

The genomics revolution: will canine atopic dermatitis be predictable and preventable?

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Background – Heritability studies suggest that atopic dermatitis (AD) involves multiple genes and interactions with environmental factors. Advances in genomics have given us powerful techniques to study the genetics of AD.

Objective – To review the application of these techniques to canine AD.

Results – Candidate genes can be studied using quantitative PCR and genomic techniques, but these are hypothesis-dependent techniques and may miss novel genes. Hypothesis-free techniques avoid this limitation. Microarrays quantify expression of large numbers of genes, although false-positive associations are common. In the future, expression profiling could be used to produce a complete tissue transcriptome. Genome-wide linkage studies can detect AD-associated loci if enough affected dogs and unaffected relatives are recruited. Genome-wide association studies can be used to discover AD-associated single nucleotide polymorphisms without relying on related dogs. Genomic studies in dogs have implicated numerous genes in the pathogenesis of AD, including those involved in innate and adaptive immunity, inflammation, cell cycle, apoptosis, skin barrier formation and transcription regulation. These findings, however, have been inconsistent, and problems include low case numbers, inappropriate controls, inconsistent diagnosis, incomplete genome coverage, low-penetrance mutations and environmental factors.

Conclusions – Canine AD has a complex genotype that varies between breeds and gene pools. Breeding programmes to eliminate AD are therefore unlikely to succeed, but this complexity could explain variations in clinical phenotype and response to treatment. Genotyping of affected dogs will identify novel target molecules and enable better targeting of treatment and management options. However, we must avoid misuse of genomic data.

Introduction

Human and canine AD

Canine atopic dermatitis (AD) is very similar to human AD;^{1–4} both are inflammatory dermatoses with characteristic clinical features. They are complex diseases involving immune dysregulation, allergic sensitization, skin barrier defects, microbial colonization and environmental factors. Human and canine AD are very common conditions, affecting up to one-third of children in Western societies and 10% of all dogs.^{1,5–8}

It's in the genes

Both the human and canine conditions have a genetic component. Family history is a major risk factor for human AD.^{1–3} Strong breed predispositions, with high prevalences in some dog breeds (e.g. up to 25% in West Highland white terrier dogs), suggest that this is also true in canine AD.^{9–11} In British guide dogs (mostly Labrador and golden retriever cross-bred dogs) the mean heritability is 0.47 (range 0.13–0.81), suggesting that the

genetic background accounts for almost 50% of the risk of developing AD.¹² Experimental laboratory colonies of dogs with conditions that mimic clinical AD have also been established.^{4,13–15}

It's not all genetic – environmental factors in AD

The risk of developing AD, the severity and the response to treatment are highly variable. This may be explained by complex genotypes, but it is also likely that environmental influences are important.⁸ Environmental factors that influence the development of canine AD are listed in Table 1.^{10,16,17} These may be important in immunity, tolerance and skin barrier function. The environmental influence, however, appears to vary with breed; for example, these factors do not affect the prevalence of AD in West Highland white terrier dogs.

Genetic studies in AD

Historically, studies have been limited to observations of breed predispositions, as well as heritability and linkage studies. Advances in genomics now allow us to study the genetics of AD in more detail. The aim of this paper is to review these techniques, the current evidence for the genetic basis of canine AD and future opportunities.

Accepted 28 June 2012

Sources of Funding: This study was self-funded.

Conflict of Interest: No conflicts of interest have been declared.

Table 1. Environmental factors that influence the development of canine atopic dermatitis

Risk of developing atopic dermatitis	Environmental factor
Increased	Urban life
	High human population density
	Increased average annual rainfall
	Living in southern Sweden
	Adoption at the age of 8–12 weeks
Reduced	Regular bathing
	Rural life
	Living with other animals
	Walking in forests
	Feeding noncommercial foods to lactating bitches
No effect	Sex
	Season of birth
	Home environment
	Vaccination
	Deworming

Investigating AD-associated genotypes

Genome-wide linkage studies

Genome-wide linkage studies have been widely used to investigate human AD.⁸ These are family-based approaches using affected individuals, their parents and nonaffected family members. The inheritance of the disease is compared with the inheritance of microsatellite markers.

This technique can evaluate the whole genome, avoiding the limitations of candidate gene approaches. Microsatellite markers have been used to identify chromosomal loci associated with human AD.⁸ However, the usefulness of this approach has been questioned, because there has been little overlap in the results from these studies. The linked loci extend over large areas of each chromosome, spanning several genes and making it difficult to identify a candidate gene without either extensive chromosomal sequencing or further genotyping of additional microsatellite markers clustered around the loci of interest. It is difficult, furthermore, to amass enough affected individuals and unaffected relatives to perform genome-wide linkage studies in dogs.

Candidate gene association studies

It is possible to genotype markers specifically associated with candidate genes of interest. This allows the use of relatively simple markers, such as single nucleotide polymorphisms (SNPs), insertions, deletions and repeats. In addition, candidate gene approaches are not limited to families, making it easier to recruit large numbers of affected individuals and control subjects.⁸ However, case-control studies can be confounded by population stratification effects, such as ethnicity or breed and geography. A major disadvantage of this approach is that it is hypothesis dependent in that the genetic analysis is restricted to genes that have been previously implicated in the pathogenesis of AD. Novel genes may therefore be missed.

Genome-wide association studies

Genome-wide association studies (GWASs) are a hypothesis-free way to discover disease-associated SNPs.⁸ This avoids the limitations of using candidate genes, while

retaining the advantages of the case-control approach. Genome sequencing has identified large numbers of genetic variants that can be read on SNP arrays. This allows identification of SNPs that are more frequent in affected individuals than control subjects. These disease-associated SNPs mark regions of the genome that may be involved in the pathogenesis of AD (Figure 1). Depending on the number and distribution of SNPs, GWASs can interrogate the entire genome. However, fine mapping relies on having many SNPs evenly distributed throughout the genome. In addition, disease-associated SNPs may be located in unknown areas of the genome. Further sequencing and functional studies are therefore required to confirm whether the disease association is causal. The functional effects, moreover, may depend on the specific combinations of SNPs within a gene and/or interactions with SNPs in other genes, or both. Another weakness of this approach is that GWASs are limited to identifying common SNPs with small effects. They cannot identify rare SNPs with large effects, untyped SNPs and some structural variations, e.g. microsatellites, variable number tandem repeats, insertions, deletions and duplications.

