

Does filaggrin expression correlate with severity of clinical signs in dogs with atopic dermatitis?

Rosanna Marsella

Department of Small Animal Clinical Sciences, University of Florida, Gainesville, FL, USA

Correspondence: Rosanna Marsella, Department of Small Animal Clinical Sciences, University of Florida, 2015 SW 16th Avenue, Gainesville, FL 32615, USA

E-mail: marsella@ufl.edu

Background – Filaggrin expression is decreased in subsets of humans and dogs with atopic dermatitis (AD). Filaggrin expression is modulated by inflammation, but it is unknown whether expression is inversely correlated with clinical signs.

Hypothesis/Objectives – To investigate the correlation between filaggrin expression and the severity of clinical signs in an experimental model of canine AD.

Animals – Eighteen atopic research beagle dogs sensitized to house dust mite allergens.

Methods – Dogs were challenged with house dust mites for 3 days consecutively (days 1–3) and skin biopsies collected on days 1 (before allergen challenge), 3 (peak of challenge) and 10 (at resolution of lesions). Clinical signs were scored using a modified version of CADESI-03, the Canine Atopic Dermatitis Extent and Severity Index. Immunohistochemical staining was measured subjectively by three investigators who graded filaggrin staining for continuity, granularity and intensity. For objective evaluation of staining, the stratum granulosum and epidermis were traced, and the percentage of filaggrin per unit stratum granulosum and epidermal area were calculated. Correlations were investigated using Pearson's product-moment correlation (for linear correlations) and Spearman's rank correlation (for nonlinear correlations), pooling all days together and then separately on each day, using total clinical scores and either subjective or objective filaggrin scores.

Results – The only significant correlation was found between the subjective filaggrin scores and total clinical scores on day 1.

Conclusions and clinical importance – No inverse correlation exists between the severity of clinical signs and filaggrin expression; the clinical relevance of filaggrin in canine AD is unknown.

Introduction

Skin barrier dysfunction in canine atopic dermatitis (AD) has striking similarities with the human counterpart.¹ Loss-of-function mutations of the filaggrin (*FLG*) gene are a major risk factor for the development of AD in humans.² In atopic dogs, various patterns of filaggrin expression have been described using an antibody that targets the C-terminal portion of the protein.³ One subgroup (approximately 20% of atopic dogs) fit the category of no staining (similar to humans with mutations), while other atopic dogs may have filaggrin expression similar to normal control dogs.³ Decreased filaggrin expression was described in an experimental model of canine AD using a polyclonal antibody directed against the C-terminal portion of canine filaggrin.⁴

Although filaggrin is one of the most important risk factors for human AD, the relationship between filaggrin and AD is not straightforward, because mutations can occur in the absence of the development of AD, and many patients develop AD without mutations.⁵ Filaggrin expression is also regulated by inflammation and cytokines,^{6,7}

and it would be reasonable to speculate that an inverse relationship exists between filaggrin expression and the severity of clinical signs. Although no studies in human medicine have specifically measured filaggrin expression in the skin of AD patients to correlate it with the severity of clinical signs, it has been documented that significantly more severe erythema exists in humans with filaggrin mutations compared with the AD patients without filaggrin mutations.⁸ In human medicine, there is an indirect suggestion that an inverse relationship may exist between filaggrin and the severity of AD lesions, which comes from the proven decreased presence of natural moisturizing factors, derived from the breakdown of filaggrin, in patients affected by moderate to severe AD. A recent study showed that the *FLG* genotype is the major determinant of natural moisturizing factors and that disease severity is a secondary modifier.⁶ Consequently, a correlation in humans may exist between the severity of clinical signs and the magnitude of the decrease of natural moisturizing factors.

The purpose of this study was to evaluate the correlation between the severity of clinical signs and filaggrin expression, as detected by immunohistochemistry, using an experimental model of canine AD.⁴ The study reported here formed part of a project reported by Marsella *et al.*¹⁴

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Materials and methods

All procedures were approved by the Institutional Animal Care and Use Committee.

