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Uncovering a Novel Pathway for Autoinflammation

With a Little Help from a Wrinkled Friend

MIA OLSSON

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Abstract
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A major challenge in medical genetics is to identify the mutations underlying heritable diseases. Dogs are excellent genetic models in the search for causative mutations, as they constitute a large library of naturally occurring heritable diseases many of which are analogous to those suffered by man. In addition, these animals have a genome structure well suited to gene mapping. The Shar-Pei dog has two breed-specific features; a strongly selected for wrinkled skin and a high predisposition to an autoinflammatory disease (AID). Abnormalities in the innate immune system cause this type of disease, presenting as spontaneous attacks of inflammation. Persistent inflammation puts an affected Shar-Pei at risk of amyloidosis, organ failure and premature death. In humans, similar AIDs occur and for a majority of cases, no underlying genetic cause has yet been identified. The aim of this thesis was to use the Shar-Pei as a genetic model for autoinflammation in order to find new genes and signalling pathways involved in disease. In paper I, a pleiotropic mutation was identified that could explain both the wrinkled skin and autoinflammation in Shar-Pei. The mutation is associated with an up-regulation of Hyaluronic Acid Synthase 2 (*HAS2*). Increased expression of *HAS2* leads to abnormal depositions of hyaluronic acid (HA) in the skin, resulting in the wrinkled appearance. When fragmented, HA also function as a *damage signal* sensed by the innate immune system which then responds with inflammation. By selecting for the wrinkled skin, the autoinflammatory disease has inadvertently been enriched in the breed. In paper II, five different inflammatory signs could be associated with the same genetic risk factor, allowing the introduction of a new terminology: *Shar-Pei autoinflammatory disease* (SPAID) to describe the whole disease complex. In addition, a modifying locus containing several biologically attractive genes was suggested to contribute to varying incidence of amyloidosis in Shar-Pei. In paper III, signs of pathological changes in HA metabolism were investigated in human AID. HA concentration was found to be both higher in subjects with no molecular diagnosis and also associated to disease activity and severity. Taken together, this suggests HA is also involved in human AID.

Keywords: autoinflammation, hyaluronic acid, amyloidosis, canine model, genetic association

Mia Olsson, Uppsala University, Department of Medical Biochemistry and Microbiology, Box 582, SE-751 23 Uppsala, Sweden.

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To Linda

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Olsson, M.**, Meadows, JRS., Truvé, K., Rosengren Pielberg, G., Puppo, F., Mauceli, E., Quilez, J., Tonomura, N., Zanna, G., Docampo, MJ,. Bassols, A., Avery, A., Karlsson, EK., Thomas, A., Kastner, DL., Bongcam-Rudloff, E., Webster, MT., Sanchez, A., Hedhammar, Å., Remmars, EF., Andersson, L., Ferrer, L., Tintle., Lindblad-Toh, K. (2011) A Novel Unstable Duplication upstream of *HAS2* predisposes to a Breed-defining Skin Phenotype and a Periodic Fever Syndrome in Chinese Shar-Pei Dogs. *PLoS Genetics*, 7(3):e1001332.

II **Olsson, M.,** Tintle, L., Kierczak, M., Perloski, M., Tonomura, N., Lindquist, A., Murén, E., Tengvall, K., Pielberg, G., Dufaure de Citres, C., Dorso, L., Abadie, J., Hanson, J., Thomas, A., Leegwater, P., Hedhammar, Å., Lindblad-Toh, K., Meadows, JRS. (2012) Thorough investigation of a canine autoinflammatory disease (AID) syndrome confirms one main risk locus and suggests a modifier locus for amyloidosis. *Manuscript*.

III **Olsson, M.,** Meadows, JRS., Blank, N., Rech, J., Hügle, B., Herrmann, M., Lindblad-Toh, K. (2012) Circulating hyaluronic acid is elevated in subjects with genetically undefined autoinflammatory disease. *Manuscript*.

