

Gene (mRNA) expression in canine atopic dermatitis: microarray analysis

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What is known about the topic of this paper

- Canine atopic dermatitis is a common and pruritic inflammatory skin disease.
- Canine atopic dermatitis shows breed associations and familial predisposition with no reports of proven genetic linkage with the disease pathology.
- Microarray technology allows large-scale sampling of gene expression in cases and controls, identifying potential candidate genes for further study.

What this paper adds to the field of veterinary dermatology

- Identifies candidate genes for further genetic study into the pathogenesis of atopic dermatitis.
- Highlights the similarities between atopic lesional and nonlesional skin and shows the marked differences in gene expression in healthy control skin, suggesting atopic dermatitis affects all skin to variable degrees.
- Genes identified potentially support the use of the dog as a model for human atopic dermatitis.

Abstract

Genes potentially involved in the pathology of canine atopic dermatitis (AD) were identified using gene expression microarrays. Total RNA extracted from skin biopsies was hybridized to an Agilent Technologies custom-designed 22K canine array. The arrays were analysed using Genedata Analyst software. Data were corrected for multiple hypothesis testing and tested for significance using the National Institute on Aging array analysis tool. For comparison, data were divided into separate groups: lesional atopic ($n = 16$), nonlesional atopic ($n = 17$) and healthy controls ($n = 9$). Fifty-four genes were differentially expressed at a significance level of 0.05 in canine AD compared to

healthy controls. Sixteen genes were differentially expressed in both nonlesional and lesional atopic skin, 26 genes only in nonlesional skin and 12 only in lesional skin. These genes were associated with innate immune and inflammatory responses, cell cycle, apoptosis, barrier formation and transcriptional regulation. The most dysregulated gene in lesional skin was S100A8, which showed an almost 23-fold increase in expression. This is a pro-inflammatory cytokine located in the epidermal differentiation complex. Microarray analysis is a novel technique in canine AD. Significant changes in gene expression were identified in atopic skin. These were relevant to skin barrier formation and the immune response, suggesting that they both participate in AD. Gene expression restricted to lesional skin may be involved in inflammatory changes, whereas those shared or restricted to nonlesional skin may reflect the atopic phenotype. Investigating gene polymorphisms in the targets identified in this study will help improve our understanding of the genetic basis of this disease.

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Introduction

Canine atopic dermatitis (AD) is a common and pruritic inflammatory skin disease. Its pathogenesis is strongly associated with immunological hyper-reactivity, although skin barrier function, and microbial colonization and infection may also play a role.^{1,2} It is thought that canine AD has become more common over the last three decades as the human prevalence has risen, and prevalence in the canine population is now estimated to be 10%.³ Management of canine AD is difficult as the pathogenesis is complex and evolving.⁴ Topical and systemic therapies have been used to date together with allergen-specific immunotherapy,⁵ glucocorticoids,⁶ antihistamines,⁷ cyclosporin,⁸ and essential fatty acid supplements.⁹ However, many of these treatments can have adverse side effects and are not always effective in all patients.⁴

Human and canine AD are characterized by a biphasic T-cell response, with both Th1 and Th2 cells present in atopic skin. The Th2 response follows an allergic inflammatory pathway that results in IgE production. High IgE is a characteristic of the majority of human AD sufferers; however, some individuals do not show increased IgE and present with atopic symptoms.¹⁰ The Th1 response is thought to lead to chronic lesion formation in patients with AD.¹¹ These responses are probably triggered by environmental allergens; it is unknown whether the immune system is

over-reacting to normal levels of allergens or whether it is responding to increased allergen levels as a result of skin barrier disruption.¹²

Canine AD exhibits well-recognized breed associations with some breeds being more predisposed than others, especially West Highland white terriers and boxers.^{13,14} Sighthounds, in contrast, are rarely affected suggesting a genetic component to canine AD.¹⁵ A British guide dog study demonstrated a heritability of 0.47 providing further support for a genetic component in canine AD.¹⁶ Stronger evidence for heritability exists in human AD where heritability is 0.72–0.77 in monozygotic twins.^{17,18} Because of the similarities of human AD with canine AD, it is thought that both diseases have a significant genetic component.