Genome-wide association studies in dogs can take advantage of their strong linkage disequilibrium (LD). In humans, LD is relatively weak (extending over about 10–100 kbp), necessitating high-density arrays (up to 1.6×10^6 SNPs) and large cohorts (at least 1000 cases and controls).¹⁸ Dog breeds are of recent origin and are highly inbred, with LD over long distances (0.8–5 Mbp),^{19,20} meaning that fewer genetic markers and smaller sample sizes can be used. For example, complete coverage of the canine genome requires only 5000 to 30,000 SNPs.²¹ However, low numbers of SNPs can result in incomplete coverage that could miss important genes. For example, the first GWAS in canine AD²² used the Illumina Canine SNP20 chip (San Diego, CA, USA). This includes 22,362 canine SNPs from the CanFam2.0 assembly based on the boxer dog, with the partial sequence of a standard poodle dog and 100,000 sequence reads from nine other breeds. Despite this, many genes of interest (e.g. filaggrin) were not included. These gaps should be covered by continued development of the Dog Genome Project.

Quantitative RT-PCR

Quantification of mRNA can be performed in any tissue to identify genes that are differentially regulated in affected individuals compared with control subjects. Microarrays can be used for hypothesis-free assessment of very large numbers of genes on single chips.²³ Gene transcription, however, does not necessarily imply causality; the change may be secondary to the disease process. Despite this, gene expression studies are useful to identify candidate genes and to confirm involvement of genes associated with AD in genomic studies. Genome-wide microarrays can produce a hypothesis-free transcriptome of all known genes in any tissue, but the high cost and complex results are barriers to widespread use at present.⁸

Bias and false results

Genomic studies are prone to bias and error that reduce the power of the analyses and affect replication of the

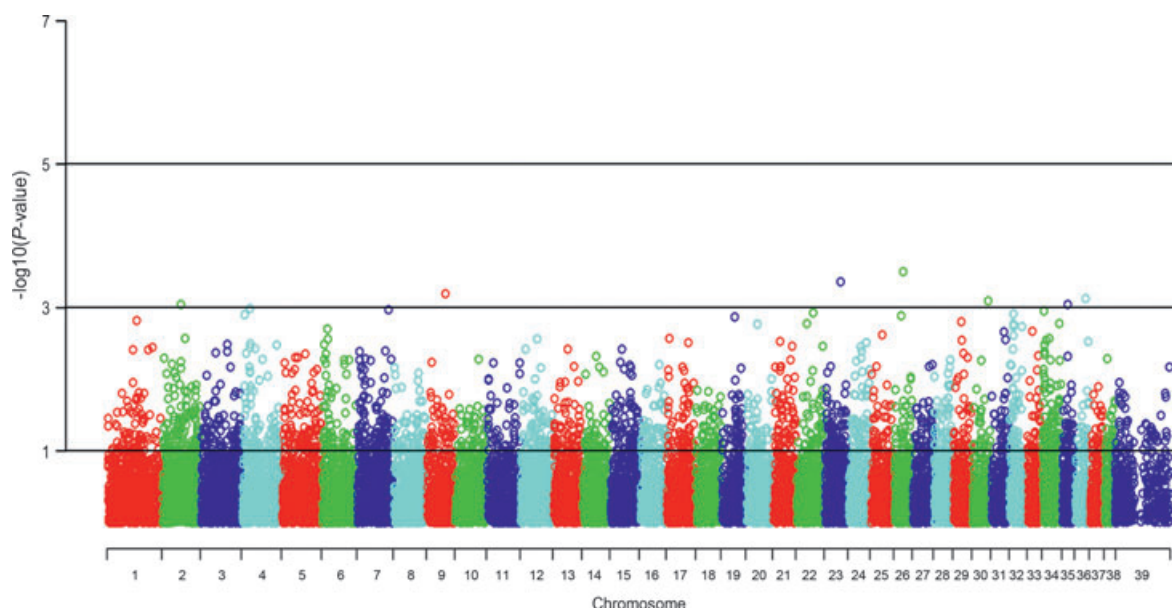


Figure 1. Whole-genome association plot of significance for canine atopic dermatitis (AD) after Wood *et al.*²² This study used a chip with approximately 22,000 single nucleotide polymorphisms (SNPs) to study SNP frequency in atopic dogs and healthy control dogs. The SNPs are plotted on the x-axis according to their position on each chromosome, with their association with AD on the y-axis shown as $\log_{10}$ P-value. The circles represent individual SNPs, which are grouped by chromosome. The SNPs that are statistically more frequent in the atopic population identify potentially AD-associated genes or loci for further studies.

results. Accurate phenotyping is critical, because any variation will have a profound impact on determining associations with AD. Other issues include failing to account for population stratification in case-control studies. Studies with relatively low numbers of cases are vulnerable to type II (i.e. false-negative) errors. Type I (i.e. false-positive) errors can occur following multiple testing unless corrections are used to reduce the false discovery rate. In addition, the effects of nonrandom mating, mutations, selection, small population effects, genetic drift etc. mean that canine populations may not be in Hardy-Weinberg equilibrium (i.e. allele and genotype frequencies in a population remain in equilibrium).

