum corneum ceramide profiles in spontaneous canine model of atopic dermatitis. *Exp Dermatol* 2011; 20: 732–736.
38. Shimada K, Yoon JS, Yoshihara T *et al.* Increased transepidermal water loss and decreased ceramide content in lesional and non-lesional skin of dogs with atopic dermatitis. *Vet Dermatol* 2009; 20: 541–554.
39. Reiter LV, Torres SM, Wertz PW. Characterization and quantification of ceramides in the nonlesional skin of canine patients with atopic dermatitis compared with controls. *Vet Dermatol* 2009; 20: 260–266.
40. Tan SP, Abdul-Ghaffar S, Weller RB *et al.* Protease–antiprotease imbalance may be linked to potential defects in profilaggrin proteolysis in atopic dermatitis. *Br J Dermatol* 2012; 166: 1137–1140.
41. Barros Roque J, O'Leary CA, Kyaw-Tanner M *et al.* Haplotype sharing excludes canine orthologous filaggrin locus in atopy in West Highland white terriers. *Anim Genet* 2009; 40: 793–794.
42. Salzmann CA, Olivry TJ, Nielsen DM *et al.* Genome-wide linkage study of atopic dermatitis in West Highland White terriers. *BMC Genet* 2011; 12: 37.
43. Gutowska-Owsiak D, Schaupp AL, Salimi M *et al.* Interleukin-22 downregulates filaggrin expression and affects expression of profilaggrin processing enzymes. *Br J Dermatol* 2011; 165: 492–498.
44. McPherson T, Sherman VJ, Aslam A *et al.* Filaggrin null mutations associate with increased frequencies of allergen-specific CD4+ T-helper 2 cells in patients with atopic eczema. *Br J Dermatol* 2010; 163: 544–549.
45. Nuttall TJ, Knight PA, McAleese SM *et al.* T-helper 1, T-helper 2 and immunosuppressive cytokines in canine atopic dermatitis. *Vet Immunol Immunopathol* 2002; 87: 379–384.
46. Törmä H, Lindberg M, Berne B. Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin *in vivo*. *J Invest Dermatol* 2008; 128: 1212–1219.
47. O'Regan GM, Sandilands A, McLean WH *et al.* Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 2009; 124: R2–R6.
48. Hvid M, Johansen C, Deleuran B *et al.* Regulation of caspase 14 expression in keratinocytes by inflammatory cytokines – a possible link between reduced skin barrier function and inflammation? *Exp Dermatol* 2011; 20: 633–636.
49. Wu Z, Hansmann B, Meyer-Hoffert U *et al.* Molecular identification and expression analysis of filaggrin-2, a member of the S100 fused-type protein family. *PLoS ONE* 2009; 4: e5227.

50. Hansmann B, Ahrens K, Wu Z *et al.* Murine filaggrin-2 is involved in epithelial barrier function and down-regulated in metabolically induced skin barrier dysfunction. *Exp Dermatol* 2012; 21: 271–276.
51. Kanda S, Sasaki T, Shiohama A *et al.* Characterization of canine filaggrin: gene structure and protein expression in dog skin. *Vet Dermatol* 2013; 24: 25–e7.
52. Burgess A, Vigneron S, Brioudes E *et al.* Loss of human Great-wall results in G2 arrest and multiple mitotic defects due to deregulation of the cyclin B-Cdc2/PP2A balance. *Proc Natl Acad Sci USA* 2010; 107: 12564–12569.

Résumé

Contexte – La filaggrine est une protéine de structure qui a présenté un intérêt croissant au cours de la dernière décennie pour son rôle dans la pathogénie de la dermatite atopique humaine (AD). Les mutations nulles dans sa séquence sont considérées comme des facteurs de risque du développement de l'AD.

Hypothèses/Objectifs – Etudier l'ARNm de la filaggrine canine et l'expression protéique dans la peau de beagles atopiques avec AD expérimentalement induite comparée avec des chiens croisés sains contrôles.