By identifying the genes involved in canine AD, it may be possible to establish the disease pathogenetic mechanisms provide candidate genes for studies of gene polymorphisms contributing to disease aetiopathogenesis and potentially develop improved treatments for the disease. Linkage analysis and association studies are two approaches commonly used to identify causative genes in a disease. Linkage analysis requires the study of a large number of families.¹⁹ Chromosomal regions are identified from linkage analysis; however, these regions can span large areas and many genes and, therefore, additional research is often required with this approach. Association studies do not require related individuals for analysis but potential candidate genes must be first identified. The selection of candidate genes is often based on literature searches and focuses on genes previously linked with the disease; this often leads to bias in the genes selected.²⁰ For an unbiased approach in candidate gene selection, gene expression microarray analysis can be used. Microarrays allow coverage of a large portion of the genome. This technique measures the levels of expression of mRNA transcripts for very large numbers of genes in a relatively small number of cases and control samples. Comparison of expression levels in cases and controls means that transcripts under- or overexpressed in the disease phenotype can be identified as potential candidate genes for involvement in the pathogenesis of the disease. Microarray analysis has the advantage of being unbiased and because of its broad sweep approach it can potentially provide novel genes for further analysis.

The purpose of this study was to identify potential candidate genes involved in canine AD by measuring mRNA expression in lesional atopic skin, nonlesional atopic skin and healthy skin using a gene expression microarray.

Methods

Case recruitment and sample collection

The diagnosis of canine AD was based on compatible history and clinical signs, and exclusion of other causes of pruritus. Coat brushing, skin scraping, *Sarcoptes*-specific immunoglobulin serology and antiparasite trial therapy were used to eliminate the possibility of an ectoparasite infestation. All dogs underwent a strict 6–8 weeks of diet trial with either a home-cooked or a commercial single, novel protein diet, or a commercial hydrolysed protein diet and water to eliminate adverse food reactions. No anti-inflammatory medication was given for at least 3 weeks prior to examination.

All dogs with a clinical diagnosis of AD were intradermally tested with 54 allergens and showed at least one clinically relevant positive result.

Skin samples from dogs with AD were taken under sedation as follows: 0.5–1.0 mL of 2% lignocaine without adrenalin was infiltrated into the subcutis at the sites marked for biopsy. Nonlesional samples were selected from clinically unaffected skin on the flank. Lesional samples were selected from areas of erythema and macular-papular dermatitis from the ventral body. Areas of grossly evident excoriation, staphylococcal pyoderma and *Malassezia* dermatitis were avoided. Six-millimetre-diameter skin biopsies were snap frozen in liquid nitrogen and placed into storage at –80 °C until processed.

Control samples were taken from healthy dogs that had been euthanized for reasons unrelated with the study. These dogs had no history and clinical signs of skin disease or conditions likely to alter immune function. All samples were taken from the flank within 30 min of euthanasia.

The dogs with AD included 12 pure breeds (Labrador retriever, German shepherd, Rhodesian ridgeback, West Highland white terrier, Staffordshire bull terrier, Scottish terrier, Jack Russell terrier, bulldog, boxer, bullmastiff, springer spaniel, Neapolitan mastiff) and five cross-breeds. The healthy control dogs included seven pure breeds (Jack Russell, German shepherd, Staffordshire bull terrier, Labrador, boxer and English bull terrier) and two cross-breeds. All controls were male dogs (18 months to 8 years), and of the 17 dogs with AD, 7 were male (13 months to 8 years and 1 month) and 10 were female (7 months to 9 years and 2 months). All animals were privately owned.

RNA extraction

Total RNA was extracted from skin samples via homogenization in Tri-reagent (Aldrich, St. Louis, MO, USA) according to the manufacturer's recommended protocol.²¹ The RNA pellet was allowed to air dry and, thereafter, was resuspended in diethyl pyrocarbonate-treated water and stored at –80 °C until use. The integrity and quality of the RNA was checked using the Agilent 2100 Bioanalyser and the concentration determined using a Nanodrop® ND-100 spectrophotometer.