Genomic studies in canine AD

Atopic dermatitis is a complex disease

Observations of atopic West Highland white terriers indicated that the inheritance patterns were consistent with a common fully penetrant dominant or recessive major locus.^{24–26} However, studies in other breeds suggest that canine AD is a multifactorial and polygenic condition with a complex mode of inheritance.^{4,12,15,27} Genomic studies in dogs have now implicated numerous genes in the pathogenesis of canine AD (Table 2), although whether these are associated with cause or effect is not always clear. These include genes involved in innate and adaptive immunity, inflammation, cell cycle, apoptosis, skin barrier formation and transcription regulation, many of which have also been implicated in human AD.

Microarray studies

An initial paper reported that 54 of 22,000 genes (Agilent 22K oligonucleotide canine array; Agilent Technologies, Palo Alto, CA, USA) showed significantly different

transcription in canine AD compared with control dogs (Table 2).²³ The genes were broadly grouped into inflammation/immunity, cell cycle/apoptosis/repair, barrier formation, transport/regulation and transcription pathways. Most of the inflammation or immunology markers were upregulated in atopic, particularly lesional, skin. Other genes were generally underexpressed, especially in nonlesional atopic skin. A large number of these downregulated genes are involved in the transport of calcium, potassium and other ions which may affect expression and function of other mediators involved in inflammation and barrier function. The most dysregulated gene was S100 calcium binding protein A8 (S100A8). This is an important pro-inflammatory molecule located on the epidermal differentiation complex. Interestingly, a recent paper correlated S100A8 expression with the clinical severity of canine AD.²⁸ Release is stimulated by tumour necrosis factor- α , the levels of which also correlate with clinical severity.²⁹ The epidermal differentiation complex comprises genes for proteins such as profilaggrin, loricrin, involucrin and S100, which are essential for keratinocyte and epidermal barrier differentiation.³⁰ These genes were not available on the canine microarray and GWAS chips used in earlier studies,^{22,23} and since these studies canine and human AD have been associated with loss-of-function filaggrin mutations and altered filaggrin expression.^{31–34}

This microarray study²³ provided a rapid, wide-ranging assessment of many genes and identified novel targets and pathways for further investigation; however, it was limited by the omission of key epidermal barrier and inflammatory cytokine genes. In addition, the study used a relative low number of dogs, which reduced its power to detect small changes. Using multiple breeds may have also confounded the results and reduced the power of the study.

Table 2. Genes that have been implicated in canine atopic dermatitis by candidate gene quantitative PCR (qPCR), microarray, genome-wide linkage studies (GWLS), candidate gene single nucleotide polymorphism (SNP) studies or genome-wide association studies (GWAS)

Gene	Study	Potential relevance to canine atopic dermatitis
Inflammation or immunology		
S100A8	Microarray ²³ GWLS ²⁶ qPCR ³⁵	Pro-inflammatory; correlated with clinical severity
INPPL1	Microarray ²³ qPCR ³⁵ SNP ³⁸	Affects allergen-specific IgE responses, allergen presentation and uptake, T-cell activation and inflammatory responses
SCCA-2	Microarray ²³	Serine protease inhibitor; marker for squamous cell carcinoma
SAA	Microarray ²³	Expressed in response to inflammatory stimuli
SAA3	Microarray ²³	
Serum amyloid A protein (<i>Canis familiaris</i>)	Microarray ²³	
TIMP1	Microarray ²³ qPCR ³⁵	Tissue inhibitor of metalloproteinase 1; promotes cell proliferation; anti-apoptosis
<i>ARTS-1</i>	Microarray ²³	Cytokine receptor regulation; antigen presentation
<i>C10orf118</i>	Microarray ²³	Unknown
<i>IL1RAPL1</i>	Microarray ²³	Synthesis of pro-inflammatory proteins
<i>DPP4</i>	qPCR ³⁵ SNP ³⁸	Regulation of inflammation; interacts with tumour necrosis factor- α and tissue inhibitor of metalloproteinases 1 (TIMP1)
PKP2	qPCR ³⁵	Upregulates T-cell activity and survival
TNF	qPCR ³⁵	Pro-inflammatory; tumour necrosis factor- α correlates with clinical severity
<i>PTPN22</i>	GWAS ²⁵	Regulation of B- and T-cell receptor signalling; regulation of innate immune response; regulation of natural killer cell proliferation; associated with immune-mediated disorders
<i>TSLP-receptor</i>	SNP ³⁸	Cytokine gene transcription; implicated in allergic inflammation
<i>MS4A2</i>	SNP ³⁸	High-affinity IgE receptor β subunit
<i>CD83</i>	GWAS ⁴⁰	Antigen presentation and humoral immunity
<i>CMA1</i>	GWAS ²²	Mast cell chymase
<i>NOD1</i>	GWAS ²²	Intracellular microbial pattern recognition receptor; innate and adaptive immunity
<i>SELP (P-selectin)</i>	GWAS ²²	Leukocyte recruitment
Cell cycle, apoptosis, repair or lesion formation		
RAD50 homologue isoform 1	Microarray ²³	Unknown
POSTN	Microarray ²³ qPCR ³⁵	Epithelial cell adhesion and migration
<i>POSTN</i>	qPCR ³⁵	
<i>CIDE-3</i>	Microarray ²³	Apoptosis and cell proliferation
<i>SDC1 (syndecan 1)</i>	Microarray ²³	Integral membrane protein; involved in cell binding, cell signalling, cytoskeleton organization, cell proliferation, cell migration and cell-matrix interaction
<i>Cadherin-13</i>	Microarray ²³	Cell signalling; cell migration and differentiation
<i>Cullin 4A</i>	Microarray ²³ qPCR ³⁵	Cell cycle regulation and development; DNA repair
<i>DCLRE1B</i>	GWAS ²⁵	Unknown
<i>BCL2L15 (BCL2-like 15)</i>	GWAS ²⁵	Regulation of apoptosis and tolerance
<i>ANGPTL4</i>	GWAS ²²	Skin wound healing; keratinocyte migration, endothelial apoptosis survival factor
Transport or regulation		
Kinectin 1	Microarray ²³	Unknown
Myosin Va	Microarray ²³	Unknown
A-kinase anchor protein 9 isoform 2	Microarray ²³	Unknown
Sperm-associated antigen 5	Microarray ²³	Unknown
<i>Canis familiaris ret proto-oncogene</i>	Microarray ²³	Unknown
<i>Nucleoprotein TPR</i>	Microarray ²³	Unknown
<i>Phospholipase C, zeta 1</i>	Microarray ²³	Unknown
<i>Potassium channel tetramerization</i>	Microarray ²³	Unknown
<i>FERM, RhoGEF, pleckstrin domain protein 2</i>	Microarray ²³	Unknown
<i>ATP-binding cassette C12e</i>	Microarray ²³	Unknown
<i>SFXN5 sideroflexin 5</i>	GWAS ²⁵	Unknown
<i>EXOC6B exocyst complex component 6B</i>	GWAS ²⁵	Exocytosis
<i>AP4B1</i>	GWAS ²⁵	Endocytosis and secretion
<i>ABCC3</i>	GWAS ²²	Protein transport; drug efflux
Barrier formation		
GOLGA4 subfamily a5	Microarray ²³	Golgi body function; glycosylation and transport of proteins and lipids
GOLGA4 subfamily a4		
SPINK5	qPCR ³⁵	Regulates proteolysis and keratinocyte differentiation; filaggrin function; T helper-2 polarization