Animals

Eighteen atopic beagles that had previously been epicutaneously sensitized to house dust mites (HDM; *Dermatophagoides farinae*) were used.

Allergen challenge

All beagles were environmentally challenged with HDM by applying 50 mg/day per dog on the floor of a plastic air kennel. Each dog was placed in the kennel for 3 h each day, for three consecutive days (days 1–3). Kennels were washed at the end of the challenge. The concentration and volume of HDM used were selected based on previous studies.⁴

Evaluation of clinical signs

All dogs were evaluated and scored before and 6 h after each exposure and daily thereafter for 5 days; an additional evaluation was performed on day 10.

Clinical signs were evaluated using a Canine Atopic Dermatitis and Extent Severity Index (CADESI),⁹ a modified version of the validated CADESI-03.¹⁰ The two modifications in relation to CADESI-03 were that papules were added as a clinical sign and that the scores for each sign ranged from 0 to 3 rather than from 0 to 5. The dog's body was divided into sections, each of which received a score based on the clinical signs evaluated. The total score was the sum of the scores for all body sites. Clinical signs evaluated included diffuse erythema, erythematous macules, papules, excoriations and alopecia. The total score was used in the statistical analysis.

Skin biopsies

Two 8 mm punch biopsies were taken from each dog on day 1 (before allergen challenge), day 3 (third day of allergen challenge) and day 10. All samples were taken from the inguinal skin, or from the ventral thoracic area when no lesions were present in the inguinal area. Biopsies were collected under local anaesthesia with 1% lidocaine and sutured routinely.

Immunohistochemistry: processing of samples

The 8 mm punch biopsies were fixed in 10% neutral buffered formalin and embedded in paraffin. Samples from days 1, 3 and 10 were embedded in the same block for each dog. Sections were stained for filaggrin as previously described.¹⁴

Immunohistochemistry: imaging and image analysis

Slides were viewed using a Nikon Lapophot microscope with $\times 20$ objective ($\times 200$ magnification), and 18 pictures per slide were taken. The pictures were representative of 18 consecutive optical fields, progressing from the left to the right side of the biopsy. Images were taken with a Nikon Coolpix 990 camera (Nikon Inc., Melville, NY) and saved as JPEG files. The resolution for the JPEG images was set at 300 d.p.i. Filaggrin expression was measured both objectively and subjectively.¹⁴

Objective measurements

Semi-quantification was performed using the public domain ImageJ program (<http://rsb.info.nih.gov/ij/>). A constant pixel-intensity threshold was set for the evaluation of every slide. Stratum granulosum and epidermis were traced. The number of positive pixels and the total pixel count for the selected area was used to calculate the percentage of immunostaining.

Subjective evaluations

Photographs were also evaluated blindly by three investigators who scored the continuity, intensity and granularity of the staining in the

stratum granulosum of each picture on a scale of 0–3 (0 = absent and 3 = severe). The scores for the extent and intensity of staining were added, and the mean of the total scores was used for analysis.

Statistics

Correlations were investigated using both Pearson's product-moment correlation (for linear correlations) and Spearman's rank correlation (for nonlinear correlations). Data were analysed for all the days pooled together and then separately on each day. Correlations were investigated using the total clinical scores and either the subjective or the objective filaggrin scores. For data analysis, JMP 9.0.3 (SAS Institute, Inc., Cary, NC, USA) was used.

Results

The only significant correlation between filaggrin measurements and the severity of clinical signs was found between the subjective scores and total CADESI on day 1 (Spearman's $\rho = 0.485$, $P = 0.0413$). Means and standard deviations for the scores at various time points are reported in Table 1.