Agilent 22K oligonucleotide canine array

The array was designed by definition of coding fragments from the 1.2x poodle genome sequence in collaboration with Celera (Celera diagnostics, Rockville, MD, USA; <http://www.celera.com/>). An in-house 54 000 protein database and the NCBI reference sequence database (<http://www.ncbi.nlm.nih.gov/RefSeq/>; release of 12 April 2002) were used to isolate match islands for dog coding sequences using BLAST.²² Nonredundant sequences matching NCBI reference sequence database (cut-off of e^{-40}) and the Protein database (cut-off of e^{-20}) were submitted for oligonucleotide design to Agilent Technologies (Palo Alto, CA, USA). Three oligonucleotides per sequence were designed and tested against mRNA derived from canine blood, testes and cell culture. Based on maximum performance on the chip and uniqueness in the gene set, one of the three oligonucleotides was selected.

Agilent 22K microarrays

Following the first round of *in vitro* transcription, the amplified RNA underwent a second round of amplification and fluorescent labelling with Cyanine 5-CTP or Cyanine 3-CTP (PerkinElmer, Bucks, UK) using the Low RNA Input Fluorescent Linear Amplification Kit protocol (Agilent Technologies). The Cyanine-labelled amplified RNA samples were subsequently purified using RNeasy spin columns (Qiagen Ltd, West Sussex, UK) and were eluted in a volume of 60 µL.

Target solutions for the microarray chips were produced by combining 8 pmol of Cyanine 3- and Cyanine 5-labelled cRNA and 1× control targets in 1× fragmentation buffer as described in the Agilent 60-mer Oligo Microarray processing protocol. The hybridization mix was loaded onto the 22K microarray which was incubated for 17 h at 60 °C with rotation at 4 r.p.m.

The microarrays were initially washed in 6× SSPE (0.75 M NaCl, 50 mM NaH₂PO₄, 5 mM EDTA, pH 7.0), 0.005% N-lauroylsarcosine followed by a 0.06× SSPE, 0.005% N-lauroylsarcosine wash and finally a wash in Acetonitrile Far UV (Fisher Scientific, Leic, UK). The microarrays were scanned at a resolution of 5 µm in an Agilent G2565AA Microarray Scanner system (G2567AA Feature Extraction

Software, version 7.1, Agilent Technologies). The images were analysed using an Agilent Technologies software package.

Statistical analysis of microarray data

Data were imported into Genedata Expressionist Analyst (Genedata AG, Basel, Switzerland), and the Cyanine 3 and Cyanine 5 fluorescence intensities normalized using lowest weighted linear regression (LOW-ESS).²³ Normalized data in tab delineated text format were uploaded into NIA array analysis (<http://lgsun.grc.nia.nih.gov/ANOVA>); this allowed a pairwise comparison of gene signal intensity (expression) with fold difference of two or greater and statistical significance of $P \leq 0.05$, as determined by single factor analysis of variance with multiple hypothesis correction by the false discovery rate.²⁴ The fold change was calculated for each sample to represent a ratio of expression between disease and control samples. This number was further corrected to provide the actual fold change in the sample by: ratio – 1 if the sample was up-regulated and by (–1/ratio) + 1 for down-regulated samples. This is stated as the true fold change.

Results

Fifty-four genes out of 22 000 were found to be significantly differentially expressed in canine AD skin when compared to healthy control skin. Sixteen of the 54 genes showed an increase or decrease in expression in both lesional skin and nonlesional skin from dogs with AD when compared to healthy controls (Table 1). Twelve of the 54 genes were only differentially expressed in lesional skin when compared to healthy controls (Table 2), and the remaining 26 were only differentially expressed in non-lesional skin (Table 3). Only one gene, S100 calcium-binding protein A8 (S100A8), showed a significant increase in expression when comparing lesional to nonlesional skin (Table 2). The true fold change in expression between healthy, lesional and nonlesional skin and statistical significance are presented in Tables 1–3, with the genes grouped according to function.

Discussion

The pathogenesis of canine AD is poorly understood and this project was set out to identify potential candidate genes for further study. Fifty-four genes were identified as being dysregulated in canine AD suggesting that a number of complex interactions lead to the atopic phenotype. Moreover, a number of these genes have functions that potentially link them with the disease.