Méthodes – Tous les chiens ont été testés pendant 3 jours consécutifs avec des allergènes auxquels les chiens atopiques étaient sensibilisés. Des biopsies cutanées ont été prélevées sur six chiens sains et sept beagles atopiques avant et après la provocation allergénique. L'ARNm de filaggrine canine a été mesurée par PCR quantitative en temps réel. L'immunofluorescence indirecte a été utilisée pour localiser les protéines de filaggrine dans la peau canine. L'analyse de variance avec test de comparaison multiple de Turkey (effet temps) et test-t de Student non-apparié (effet traitement) ont été utilisés. Les valeurs de $P \leq 0.05$ étaient considérées significatives.

Résultats – L'analyse de la variance montre une expression significativement plus importante d'ARNm de filaggrine chez les chiens atopiques comparé aux chiens contrôles sains ($P = 0.004$ à jour 3 et $P = 0.01$ à jour 10) et une expression diminuée d'ARNm à jour 3 chez les chiens contrôles sains (effet temps, $P = 0.006$). A l'évaluation en aveugle, l'immunofluorescence de filaggrine était distribuée de façon homogène dans le *stratum granulosum* et le *stratum corneum* chez les chiens sains. Les chiens atopiques présentaient une distribution immunofluorescente inégale qui était accentuée après provocation environnementale.

Conclusions et importance clinique – Une expression épidermique d'ARNm et de distribution protéique de filaggrine altérées ont été mises en évidence dans ce modèle expérimental.

Resumen

Introducción – La filagrina es una proteína estructural que ha atraído un interés creciente en la última década dado su papel en la patogenia de la dermatitis atópica humana (AD). Mutaciones nulas en su secuencia son considerados factores de riesgo en el desarrollo de AD.

Hipótesis/objetivos – Investigar la expresión de mRNA y de proteína filagrina en la piel de perros Beagle atópicos con AD inducida de forma experimental y compararla con perros control sanos de la misma raza y similares.

Métodos – Todos los perros fueron expuestos en su ambiente durante tres días a alérgenos frente a los cuales los animales habían sido sensibilizados. Se tomaron muestras de piel de seis perros sanos y siete perros atópicos antes y tras la exposición al alérgeno. Se midió el mRNA de filagrina canine mediante PCR cuantitativa a tiempo real. Se utilizó inmunofluorescencia indirecta para localizar la proteína filagrina en la piel de perro. El análisis estadístico se llevó a cabo mediante el análisis de varianza con la prueba de comparaciones múltiples de Tukey's (efecto a lo largo del tiempo) y la prueba no pareada t de Student (efecto del tratamiento). Valores de $P \leq 0,05$ se consideraron significativos.

Resultados – El análisis de varianza demostró una expresión significativamente mayor de mRNA de filagrina en perros atópicos comparada con los perros control ($P = 0.004$ al día 3 y $P = 0.01$ al día 10) y una expresión menor de mRNA al día tres en perros control sanos (efecto a lo largo del tiempo, $P = 0.006$). En evaluación ciega, la inmunofluorescencia de filagrina estaba distribuida de forma homogénea en los estrato granuloso y corneo en perros sanos. Los perros atópicos mostraron una tinción discontinua que se exacerbó tras la exposición al alérgeno.

Conclusiones e importancia clínica – En este modelo experimental se observaron expresión anormal de mRNA de filagrina y distribución alterada de la proteína.

Zusammenfassung

Hintergrund – Filaggrin ist ein bauliches Protein, welches in den letzten zehn Jahren aufgrund seiner Rolle bei der Pathogenese der atopischen Dermatitis (AD) des Menschen ein zunehmendes Interesse erfahren hat. Nullmutationen in seiner Sequenz werden als Risikofaktoren bei der Entwicklung von AD betrachtet.

Hypothese /Ziele – Eine Untersuchung der mRNA und der Proteinexpression des caninen Filaggrin in der Haut atopischer Beagles mit experimentell induzierter AD im Vergleich zu Rasse-gleichen gesunden Kontrollhunden.

Methoden – Alle Hunde wurden an 3 aufeinander folgenden Tagen mit Allergenen, gegen die sie sensibilisiert worden waren, einer Provokation in der Umwelt ausgesetzt. Hautbiopsieproben wurden von sechs gesunden und sieben atopischen Beagles vor und nach der Allergenprovokation genommen. Die canine mRNA wurde mittels quantitativem real-time PCR gemessen. Die indirekte Immunfluoreszenz wurde verwendet, um das Filaggrinprotein in der Hundehaut zu lokalisieren. Eine Varianzanalyse mit Tukey multiple Vergleichstest (Auswirkung der Zeit) und ein ungepaarter Student t-Test (Auswirkung der Behandlung) wurde eingesetzt. Werte von $P \leq 0,05$ wurden als signifikant angesehen.