Discussion

The results of this study in atopic beagle dogs do not support the existence of an inverse correlation between filaggrin expression and the severity of clinical signs. Interestingly, a study in humans by Nemoto-Hasebe *et al.*¹¹ showed that there was no correlation between the severity of clinical signs and filaggrin expression. Furthermore, the results of that study showed that the clinical severity of AD in patients with filaggrin mutations was comparable with that in patients without filaggrin mutations.¹¹ Thus, the question of the relevance of a reduction in filaggrin for the severity of clinical signs in AD is still open. It is likely that the severity of dermatitis is influenced by a variety of factors, including skin barrier function, to which filaggrin is one of many contributors. One clinically relevant question would be whether stimulators of filaggrin expression would be of any clinical benefit. Given that the morphology of filaggrin in some subsets of AD dogs is abnormal, with large granules in category III in the study by Chervet *et al.*,³ it is possible that even an increase may not lead to appreciable clinical improvement, because the issue may not be a matter of sheer quantity but also one of the quality of the protein.

Table 1. Summary of the mean $\pm$ SD of the clinical and filaggrin scores (subjective and objective) at different time points (days 0, 3 and 10)

	Day 0	Day 3	Day 10
Total CADESI	21.8 $\pm$ 7.9	57.8 $\pm$ 7.7	23.5 $\pm$ 7.5
Total subjective filaggrin score	6.7 $\pm$ 0.7	6.8 $\pm$ 1.1	6.6 $\pm$ 0.8
Objective filaggrin			
Percentage of filaggrin per unit stratum granulosum	22.3 $\pm$ 8.8	24.48 $\pm$ 12.6	24.58 $\pm$ 10.3
Percentage of filaggrin per unit epidermis	7.36 $\pm$ 4.3	9.15 $\pm$ 7.5	7.56 $\pm$ 3.3

Controversy still exists in veterinary medicine about the relevance of filaggrin in canine AD and whether mutations in the filaggrin gene play a role or not. The results of initial studies have been mixed, probably reflecting the fact that different mechanisms may be important in different subsets of AD dogs and that breed and geographical variations exist.^{12,13} The conclusions of this study relate only to a specific experimental model of AD and may not be applicable to other breeds or to atopic dogs in other geographical locations. The main advantage of using an experimental model is the ability to control the environment, the diet and the amount of allergen exposure. One disadvantage is that a model may not always be representative of all aspects of the naturally occurring disease.

It is important to note that when correlations are considered in human medicine, complex scoring systems are used, such as the Nottingham Eczema Severity Score,¹¹ which also captures information relating to the duration of the disease, pruritus and quality-of-life parameters. All of these factors are not currently reflected in the scoring system of CADESI, regardless of the version used. Thus, in the future, efforts need to be made to elaborate and validate a scoring system that will include the extent and severity of clinical signs as well as other important aspects of disease severity, such as the quality of life.

It is concluded that in this experimental model of canine AD, a reduction in filaggrin does not correlate with the severity of signs. Given that filaggrin is one of the many components of the skin barrier, it is possible that in dogs other proteins may play a key role.

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Résumé

Contexte – L'expression de la filaggrine est diminuée dans les groupes d'hommes et de chiens atteints de dermatite atopique. L'expression de la filaggrine est modulée par l'inflammation mais on ne sait pas si l'expression est inversement corrélée aux signes cliniques.

Hypothèses/Objectifs – Étudier la corrélation entre l'expression de filaggrine et la sévérité des signes cliniques dans un modèle expérimental de dermatite atopique canine.

Sujets – Dix huit beagles expérimentaux sensibilisés aux acariens de la poussière de maison.

Méthodes – Les chiens ont été testés avec les acariens de poussière de maison pendant 3 jours consécutifs (jours 1 à 3) et des biopsies cutanées ont été prélevées à jour 1 (avant le test allergénique), à jour 3 (au pic du test) et à jour 10 (à la résolution des lésions). Les signes cliniques ont été notés à l'aide d'une version modifiée du CADESI-03 (Canine Atopic Dermatitis Extent and Severity Index). La coloration immunohisto-chimique a été évaluée subjectivement par trois investigateurs qui ont noté la coloration de la filaggrine selon la continuité, la granularité et l'intensité. Pour l'évaluation objective du marquage, la couche granuleuse et les aires épidermiques ont été calculées. Les corrélations étaient étudiées par la corrélation produit-moment de Pearson (pour les corrélations linéaires) et la corrélation de rang de Spearman (pour les corrélations non-linéaires), mises en commun de tous les jours ensemble et ensuite séparément chaque jour, utilisant des scores cliniques totaux et des scores de filaggrine, soit objectifs soit subjectifs.