Table 2. (continued)

Gene	Study	Potential relevance to canine atopic dermatitis
SGPL1	qPCR ³⁵	Skin barrier regulation; antimicrobial activity; keratinocyte proliferation and differentiation
Mucin-15	Microarray ²³	Cell adhesion to matrix
<i>Mucin-2</i>	Microarray ²³	Mucosal mucin protection
<i>Tight junction protein 3</i>	Microarray ²³	Signal transduction; cell adhesion
<i>PPAR gamma</i>	qPCR ³⁵	Reduced lamellar body formation and lipid processing; anti-inflammatory
FLG (Filaggrin)	SNP ³⁸	Alterations in filaggrin expression and function associated with human and canine atopic dermatitis
RAB3C	GWAS ²²	Implicated in epidermal lipid layer and skin barrier formation
Transcription and translation regulation		
eIF-5B	Microarray ²³	Unknown
<i>STAT2</i>	Microarray ²³	Cell growth, survival and differentiation; inflammation and immunity; interferon- γ regulation; tolerance
<i>CGGBP1 (CGG repeat binding protein 1)</i>	Microarray ²³	Unknown
<i>FUSE binding protein 2</i>	Microarray ²³	Unknown
<i>FOXO4 (foxhead box)</i>	Microarray ²³	Cell metabolism and proliferation; tolerance
Empty spiracles homologue 1	GWAS ²⁵	Unknown
ZNF638 zinc finger protein 638	GWAS ²⁵	Unknown
PAIP2B	GWAS ²⁵	Unknown
PHTF1	GWAS ²⁵	Unknown
HIPK1	GWAS ²⁵	Unknown
TRIM33	GWAS ²⁵	Regulation of transforming growth factor- β receptor signalling
Miscellaneous		
EEA1	Microarray ²³	Unknown
CG15747-PA	Microarray ²³	Unknown
HIF1a (Hypoxia-induced gene 1a)	Microarray ²³	Unknown
<i>C6orf142</i>	Microarray ²³	Unknown
<i>Sushi-repeat-containing protein SRPX</i>	Microarray ²³	Antioxidant; cytokine regulation
<i>RING-H2 protein</i>	Microarray ²³	Unknown
<i>ATRX1</i>	Microarray ²³	Unknown
<i>C1orf163</i>	Microarray ²³	Unknown
<i>FBXL10</i>	Microarray ²³	Unknown
<i>FOLH1</i>	Microarray ²³	Unknown
<i>S6K-alpha 6</i>	Microarray ²³	Serine kinase; cell growth and differentiation
<i>mSin3A-associated protein 130</i>	Microarray ²³	Unknown
<i>PH domain leucine-rich repeat protein phosphatase-like</i>	Microarray ²³	Inhibitor of kinases and growth factor-induced signalling
<i>Spag6</i>	Microarray ²³	Unknown
<i>Ecotropic viral integration site 1</i>	Microarray ²³	Unknown
SPR sepiapterin reductase	GWAS ²⁵	Unknown
Cytochrome P450 26B1	GWAS ²⁵	Lipid metabolism and barrier function; cytochrome P450 activity
DYSF dysferlin	GWAS ²⁵	Unknown
NAGK N-acetylglucosamine kinase	GWAS ²⁵	Unknown
RSBN1L	GWAS ²⁵	Unknown
OLFML3	GWAS ²⁵	Unknown
SYT6 synaptotagmin VI	GWAS ²⁵	Endocytosis; cell adhesion
PROM1	GWAS ²²	Unknown
RAB7A	GWAS ²²	Melanocyte function and melanogenesis
SORCS2	GWAS ²²	Neuropeptide receptor activity

The bold and italic refers to genes that were differentially expressed in atopic skin compared with healthy control dogs; bold, upregulated and italic, downregulated.

Candidate gene studies

One study using atopic and healthy skin quantified mRNA for 20 genes identified from the microarray study²³ and/or earlier literature.³⁵ Significant differences were seen for 11 genes, involving immune responses, regulation and skin barrier function. Seven of these have been associated with human AD. S100A8 was again the most dysregulated gene. Three genes correlated with Canine Atopic Dermatitis Extent and Severity Index (CADESI)-03 scores (S100A8, SAA-1 and PKP2), and four genes correlated with intradermal test results (CMA1, SAA-1, SPINK5 and

S100A8); these have been associated with inflammation, T-cell survival and skin barrier function.^{28,36,37} Weaknesses of this study included the relatively small sample size, use of multiple dog breeds and the variable time scale of the lesions at presentation.