Ergebnisse – Die Varianzanalyse zeigte bei den atopischen Hunden eine signifikant höhere mRNA Filaggrinexpression als bei den gesunden Kontrollhunden ($P = 0.004$ am Tag 3 und $P = 0.01$ am Tag 10) und eine verminderte mRNA Expression am Tag 3 bei den gesunden Hunden (Auswirkung der Zeit, $P = 0.006$). Bei blinder Evaluierung war die Filaggrin Immunfluoreszenz im Stratum granulosum und im Stratum corneum bei gesunden Hunden homogen verteilt. Die atopischen Hunde zeigten ein fleckiges Immunfluoreszenzmuster, welches sich nach Umweltprovokation verstärkte.

Schlussfolgerungen und klinische Bedeutung – In diesem experimentellen Modell wurde eine veränderte Filaggrin mRNA Expression und Proteinverteilung festgestellt.

要約

背景 – フィラグリンはヒトアトピー性皮膚炎 (AD) の病因における役割のために過去10年間以上にわたり関心が高まっている構造タンパクである。その配列のヌル変異はAD発生のリスク因子と考えられている。

仮説/目的 – 犬種が一致する健康犬ならびに実験的にADを誘発したアトピービーグルのイヌフィラグリンmRNAおよび皮膚のタンパク発現を調査すること。

方法 – すべてのイヌにアトピー犬を感作したアレルゲンを3日間連続で環境中に暴露させた。皮膚生検標本を6頭の健康犬と7頭のアトピー犬でアレルゲン暴露前後に採取した。イヌフィラグリンmRNAを定量的リアルタイムPCRを用いて測定した。間接免疫蛍光法をイヌ皮膚でのフィラグリンタンパクの位置を特定するのに用いた。分散解析にTukey法多重比較検定 (延長時間効果) およびStudentのt検定 (処理効果) を用いた。P値は ≤ 0.05 を有意差ありとみなした。

結果 – 分散解析は正常犬 (第3日目 $P = 0.004$ および第10日目に $P = 0.01$) と比較しアトピー犬のフィラグリンmRNA発現は有意に高く、健康犬3日目のmRNA発現は減少していた (効果時間, $P = 0.006$)。盲検下ではフィラグリン免疫蛍光は健康犬で不均一に表皮顆粒層と角層に分布していた。アトピー犬では環境中暴露後悪化した際に、斑状の免疫蛍光を示していた。

結論と臨床的重要性 – 表皮フィラグリンmRNA発現の変化とタンパクの分布がこの実験モデルで検出された。

摘要

背景 – 丝聚合蛋白是一种结构蛋白质, 由于参与人异位性皮炎的发病机制, 人们对它的兴趣近十年来与日俱增。它的排序的无效突变可能是AD的致病因素。

假设/目的 – 制造异位性比格犬实验性模型, 诱发AD。调查其皮肤丝聚合蛋白的 mRNA和蛋白表达, 并与同种健康对照犬相比较。

方法 – 让异位性犬在环境中接触致敏抗原。从 6 只健康犬和 7 只异位性比格犬身上, 分别采集过敏原接触前、后皮肤活检样本。使用实时PCR定量测定犬丝聚合蛋白mRNA。使用间接免疫荧光法定位犬皮肤中的丝聚合蛋白。使用Tukey多重比较试验 (超时效应) 和不配对学生t试验 (治疗效应) 进行方差分析。 $P \leq 0.05$ 时确认为结果差异显著。

结果 – 方差分析显示, 异位性犬的丝聚合蛋白mRNA表达, 相比于健康对照犬显著升高 (第3天 $P = 0.004$ 和第10天 $P = 0.01$), 健康对照犬mRNA表达第3天降低 (时间效应, $P = 0.006$)。盲法评估发现, 丝聚合蛋白免疫荧光在健康犬的表皮颗粒层和角质层均匀分布。异位性犬的免疫荧光分布不均, 接触环境过敏原后尤甚。

结论和临床价值 – 实验室模型的表皮丝聚合蛋白mRNA表达和蛋白分布发生变异。