Résultats – La seule corrélation significative a été trouvée entre les scores subjectifs de filaggrine et les scores cliniques totaux à jour 1.

Conclusion et importance Clinique – Il n'existe pas de corrélation inverse entre la sévérité des signes cliniques et l'expression de filaggrine; on ne connaît pas la pertinence clinique de la filaggrine dans la dermatite atopique canine.

Resumen

Introducción – La expresión de filagrina está disminuida en ciertos grupos de humanos y perros con dermatitis atópica (AD). La expresión de filagrina es modulada por la inflamación, pero se desconoce si su expresión está inversamente correlacionada con los signos clínicos.

Hipótesis/objetivos – Investigar la correlación entre la expresión de filagrina y la severidad de los signos clínicos en un modelo experimental de AD canina.

Animales – Dieciocho perros de investigación de raza Beagle sensibilizados a alérgenos de ácaros del polvo.

Métodos – Los perros fueron expuestos a ácaros del polvo durante tres días consecutivos (días 1-3) y se tomaron biopsias de piel en los días 1 (antes de la exposición a alérgeno), 3 (pico de exposición) y 10 (al resolver las lesiones). Los signos clínicos se evaluaron utilizando una versión modificada del CADESI-03, el Índice de Extensión y Severidad de Dermatitis Atópica Canina. La tinción inmunohistoquímica para filagrina se evaluó subjetivamente por tres investigadores que evaluaron la tinción de filagrina en términos de continuidad, granularidad e intensidad. Para realizar una evaluación objetiva se trazaron el estrato granuloso y la epidermis, y se calculó el porcentaje de filagrina en el estrato granuloso y la epidermis por unidad de área. La correlación se evaluó utilizando la correlación del producto-momento de Pearson (para correlaciones lineales) y la correlación de rango de Spearman (para correlaciones no lineales), agrupando todos los días y después separándolos, utilizando los valores clínicos totales y los valores subjetivos u objetivos de filagrina.

Resultados – La única correlación significativa se encontró entre los valores subjetivos de filagrina y los valores clínicos totales en el día 1.

Conclusiones – No existe una correlación inversa entre la severidad de los signos clínicos y la expresión de filagrina; se desconoce la relevancia clínica de la filagrina en AD canina.

Zusammenfassung

Hintergrund – Die Fillagrin Expressierung ist in einzelnen Untergruppen von Menschen und Hunden mit atopischer Dermatitis (AD) vermindert. Die Fillagrin Expressierung wird durch Entzündung moduliert, aber es ist nicht bekannt, ob die Expressierung mit klinischen Symptomen invers korreliert ist.

Hypothese/Ziele – Eine Untersuchung der Korrelation zwischen der Fillagrin Expressierung und dem Schweregrad der klinischen Symptome in einem experimentellen Modell caniner AD.

Tiere – Achtzehn atopische Versuchshunde wurden mit Hausstaubmilbenallergenen sensibilisiert.

Methoden – Die Hunde wurden Hausstaubmilben an 3 aufeinander folgenden Tagen (Tage 1-3) ausgesetzt und Hautbiopsien an den Tagen 1 (vor Allergensensibilisierung), 3 (Höhepunkt der Sensibilisierung) und 10 (Verschwinden der Hautveränderungen) entnommen. Die klinischen Symptome wurden mittels einer modifizierten Version des CADESI-03, dem Canine Atopic Dermatitis Extent and Severity Index, bewertet. Die immunhistochemischen Färbungen wurden subjektiv von drei UntersucherInnen bewertet, die die Färbung für Fillagrin auf Kontinuität, Granularität und Intensität einstufen. Zur objektiven Evaluierung der Färbung wurden das Stratum granulosum und die Epidermis durchgesehen und der Prozentanteil von Fillagrin pro „Einheit“ Stratum granulosum und Epidermis kalkuliert. Die Korrelationen wurden mittels Produkt-Moment Korrelation nach Pearson (für lineare Korrelationen) und Spearman's Rangkorrelationskoeffizient (für nicht-lineare Korrelationen) untersucht, wobei zuerst alle Tage zusammengekommen wurden und danach jeder Tag für sich beurteilt wurde. Es wurden dafür die gesamten klinischen Werte und entweder die subjektiven oder die objektiven Fillagrin Werte verwendet.