Analysis of 97 SNPs in 25 candidate genes in 659 dogs of eight breeds from the UK, USA and Japan found that six were significantly associated with canine AD.³⁸ A SNP in the thymic stromal lymphopoietin (TSLP)-receptor, which has been implicated in allergic inflammation,³⁹ was seen in all eight breeds. Other canine AD-associated

SNPs, however, were restricted to certain dog breeds and locations (e.g. filaggrin with UK Labrador retriever, and INPPL1 and MS4A2 with Japanese shiba inu).

A linkage study in West Highland white terrier dogs²⁴ used specific microsatellite markers for fine mapping of the *FLG* locus. There were, however, no haplotypes that associated significantly with canine AD. This makes a primary role for filaggrin defects in AD in these dogs unlikely, although this has been implicated in other breeds.^{31–33}

Genome-wide linkage studies

A genome-wide family-based linkage approach using microsatellites [256 markers from the Minimal Screening Set 2 (MSS-2) that covered the genome with an average intermarker distance of 8.59 cM] in 90 West Highland white terrier dogs from families with AD did not detect any chromosomal regions that were significantly linked to canine AD.²⁶ However, some chromosomal regions were not covered at high density, reducing the power of this study. The highest linkage score was for a region on CFA7 that contains the *S100A8* gene, which has been associated with canine AD.^{23,28,35} This study excluded linkage to the *FLG* locus, corroborating the results of other reports.^{24,38,40} This suggests that primary defects in filaggrin expression are not important in the pathogenesis of canine AD in West Highland white terrier dogs.

Genome-wide association studies

The first GWAS in canine AD²² evaluated DNA from 242 atopic and 417 control dogs of eight breeds from the UK, US and Japan. The top 40 SNPs were selected for validation (Figure 2). Nine chromosomes expressed multiple SNPs, suggesting a haplotype effect. Subsequent quantitative PCR with quality assurance testing and corrections for the false discovery rate and population structure revealed that 13 SNPs had a significant association with canine AD; however, a number were intergenic, with unknown functions and interactions.

Two independent intergenic SNPs were linked with canine AD in all eight breeds; rs22114085 (CFA10) was associated with susceptibility, while rs23472497 (CFA29) was protective. These may represent novel loci for sequencing and fine mapping.²² There was a lack of interbreed correspondence between the AD-associated SNPs, suggesting that the atopic genotype varies among breeds. However, the numbers of each individual breed in this study were relatively low, which reduced the statistical power. Furthermore, the relevance of the intergenic SNPs is unclear, and fine mapping of these areas on the genome is required.

Some breed-specific gene-associated SNPs were identified. AD in golden retriever dogs was associated with SNPs in *RAB3C* (rs22859255) and *PROM1* (rs23602938). This *RAB3C* SNP also formed a significant haplotype with another *RAB3C* SNP (rs22784610). Atopic Labrador retriever dogs and West Highland white terrier dogs from the UK showed associations with a SNP within *RAB7A* (rs22915894), as well as the intergenic SNP rs8806978, which is downstream of *RAB7A*. German Shepherd dogs from the UK showed an association with *SORCS2*. Significant associations were, however, variable between

breeds and within breeds from different geographical locations. However, the fact that a SNP is associated with AD in one breed, but not another, does not necessarily mean that the SNP is not a risk factor for canine AD, because a SNP may have different levels of penetrance in different breeds.⁴¹ However, breed variation in genotype may explain breed-specific phenotypes in canine AD.⁹

A further GWAS was performed on 35 atopic and 25 nonatopic West Highland white terrier dogs using an Affymetrix Canine SNP V2 array with approximately 42,800 SNPs.²⁵ This reported significant association with a locus on CFA17. Nineteen genes less than 0.5 Mbp from this region were identified. Two other main linkage peaks for canine AD were also found on CFA6 and CFA9. These candidate genes covered a range of functions, including innate and adaptive immunity, skin barrier function, and transcription and regulation. Another GWAS using this array found a significant association between serum *Dermatophagoides farinae*-specific IgE levels in West Highland white terriers and a 2.3 Mbp area on CFA35.⁴⁰ *CD83*, a gene closely associated with this region, is important in antigen presentation and humoral immunity. Sequencing detected an intronic polymorphic repeat sequence, but this did not explain the GWAS association in these dogs.

Finally, a very recent GWAS used allergen-specific immunoglobulin levels (IgE, IgG1 and IgG4 specific for *D. farinae*, and IgE specific for *Dermatophagoides pteronyssinus*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Acarus siro*, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Penicillium*, cat epithelium, flea saliva and *Blattella germanica*) as phenotypic markers for AD in Labrador retriever dogs.⁴² The GWAS analysed 113,021 SNPs in 135 affected dogs and 24 control animals, with correction for false discovery rate, population stratification and relatedness. The 27 most associated SNPs were clustered on CFA5 from 79.0 to 82.5 Mbp, with the 28th SNP on CFA37. However, only two of the SNPs on CFA5 were significantly associated with *A. siro*-specific serum IgE levels (BICF2S2297212 and BICF2P1022237). A further SNP on CFA5 (BICF2G630182288) was significantly associated with *T. putrescentiae*-specific serum IgE levels. No genes involved in IgE regulation are known to reside in this area on CFA5 or its human equivalent. These results may therefore indicate the presence of a novel gene, and these loci warrant further investigation and fine mapping. However, the relevance of *Acarus*- and *Tyrophagus*-specific serum IgE in the pathogenesis of canine AD is unclear,^{43,44} and the use of surrogate markers such as serum IgE levels will identify only a subset of atopic dogs.