S100 calcium-binding protein A8 (S100A8) showed the highest increase of all the genes, nearly 23-fold in lesional skin. The S100 family are pro-inflammatory molecules released by phagocytes in inflammatory conditions²⁵ and levels have been shown to 'track' the severity of inflammatory diseases.²⁶ Release is stimulated by tumour necrosis factor- α (TNF- α), a cytokine expressed in both canine and human AD.²⁵ S100A8 has been linked to leucocyte recruitment, suggesting that it is a key factor in the inflammatory response.²⁵ S100A8 was not significantly overexpressed in nonlesional skin, suggesting that it has a purely inflammatory role in lesional skin. A positive feedback loop via RAGE/NF- κ B pathway is thought to maintain release of TNF- α and subsequent release of S100.²⁷ This is comparable with previous evidence correlating TNF- α expression and the degree of inflammation in both canine and human AD.^{10,28}

Serum amyloid A is also produced in response to inflammation and its mRNA transcription was increased in lesional skin. Serum amyloid A is an apolipoprotein that recruits immune cells to inflammatory sites and are regulated by interleukin-1 (IL-1), IL-6 and TNF- α .^{29,30} Squamous cell carcinoma antigen 2 (SCCA2) is another gene induced by inflammation in response to IL-4 and IL-13. It has been shown to disrupt the enzyme function of *Dermatophagoides* house dust mites allergens which are

Table 1. Genes differentially expressed in both lesional and nonlesional skin compared to healthy controls grouped according to function

Gene name	Accession number	Lesional		Nonlesional	
		True fold change*	FDR†	True fold change*	FDR†
Inflammation/immunology					
LOC488898 (ARTS-1)	XP_546015.1	–1.60	0.00	–3.4	0
Cell cycle/apoptosis/repair/lesion formation					
LOC477298 (POSTN)	XP_534490.1	4.52	0.00	2.85	0
LOC478537 (down-regulated in ovarian cancer 1 isoform 2)	XP_535715.2	2.53	0.04	3.07	0.01
Translational control					
LOC474556 (eIF-5B)	XP_531784.1	3.36	0.00	3.03	0
Transport/regulation					
LOC480332 (kinectin 1)	XP_537455.1	2.19	0.00	1.66	0.01
LOC478312 (Myosin Va)	XP_535487.1	2.05	0.02	1.98	0.02
LOC475224 (A-kinase anchor protein 9 isoform 2)	XP_532456.1	1.65	0.05	1.61	0.04
<i>Canis familiaris</i> ret proto-oncogene (RET)	XP_543915.1	–2.31	0.03	–5.3	0
Barrier formation					
GOLGA4 subfamily a, 5	CAA54261.1	2.09	0.00	2.19	0
GOLGA4 subfamily a, 4	AAC51791.1	1.41	0.02	1.39	0.01
GOLGA4 subfamily a, 5	NP_002069.2	1.41	0.04	1.85	0
Miscellaneous					
LOC475424 (EEA1)	XP_532649.1	2.42	0.00	2.23	0
LOC610752 (CG15747-PA)	XP_868363.1	2.02	0.00	1.54	0.01
C6orf142 (chromosome 6 open reading frame 142)	NP_612636.1	–2.28	0.00	–5.4	0
LOC492014 (sushi-repeat-)	XP_549134.1	–2.50	0.00	–6.8	0
Hypothetical protein LOC55086	AAH70110.1	–2.70	0.03	–8.5	0

*True fold change represents the actual change in expression. †FDR is the false discovery rate for these findings. The P -value is < 0.05 for all genes. The results show 5 significantly down-regulated genes and 11 significantly up-regulated genes.

Table 2. Genes differentially expressed only in lesional skin when compared to healthy controls

Gene name	Accession number	True fold change*	FDR†
Inflammation/immunology			
LOC490461 (S100A8)‡	XP_547583.1	22.81	0.00
LOC 485209 (INPPL1)	NP_001558.2	7.49	0.00
LOC483954 (SCCA-2)	XP_541074.1	3.11	0.00
LOC480749 (SAA3)	XP_537868.1	3.07	0.01
Serum amyloid A protein (<i>Canis familiaris</i>) – no records	AAA62765.1	2.91	0.00
LOC403816 (TIMP1)	BAA32393.1	2.72	0.04
LOC751814 (SAA)	AAA62762.1	2.67	0.01
Cell cycle/apoptosis/repair/lesion formation			
LOC474674 (similar to RAD50 homologue isoform 1)	XP_531901.1	2.00	0.04
LOC484662 (CIDE-3)	XP_541777.1	-1.99	0.00
Transport/regulation			
LOC480623 [sperm-associated antigen 5 (Astrin)]	XP_537743.1	1.56	0.05
Barrier formation			
LOC143662 (Mucin-15)	AAH20912.2	1.29	0.05
Miscellaneous			
LOC477037 (hypoxia-induced gene 1)	XP_534235.1	1.42	0.05