Ergebnisse – Die einzige signifikante Korrelation wurde zwischen den subjektiven Fillagrin Bewertungen und dem Gesamtwert der klinischen Bewertungen von Tag 1 gefunden.

Schlussfolgerungen und klinische Bedeutung – Es besteht keine inverse Korrelation zwischen dem Schweregrad der klinischen Symptome und der Fillagrin Expressierung; die klinische Bedeutung von Fillagrin bei der caninen AD ist unbekannt.

要約

背景 – 仮説—ヒトと犬のアトピー性皮膚炎(AD)ではフィラグリンの発現が低下している。フィラグリンの発現は炎症に左右されるが、その発現が臨床症状と逆相関をいめすかどうか知られていない。

目的 – フィラグリン発現と犬のAD実験モデルの臨床症状の重症度の間に相関があるか調査すること。

供与動物 – ハウスダストマイトアレルゲンに感作した18頭のアトピー研究用ビーグル犬

方法 – 供与犬はハウスダストマイトに3日間連続して感作させ(1-3日目)、皮膚生検材料は1日目(アレルゲン感作前)、3日目(感作のピーク)と10日目()に採取した。臨床症状はthe Canine Atopic Dermatitis Extent and Severity Index であるCADESI-03の修正版を用いてスコア化した。免疫組織化学染色を連続性、粒度、濃さに関して3人の調査者が主観的に測定した。客観的な染色の評価は顆粒層と表皮を画像解析し、顆粒層と表皮の単位あたりのフィラグリンの割合を計算した。相関は(線形相関に)ピアソンの積率相関と(非線形相関に)スピアマンの順位相関を用いて

調査を行い、総臨床スコアと主観的あるいは客観的フィラグリンスコアを用いてすべての日を合算してからそれぞれの日
に区別した。

結果 — 主観的なフィラグリンスコアと1日目の総臨床スコアの間でのみ有意な差がみられた。

結論と臨床的な重要性 — 臨床症状の重症度とフィラグリン発現の間に逆相関が存在しなかった; 犬のADにおけるフィラ
グリンの臨床的な妥当性は未知である。

摘要

背景 — 丝聚蛋白表达在人和犬异位性皮炎(AD)中减少。丝聚蛋白表达通过炎症调节,但未知表达是否与临床
症状负相关。

假设/目的 — 研究在异位性皮炎犬的试验模型上,丝聚蛋白表达和临床症状严重性之间的相互关系。

动物 — 18只用户尘螨过敏原致敏的异位性疾病研究用比格犬。

方法 — 犬连续3天(1-3日)接受户尘螨刺激,在1日(接受过敏原刺激之前)、3日(刺激峰值)和10日(病变恢复时)
收集皮肤活检样本。临床症状使用CADESI-3改良版进行评分,犬异位性皮炎程度和严重指数。免疫组化染色
通过丝聚蛋白着色连续性、颗粒度和强度等级这三个研究方面进行主观判断。客观评估染色,对颗粒层和表
皮进行示踪,计算颗粒层和表皮区域每单位丝聚蛋白百分比。使用皮尔逊积差相关法进行研究统计(对线性相
关性分析)和斯皮尔曼等级相关法(用于非线性相关分析),汇集所有日,然后分别对单日,使用总临床评分和主
观或客观丝聚蛋白评分。

结果 — 仅发现在1日丝聚蛋白主观评分和总临床评分间显著相关。

结论和临床价值 — 在临床症状严重程度和丝聚蛋白表达间不存在负相关;未知犬异位性皮炎的丝聚蛋白与临
床症状的相关性。