Contents

Abbreviations

DNA	deoxyribonucleic acid
kb	kilo basepair (1000 bases)
Mb	mega basepair (10^6 bases)
LD	linkage disequilibrium
SNP	single nucleotide polymorphism
RNA	ribonucleic acid
GWAS	genome-wide association studies
AID	autoinflammatory diseases
CNV	copy number variant
PRR	pattern recognition receptor
TLR	toll-like receptor
NLR	NOD-like receptor
PAMP	pathogen associated molecular pattern
DAMP	danger associated molecular pattern
FSF	Familial Shar-Pei fever
SPAID	Shar-Pei autoinflammatory disease
HA	hyaluronic acid (hyaluronan)
LMW-HA	low molecular weight hyaluronic acid
HMW-HA	high molecular weight hyaluronic acid
HYALs	hyaluronidases
HAS2	hyaluronic acid synthase 2
kDa	kiloDalton
ECM	extracellular matrix

Introduction

Within the hereditary material of life; deoxyribonucleic acid (DNA), every individual carries the instructions needed for the body to grow, function and reproduce. From one generation to the next, the DNA transferred will carry a few new changes (mutations) that will contribute to the uniqueness of each individual. While most mutations have no functional consequence, some will introduce changes and this random process is sufficient for evolution to operate and to introduce genetic variation to populations. The faith of a mutation will depend on genetic drift and selection, of which *artificial selection* plays the central role in this thesis. Beneficial mutations might increase in frequency over time and become common genetic variants (polymorphisms), whilst others might give rise to a disease state if altering the functions of the body in a non-beneficial way. If such a mutation occurs within a germ-line cell, it might be transmitted to the next generation and the disease becomes heritable.

In the scientific discipline of medical genetics the endeavour is to understand the underlying genetic factors of heritable diseases and how they adjust functions at the molecular level. This knowledge is fundamental for fast and accurate diagnostics and targeted treatments/medications. To achieve this understanding, the first step is to identify which genetic variant(s) are connected to the development of disease by using different genetic methods and disease models. In the past decade we have witnessed the completion of numerous genome sequences from many different animal species. One implication of the sequencing projects has been the development of commercially available SNP-arrays that allow genotyping of a great proportion of the common variation in the species it was developed for. By comparing the allele frequencies between groups of individuals affected and unaffected by the trait (disease) of interest, it is possible to identify the genomic regions within which the causative mutation is located. These so called case-control studies (or genome-wide association studies, GWAS) have proven to be excellent tools for mapping traits relevant to human disease, and many important medical discoveries have been made in genetic model systems such as animal models.

As a result of strong selective breeding, the domestic dog (*Canis lupus familiaris*) has lower genetic complexity than humans and thus biologically important signals are easier to detect. Dogs also constitute the largest cata-

logue of naturally occurring genetic diseases in a non-human species, many of which are homologous to human disease with similar manifestations.

In the studies included here, the Shar-Pei dog has served as a genetic model in case-control studies in order to understand the phenomena of auto-inflammation. The group of syndromes referred to as autoinflammatory diseases (AID) are characterized by self-limiting flares of fever, arthritis and systemic inflammation with no infectious cause. The underlying genetics of AID is poorly understood and in combination with many overlapping symptoms among the syndromes, diagnostic procedures and treatment of human AID becomes challenging. The Shar-Pei is the only non-human animal in which autoinflammation is reported to occur spontaneously with symptoms that resemble the human disease. I will present two studies where the Shar-Pei dog has served as a model to elucidate the genetics behind autoinflammation. The canine AID as well as a breed-defining wrinkled skin phenotype have been successfully mapped by using a strategy of combining selective sweep- and genome-wide association mapping. In the third study, the knowledge gained from the canine disease model was taken one step further and the significance of the new finding was evaluated in human AID patients with similar pathology as the Shar-Pei.

Mutations and heritable diseases

Disease causing mutations can occur in virtually all molecular processes including DNA replication, gene regulation, protein synthesis and folding. A coding mutation will be located within a gene and might change the biochemical properties of the protein encoded by the gene if altering the chain of amino acids. Such a mutation will have an effect in all tissues if the gene is expressed. Less studied are the mutations located outside of genes in the *cis*- or *trans* acting regulatory elements that are spread across every genome. These stretches of DNA do not encode a protein themselves, but act as binding sites for RNA-polymerase, its accessory molecules and a wide range of transcription factors that together control the timing, level and location of gene expression (1) (**Figure 1**). Mutations located in these elements might therefore be expressed in one cell-type but not another and it has become clear that they frequently influence phenotypic traits such as complex diseases (2). In terms of size and type, mutations also occur in great variation. Smaller mutations involve the single nucleotide polymorphisms (SNPs) and one base pair insertion or deletions. Some mutations are larger and structural and include whole chromosomes, transposable elements, large duplicated or deleted regions and DNA segments that vary in copy number (CNVs) or orientation.