*True fold change represents the actual change in expression. †FDR is the false discovery rate for these findings. The *P*-value is < 0.05 for all genes. The results show 1 significantly down-regulated gene and 11 significantly up-regulated genes. ‡Represents significant difference between lesional and nonlesional skin.

Table 3. Genes differentially expressed only in nonlesional skin when compared to healthy controls

Gene name	Accession number	True fold change*	FDR†
Inflammation/immunology			
C10orf118 [CTCL (cutaneous T-cell lymphoma) tumour antigen L14-2]	AAM44457.1	2.16	0.03
IL1RAPL1 (interleukin 1 receptor accessory protein-like 1)	NP_001009038.1	-1.24	0.03
Cell cycle/apoptosis/repair/lesion formation			
LOC482986 (SYND1)	XP_540099.1	-1.13	0.04
LOC489256 (Cadherin-14)	XP_546374.1	-1.54	0.00
LOC8451 (Cullin 4A)	AAR13072.1	-1.60	0.03
Transport/regulation			
LOC480045 (Nucleoprotein TPR)	XP_537167.1	2.21	0.05
LOC479689 (potassium channel tetramerization)	XP_536820.2	-1.18	0.04
LOC486205 (FERM, RhoGEF and pleckstrin domain protein 2)	XP_543330.1	-1.26	0.01
LOC487294 (ATP-binding cassette C12e)	XP_544420.1	-1.58	0.01
TJP3 (tight junction protein 3)	NP_001003202.1	-1.63	0.00
LOC486657 (phospholipase C, zeta 1)	XP_543784.1	-1.65	0.05
Barrier formation			
LOC483653 (Mucin-2)	XP_540774.1	-1.47	0.03
Transcription factor			
LOC481111 (STAT2)	XP_538232.1	-1.21	0.02
CGGBP1 (CGG triplet repeat binding protein 1)	AAD04161.1	-1.70	0.00
LOC485022 (FUSE binding protein 2)	XP_542140.1	-1.44	0.03
FOXO4 (foxhead box)	XP_549066.1	-1.66	0.03
Miscellaneous			
LOC477332 (RING-H2 protein)	XP_534526.1	2.49	0.03
LOC480963 (ATRX1)	XP_538084.1	1.83	0.01
C1orf163 (hypothetical protein LOC65260)	AAH15313.1	-1.47	0.02
LOC 465184 (FBXL10)	XP_520652.2	-1.50	0.01
LOC2346 (FOLH1)	AAC83972.1	-1.51	0.02
LOC491989 (S6K-alpha 6)	XP_549109.1	-3.71	0.00
LOC476102 (mSin3A-associated protein 130)	XP_533311.1	-1.66	0.02
LOC489724 (PH domain leucine-rich repeat protein phosphatase-like)	XP_546844.1	-1.72	0.02
LOC488148 (similar to ecotropic viral integration site 1)	XP_545272.1	-1.82	0.01
LOC50525 (Spag6)	NP_056588.1	-3.94	0.00

*True fold change represents the actual change in expression. †FDR is the false discovery rate for these findings. The *P*-value is < 0.05 for all genes. The results show 22 significantly down-regulated and 4 significantly up-regulated genes.

known triggers of an allergic responses.³¹ SCCA2 transcription was only increased in lesional skin, however, indicating that it will have little impact on early responses to dust mite allergen exposure.