Conclusions

These studies have greatly improved our understanding of canine AD. Despite this, the genetic background for canine AD is still far from clear. There has been little correlation between the studies published so far. This variation could be due to the complexity of the genotype, although it is also likely to reflect environmental factors, phenotypic differences between dogs in the studies, different study techniques, mutations with low pene-

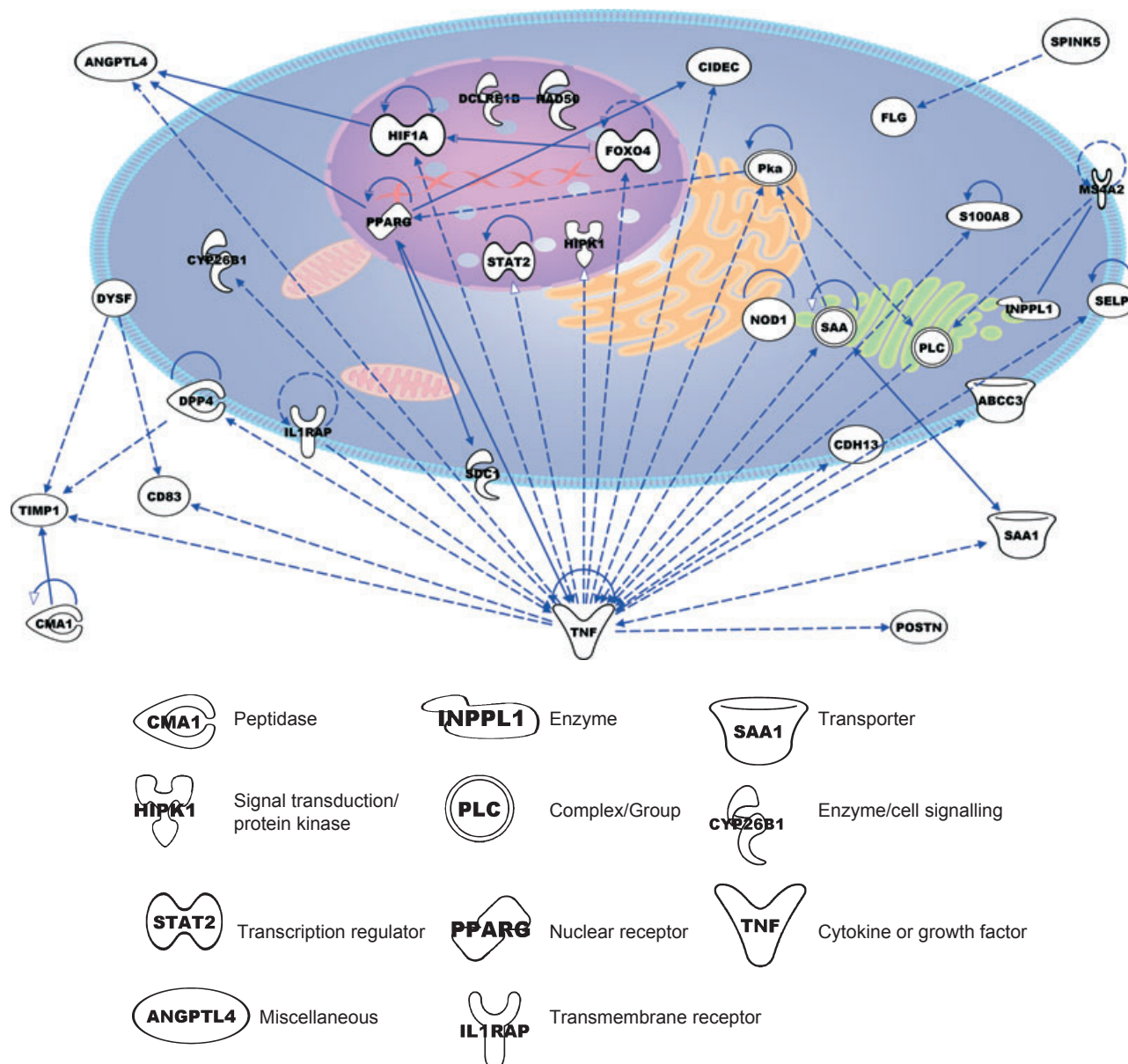


Figure 2. Network pathway of genes associated with canine atopic dermatitis supported by at least one reference from the literature or from canonical information stored in the Ingenuity Knowledge Base (IPA; Ingenuity Systems, <http://www.ingenuity.com>). Nodes are displayed using various shapes that represent the functional class of the gene product.

trance, and incomplete genome coverage. Genome-wide association studies appear to be the most powerful technique to study the genotype of canine AD, but future studies will require larger cohorts of individual breeds from defined geographical areas.

Atopic dermatitis involves a complex network of many genes, with multiple variants affecting gene function and expression (Figure 2). The effect of any one polymorphism is likely to be relatively small, and the final phenotype will depend on their interactions across the genome. These findings also suggest that the genetic background to canine AD varies between breeds and geographical gene pools. This could explain variations in clinical phenotype and response to treatment between individuals and breeds.

This complexity, and the high prevalence of canine AD, means that a screening and breeding programme to elimi-

nate the condition is unlikely to succeed. Despite this, the genomics revolution has huge potential. These techniques will allow identification of target molecules for novel treatments. In addition, we should be able to genotype atopic dogs and relate this to their phenotype. Understanding of the genotype will enable better targeting of treatment options; for example, some dogs may respond well to skin barrier therapy, whereas others would benefit more from allergen-specific immunotherapy. We should also be able to identify dogs that may have a poor response or have an increased risk of adverse effects to certain anti-inflammatory drugs. Finally, it may be possible to discover atopic genotypes in young dogs and manage environmental and other factors to minimize their risk of developing clinical AD. However, we must be careful to avoid misuse of genomic data in diagnosis and by breeders or insurance companies.