Several other genes differentially expressed only in lesional skin (Table 2) were mainly inflammatory genes and, with the exception of cell death-inducing DFF45-like effector-3 (CIDE3), were overexpressed. Genes expressed

exclusively in nonlesional skin, in contrast, were generally underexpressed (Table 3) and represent a range of functions. A large number of these down-regulated genes are involved in the transport of calcium, potassium and other ions. Calcium transport is increased in inflammatory conditions and is essential for the function of some pro-inflammatory molecules. The down-regulation of calcium transport may represent a lack of an anti-inflammatory stimulus in nonlesional skin, although the difference with healthy control skin was unexpected. Therefore, it is possible that the calcium transport mechanism may have a role in the pathogenesis of canine AD, possibly in epidermal barrier function. Signal transducer and activator of transcription 2 (STAT2) was also underexpressed in nonlesional skin. Increased levels of STAT2, however, have been linked to allergic inflammation.³² Altered expression of STATs have been linked with a number of inflammatory barrier-related diseases, e.g. irritable bowel syndrome and psoriasis,^{33,34} and with atopic diseases such as rhinitis and asthma in humans.^{35,36} STAT is part of the JAK-STAT pathway³⁷ which is used by interleukins to transduce the signals that initiate cytokine-specific gene transcription. Only STAT2 was included in this array. There are another six STATs within the family that perform a range of functions, some antagonistic to each other. To understand the role of STATs in AD, all functions within the family must be measured and the interrelationships elucidated.

Forkhead box O4 (FOXO4), a transcription factor, was down-regulated in nonlesional skin. Forkhead box P3 (FOXP3) is a related gene that is expressed on human T-regulatory cells³⁸ and whose expression leads to inhibition of the immune response.³⁹ Defects in T-regulatory cells are associated with immune pathologies, for example FOXP3-deficient mice develop allergic airway inflammation and exaggerated IgE responses.⁴⁰ If FOXO4 performs similar functions, initial down-regulation may lead to the poor T-regulatory cell function hypothesized in canine AD²⁸ resulting in increased levels of IgE, chronic inflammation and lesion formation.

Not all human and canine cases present with increased IgE,¹⁰ indicating that there is more to AD than allergic hypersensitivity. It has been suggested that skin barrier formation may play a role in the development of human and canine AD.^{12,41} A recent paper suggested how differences in barrier function could be involved in both IgE- and non-IgE-associated human AD.⁴² Genes relating to skin barrier/epidermis formation could therefore be important in the pathogenesis of canine AD.

Strong linkage to human AD has been previously reported within the epidermal differentiation complex on chromosome 1q21.^{19,42,43} The epidermal differentiation complex comprises genes such as profilaggrin, loricrin, involucrin, S100 and small proline-rich proteins, which are essential for keratinocyte differentiation and epidermal barrier formation.^{43,44} A large-scale microarray study into human AD⁴⁵ found that S100 was up-regulated in lesional skin, as observed in this study, and filaggrin and loricrin were down-regulated, although we were not able to study these with the present canine array. Since the canine array was designed, evidence has arisen illustrating the importance of filaggrin in human AD. Two loss of function variants were identified in filaggrin⁴⁶ and other mutations

and associations have been found.^{47,48} Moreover, there is evidence further linking AD to 1q21 and the associated cornified envelope proteins.⁴⁹

Ceramide is a lipid essential for barrier function and formation; it covalently binds to involucrin, envoplakin, elafin, small proline-rich proteins and loricrin to form a lipid layer and the cornified envelope.⁵⁰ Ceramide maintains the skin moisture by limiting *trans*-epidermal water loss⁵¹ and lack of this lipid may lead to the characteristic dry skin seen in human and canine AD. Ceramide was not on the array used but the Golgi autoantigen Golgi subfamily a4 (GOLGA4) was present. This protein has been shown to play a role in transport of ceramide from the Golgi apparatus to the cell surface.⁵² Our results, contrary to expected, showed that GOLGA4 was up-regulated in lesional and nonlesional atopic skin. However, it has been noted that the low levels of ceramide in atopic skin is due to increased bacterial colonization of atopic patients,⁵³ suggesting that although GOLGA4-associated ceramide transport is enhanced, ceramide levels will still be low in atopic skin.

Both human and canine AD are characterized by spongiosis of the epidermis, which is most severe at lesional sites although it is also present at nonlesional sites.⁵⁴ Syndecan-1 is present in the keratinocyte membrane and functions in cellular adhesion to regulate the normal cell architecture,⁵⁵ and down-regulation is associated with spongiosis.⁵⁶ In this study, syndecan-1 was down-regulated in nonlesional skin but not in lesional skin. The reason for this is unclear although other inflammatory factors may influence expression. If the levels of TNF- α were increased at non-lesional sites compared to lesional, this may have caused greater down-regulation of syndecan-1 as seen in irritable bowel syndrome.⁵⁷

Chronic lesions in human and canine AD are characterized by fibrosis and lichenification. Periostin and tissue inhibitor of metalloproteinase 1 (TIMP1) promote this^{58,59} and may play a role in lesion formation in AD. On our array, periostin was overexpressed in both lesional and nonlesional skin. Although not statistically significant, periostin expression was increased in lesional skin. TIMP1, in contrast, was only overexpressed in lesional skin. In human studies, serum levels of TIMP1 were greater in atopic patients and levels correlated with the chronicity of lesions and degree of lichenification.⁵⁸

This study has helped identify genes expressed in atopic lesional and nonlesional skin compared to normal healthy skin. To further elucidate the genetics of canine AD, it would have been interesting to identify IgE responders and non-IgE responders and include them as discrete groups on the array. It would have also been beneficial to include genes such as filaggrin, involucrin and inflammatory cytokines on the array as these have been implicated in human AD.

Gene expression microarray provides a rapid, wide-ranging assessment of many genes. However, it is relatively insensitive to small changes in expression and, therefore, important genes could be missed. Quantitative PCR is more sensitive to small changes in expression and is often used to confirm microarray results although quantitative PCR cannot identify novel genes in the pathogenesis of AD. Microarray studies are also prone to false-positive results and to minimize this, the Benjamini and Hochberg correction²⁴ was used. This gives a false discovery rate

and represents the probability the findings were due to chance. In this study, all false discovery rates were between 0 and 0.05, which statistically is very strong and indicates the findings in this study were robust.

In conclusion, a number of candidate genes with both barrier function and immunological activity have been identified (Table 1). The similarities found on this array with human AD provide more evidence that canine AD is a similar condition and suitable model. Further confirmation and investigation of the target genes identified in this study by quantitative PCR and immunohistochemistry is now required. It is hoped that research into the genetic basis of canine AD will improve our understanding of the disease and allow development of better diagnosis and management options.

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Résumé Les gènes potentiellement impliqués dans la pathogénie de la dermatite atopique canine (AD) ont été identifiés. L'ARN extrait de biopsies cutanées a subi une hybridation. Les prélèvements obtenus ont été analysés en un logiciel Genedata Analyst software. Les données ont été corrigées pour test d'hypothèses multiples. Par comparaison, les données ont été divisées en groupes séparés : peau lésionnelle atopique ($n = 16$), peau non-lésionnelle atopique ($n = 17$) et peau saine contrôle ($n = 9$). Cinquante quatre gènes ont été exprimés à un niveau de signification de 0.05 pour les chiens à AD comparés aux contrôles sains. Seize gènes étaient exprimés différemment dans la peau lésée, 26 dans la peau non lésée et 12 seulement dans la peau saine. Ces gènes étaient associés avec les réponses immunes innées, les réponses inflammatoires, le cycle cellulaire, l'apoptose, la formation de barrière et la régulation transcriptionnelle. Le gène le plus dérégulé dans la peau lésée était S100A8, qui présentait une augmentation de presque 23 fois son expression. Il s'agit d'une cytokine pro-inflammatoire localisée dans le complexe de différenciation épidermique. L'analyse en microarray est nouvelle pour la dermatite atopique canine. Des modifications significatives d'expression des gènes ont été observées. Elles étaient en corrélation avec la formation de la barrière cutanée et de la réponse immunitaire, suggérant que ces deux aspects participent à l'AD. L'expression des gènes limitée à la peau lésée peut être associée aux modifications inflammatoires, alors que celle partagée ou limitée à la peau non lésée pourrait être le reflet du phénotype atopique. L'étude du polymorphisme génique dans les cibles identifiées ici aideront à mieux comprendre la génétique de cette maladie.