Glossary

Allele	One of two or more forms of a gene or DNA sequence on a chromosome that may be associated with a phenotypic trait. Diploid organisms can be homozygous (i.e. two copies of the same allele) or heterozygous (i.e. two different alleles).
Exon	DNA sequences within a gene that make up the final RNA transcript. Exons are usually separated by one or more introns in a gene.
Genotype	The specific genetic makeup of an individual. The genotype, epigenetic factors (i.e. changes in gene expression not associated with the DNA sequence) and environmental influences determine the phenotype.
Haplotype	A combination of alleles at adjacent loci on the chromosome that are inherited together. Haplotype also refers to a set of single nucleotide polymorphisms on a single chromosome that are statistically associated.
Intron	DNA sequence within a gene that is removed during transcription to produce the final RNA sequence.
Linkage disequilibrium	Nonrandom association of alleles at two or more loci, which may be on the same or different chromosomes.
Locus	The specific location of a gene or DNA sequence on a chromosome.
Microsatellite markers [simple sequence repeats (SSRs) or short tandem repeats (STRs)]	Highly polymorphic repeating sequences of two to six base pairs (bp) throughout the genome used in inheritance studies.
Penetrance	The proportion of individuals with a particular genotype that develop the associated phenotype.

Glossary (Continued)

Phenotype	An individual's observable characteristics or traits. These may include morphology, development, physiology, behaviour and/or disease states.
Population stratification	Differences in allele frequencies between groups in a study population that are associated with ancestry (e.g. race, breed, geography) rather than a disease condition.
Single nucleotide polymorphisms (SNPs)	A difference in single nucleotide (A, T, C or G) in a DNA sequence; for example, AGCCTA and AGCTTA.

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Résumé

Contexte – Les études d'hérabilité suggèrent que la dermatite atopique (AD) implique plusieurs gènes et interactions avec des facteurs environnementaux. Les avancées en génomique nous ont fourni des techniques puissantes d'étude de la génétique de l'AD.

Objectifs – Examiner l'application de ces techniques à l'AD canine.

Résultats – Les gènes candidats peuvent être étudiés par PCR quantitative et techniques génomiques mais celles-ci sont dépendantes des hypothèses de départ et peuvent manquer de nouveaux gènes. Les techniques indépendantes des hypothèses évitent cette limitation. Les puces quantifient un grand nombre de gènes, bien que des associations de faux-positifs soient fréquentes. À l'avenir, les profils d'expression pourraient être utilisés pour établir un transcriptome tissulaire complet. Les études de liaison du génome global peuvent détecter les loci associés à l'AD si un nombre suffisant de chiens atteints et sains sont recrutés. Les études d'association du génome global peuvent être utilisées pour découvrir les polymorphismes de nucléotides uniques associés à l'AD sans avoir recours à des chiens. Les études génomiques chez le chien ont impliqué de nombreux gènes dans la pathogénie de l'AD, y compris ceux impliqués dans l'immunité innée et adaptative, l'inflammation, le cycle cellulaire, l'apoptose, la formation de la barrière cutanée et la régulation de la transcription. Ces données, en outre, ont été inconstantes et les problèmes comprennent un faible nombre de cas, des contrôles inappropriés, des diagnostics incompatibles, une couverture incomplète du génome, une pénétrance faible des mutations et des facteurs environnementaux.

Conclusions – La dermatite atopique canine a un génotype complexe qui varie entre les races et les groupes génétiques. Les programmes de reproduction pour éliminer les atopiques ont donc peu de chances de réussir et cette complexité pourrait expliquer les variations du phénotype clinique et des réponses au traitement. Le typage génétique des chiens atteints pourra identifier de nouvelles molécules cibles et permettre de mieux cibler les traitements et les options de gestion. Cependant, les excès de données génomiques doivent être évités.

Resumen

Introducción – los estudios de herencia sugieren que en la producción de la dermatitis atópica (AD) se implican numerosos genes y la interacción con factores ambientales. Los avances en estudios de expresión genómica proporcionan técnicas de capacidad para estudiar la genética de la AD.

Objetivo – repasar el uso de estas técnicas en la AD canine.

Resultados – los genes candidatos se pueden estudiar usando PCR cuantitativo y técnicas genómicas, pero éstas son técnicas dependientes de hipótesis y se pueden perder otros genes novedosos. Las técnicas libres de hipótesis pueden evitar esta limitación. Las micromatrices (microarrays) cuantifican la expresión de una gran cantidad de genes, aunque son comunes las asociaciones con falsos positivos. En el futuro, el perfil de expresión se podía utilizar para producir un transcriptoma completo del tejido. Los

estudios genómicos extensos de asociación pueden detectar loci del genoma asociadas con AD si se obtienen bastantes perros afectados y parientes inafectados. Los estudios genómicos extensos de asociación se pueden utilizar para descubrir polimorfismos de un solo nucleótido asociados con AD sin tener que estudiar perros relacionados. Los estudios genómicos en perros han implicado numerosos genes en la patogenia de la AD, incluyendo genes que participan en inmunidad natural y adquirida, inflamación, ciclo celular, apoptosis, la formación de la barrera de la piel y la regulación de la transcripción. Estos resultados, sin embargo, han sido poco constantes, siendo algunos problemas el bajo número de animales, controles no apropiados, diagnóstico poco acertado, cobertura incompleta del genoma, mutaciones de baja penetración y factores ambientales.

Conclusiones – la AD canina tiene un genotipo complejo que varía entre razas y grupos de genes. Los programas de cría para eliminar la AD tienen pocas posibilidades de éxito, pero esta complejidad podría explicar las variaciones en el fenotipo y la respuesta clínica al tratamiento. El genotipado de perros afectados identificará nuevas moléculas diana de tratamientos y permitirá una mejor dirección de las opciones de tratamiento y manejo de la enfermedad. Sin embargo, debemos evitar en la medida de lo posible el uso equivocado de los datos genómicos.