Resumen Se identificaron genes potencialmente implicados en la patología de la dermatitis atópica canina (AD) mediante microórdenes de expresión génica. RNA total extraído de biopsias de piel se hibridó en un orden de DNA canino de 22K procedente de la compañía Agilent Technologies de diseño exclusivo. Los microórdenes se analizaron con el programa Genedata Analyst. Los datos obtenidos se corrigieron para pruebas de hipótesis múltiples y se analizaron para ver su significado utilizando la herramienta de análisis de

orden del Instituto Nacional de la Edad. Con motivos comparativos, los datos se dividieron en grupos separados: atópicos con lesiones ($n = 16$), atópicos sin lesiones ($n = 17$) y controles sanos ($n = 9$). Cincuenta y cuatro genes fueron expresados de forma diferencial y significativamente a un nivel de 0.05 en perros atópicos comparados con perros sanos. Dieciséis genes se expresaron de forma diferencial tanto en piel con lesiones de perros atópicos como en piel sin lesiones. 26 genes sólo en piel sin lesiones y 12 sólo en piel con lesiones. Estos genes se asociaron con inmunidad innata y respuestas inflamatorias, ciclo celular, apoptosis, formación de barrera defensiva y regulación de transcripción. El gen más disregulado en la piel con lesiones fue S100A8, que mostró casi un aumento de 23 veces la expresión basal. Esta es una citoquina proinflamatoria localizada en el complejo de diferenciación epidermal. El análisis de los microórdenes es una técnica novedosa en el estudio de la dermatitis atópica canina. Cambios significativos en la expresión génica fueron identificados en la piel de perros atópicos. Los cambios observados fueron de importancia para la formación de la barrera defensiva de la piel y la respuesta inmune, lo que sugiere que ambas funciones participan en la AD. La expresión génica restringida a la piel con lesiones podría estar implicada en cambios relacionados con la inflamación, mientras que aquellos comunes o restringidos a piel sin lesiones podrían ser reflejo de un fenotipo atópico. La investigación de polimorfismos genéticos en los genes identificados en este estudio contribuirá a mejorar nuestro conocimiento en la regulación génica de esta enfermedad.

Zusammenfassung Gene, die möglicherweise bei der Pathologie der caninen atopischen Dermatitis (AD) eine Rolle spielen, wurden durch Genexpression mit Hilfe der Microarray-Technologie identifiziert. Totale RNA wurde von Hautbiopsien extrahiert und zu einem anwendungsspezifischen caninen Array (Agilent Technologies 22k) hybridisiert. Die Arrays wurden mittels Genedata Analyst Software analysiert. Die Daten wurden für multiple Hypothesentestung korrigiert und auf ihre Signifikanz mit Hilfe des Array Analysegeräts des National Institute on Aging getestet. Zum Vergleich wurden die Daten in separate Gruppen unterteilt: atopisch mit Läsionen ($n = 16$), atopisch ohne Läsionen ($n = 17$) und gesunde Kontrollen ($n = 9$). Vierundfünfzig Gene wurden bei der caninen AD im Vergleich zu den gesunden Kontrolltieren mit einer Signifikanz von 0.05 differentiell ausgedrückt. Sechzehn Gene wurden in nicht-läsionaler wie auch in läsionaler atopischer Haut unterschiedlich ausgedrückt, 26 Gene nur in nicht-läsionaler Haut und 12 nur in läsionaler Haut. Diese Gene wurden mit angeborenen Immun- und Entzündungsreaktionen, Zellzyklus, Apoptose, Bildung einer Barriere und transkriptionaler Regulierung in Zusammenhang gebracht. Das am meisten dysregulierte Gen in läsionaler Haut war S100A8, welches eine fast 23-fache Zunahme der Expression zeigte. Es handelt sich dabei um ein pro-inflammatorisches Zytokin, welches im epidermalen Differenzierungskomplex lokalisiert ist. Die Microarray Analyse ist eine neue Technik bei der caninen AD. Es wurden signifikante Veränderungen bei der Genexpression in atopischer Haut gefunden. Diese waren relevant im Bezug auf die Barrierebildung und die Immunantwort, was darauf hinweist, dass beide an AD teilhaben. Gene, deren Expression sich auf läsionale Haut beschränkt, könnten an entzündlichen Veränderungen beteiligt sein, während jene, die gleichzeitig – oder nur – in nicht-läsionaler Haut ausgedrückt werden, den atopischen Phänotyp reflektieren könnten. Eine Untersuchung des Gen-Polymorphismus der Zielgruppen, die in dieser Studie identifiziert worden waren, wird uns helfen, die genetische Grundlage dieser Erkrankung zu verstehen.