Zusammenfassung

Hintergrund – Studien über die Heritabilität weisen darauf hin, dass an der atopischen Dermatitis (AD) multiple Gene und Interaktionen mit Umweltfaktoren beteiligt sind. Die Fortschritte bei den Studien von Genomen haben uns gewaltige Techniken geliefert, um die Genetik der AD zu erforschen.

Ziel – Eine Review dieser Techniken im Bezug auf ihre Anwendungsmöglichkeit bei caniner AD.

Ergebnisse – Kandidatengene können mittels quantitativer PCR und genomischer Techniken bestimmt werden, allerdings basieren diese Techniken auf Hypothesen und könnten neue Gene übersehen. Von Hypothesen unabhängige Techniken sind dadurch nicht limitiert. Mikroarrays quantifizieren eine große Anzahl an Genen, wobei falsch-positive Zusammenhänge häufig sind. In der Zukunft könnte Expressionsprofiling verwendet werden, um die komplette Transkription eines Gewebes zu produzieren. Verknüpfungsstudien der Genome können Loci, die mit AD in Verbindung gebracht werden, nachweisen, wenn genug betroffene Hunde und nicht betroffene Verwandte dafür rekrutiert werden. Assoziationsstudien der Genome können verwendet werden, um Polymorphismen von einem Nukleotid, welches mit AD im Zusammenhang steht, festzustellen ohne dabei auf verwandte Hunde zurückgreifen zu müssen. Studien des Genoms der Hunde haben zahlreiche Gene bei der Pathogenese der AD impliziert, wozu auch jene der angeborenen und erworbenen Immunität, der Entzündung, des Zellzyklus, der Apoptose, der Bildung der Hautbarriere und der Regulierung der Transkription gehören. Diese Ergebnisse waren jedoch unterschiedlich und die Probleme dabei sind eine niedrige Anzahl an Fällen, unzureichende Kontrolltiere, uneinheitliche Diagnosen, unvollständige Abdeckung der Genome, Low Penetrance Mutationen und Umweltfaktoren.

Schlussfolgerungen – Der Genotyp der caninen AD ist komplex und variiert zwischen den Rassen und Genpools. Es ist daher unwahrscheinlich, dass spezielle Zuchtprogramme bei der Eliminierung der AD erfolgreich sein könnten, aber diese Komplexität könnte die Variationen beim klinischen Phänotyp und bei der Reaktion auf eine Behandlung erklären. Eine Genotypisierung von betroffenen Hunden wird neue Zielmoleküle identifizieren und die Möglichkeit schaffen, Behandlungs- und Managementoptionen zielgerichteter einzusetzen. Nichtsdestotrotz müssen wir die falsche Verwendung von Daten des Genoms verhindern.

要約

背景—遺伝率研究はアトピー性皮膚炎（AD）が多数の遺伝子と関与し、環境要因と相互作用していることを示唆する。ゲノム学の進歩はADの遺伝的問題を研究するのに有力な技術を提供する。

目的—犬のADに対してそれらの技術の適応を再検討すること

結果—候補遺伝子を定量的PCRとゲノム技術によって調査した、しかしこれらは仮説に基づいた技術であるため、新奇の遺伝子を見逃す可能性がある。仮説によらない技術であればこの限界からは回避される。マイクロアレイは多数の遺伝子の発現を定量する技術であるが、擬陽性が一般的に生じる。将来的には、発現プロファイルから完全な組織のトランスクリプトームを作製できる可能性がある。ゲノムワイド関連研究はもし十分な数の罹患犬と非罹患の対照群がいれば、AD関連遺伝子座を特定できる可能性がある。ゲノムワイド関連解析はADと関連する一塩基多型に関連した犬に頼ることなく発見するのに利用できるかもしれない。犬のゲノム研究は先天的または後天的な免疫力、炎症、細胞周期、アポトーシス、皮膚バリア形成、転写調節に関与しているものも含めて、ADの病因が複数の遺伝子に関連していることを示唆している。これらの所見はしかし、一貫性がなく、症例数が少ない、対照が適切でない、矛盾のある診断、ゲノムの被覆率が不完全、低い浸透率の変異や環境要因を含む問題がある。

結論—イヌADは複雑な遺伝型を示し、それらは犬種間や遺伝子プールの中で変化する。したがって、ADを排除する繁殖プログラムが成功しそうではないが、しかしこの複雑さによってADの臨床的な表現型と治療への反応の違いが説明できる可能性がある。罹患した犬の遺伝子型決定により新奇の標的分子が特定され、よりよい治療の目標や管理の選択肢が可能になる。しかし、我々はゲノム情報を誤って用いないようにしなければならない。

摘要

背景 - 遗传性研究认为，异位性皮炎（AD）涉及多个基因和环境因素的互作。基因组学的进步为我们研究异位性皮炎的基因提供了强大的技术支持。

目的 - 回顾犬异位性皮炎的应用技术。

结果 - 定量PCR和基因组技术研究候选基因，但这些技术只检测假设基因，很可能错过新基因。无假设技术避免了这种局限性。基因芯片定量表达大量的基因，尽管可能存在假正相关。在未来，转录组能用于绘制完整的组织转录组。只有征集到足够的患犬和与其有血缘关系的健康犬，基因组连锁分析才能探测与AD相关的轨迹。全基因组关联分析不需要依靠血缘关系犬，就能发现与AD相关的单核苷酸多态性。基因组研究犬的异位性皮炎发病机理涉及众多基因，包括相关的先天性和获得性免疫、炎症、细胞周期、细胞凋亡、皮肤屏障构成和基因转录调控。然而，这些发现不一致，问题包括病例数低，不适当控制，不一致的诊断，不完整的基因覆盖，低外显率突变和环境因素。

结论 - 由于品种和基因库不同，犬AD的基因型复杂。因此，通过育种消除异位性皮炎很难成功，但这种复杂性恰能说明临床表型和治疗反应的多变性。患犬的基因型将确定新的分子靶点，更好地选择治疗和管理方法。然而我们必须避免滥用基因数据。