The genetic contribution to a disease can vary from a straightforward connection between one single mutation and one disease (*e.g.* Huntington's

disease, cystic fibrosis) to a scenario when a combination of common genetic variants in concert influences the susceptibility for an infectious disease (*e.g* malaria, hepatitis). Many diseases are not monogenic but complex and influenced by a combination of genetic variants as well as the environment. The mode of inheritance also differs depending on whether the genetic variant is recessive, dominant or sex-linked. In other cases involving genomic imprinting, the inheritance pattern can be difficult to distinguish as either the maternal or paternal allele is silenced.

In the last decade, >1200 loci associated with > 165 common human diseases have been identified with genome-wide association studies (based on allelic comparisons between individuals affected and unaffected by disease). Despite this progress, the majority of diseases cannot be fully explained by the identified genetic variants. This is referred to as the "missing heritability" and its origin is continually debated. A common argument for the missing heritability is that many more genetic variants are yet to be identified; both common alleles with small influence and rare alleles with a major impact. Interaction between genes has also been suggested as a main factor that will affect the heritability (3). In human genetics, the high levels of heterogeneity that characterize the human genome complicate the identification of loci associated with disease. In genome-wide association studies, thousands of patients as well as healthy controls are required in order to reach statistically significant signals as well as extensive molecular tools with many genetic markers. The functional characterization of candidate mutations is also complicated particularly when mutations fall outside coding genes.

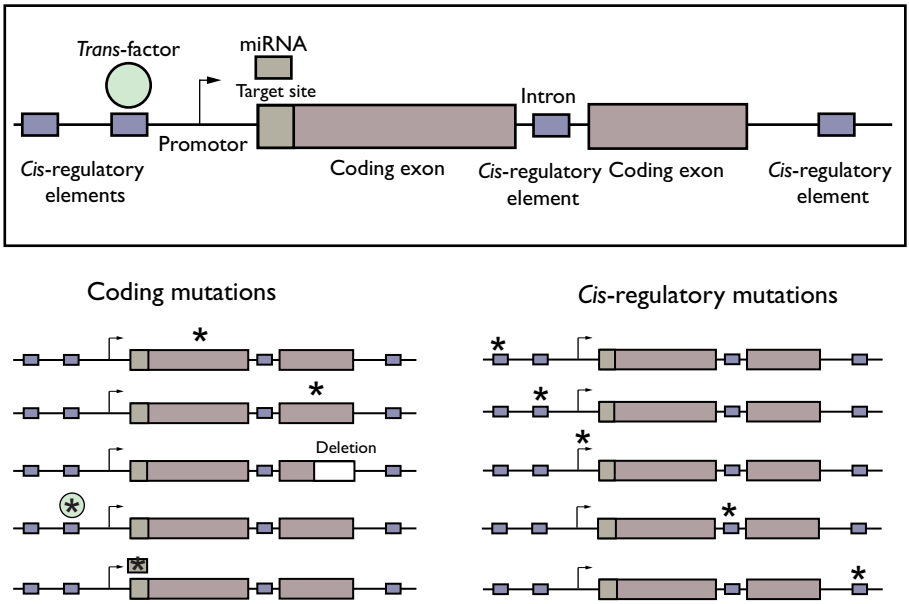

Figure 1. Coding and *cis*-regulatory mutations.

Scattered throughout the genome are regulatory elements (enhancers, silencers, insulators and promoters) that in concert with transcription factors control the expression of genes. Mutations (∗) located in these regions have been suggested to contribute greatly to phenotypic variation. The figure is kindly provided by Dr J. Eriksson and modified from Coyne and Hoekstra (2007) (4).

Disease models

In medical research, simplified model systems are often needed to understand the basic principles that operate in a surprisingly similar way in most living organisms. The foremost recourse in genetics, in terms of animal models, is the mouse (*Mus musculus*) with apparent benefits when it comes to gene targeting and when characterizing the effects of mutations. During the last decade many animal genomes have been sequenced based on their importance for medical genetics. The mouse genome was one of the first to be completed (5) closely followed by the rat (*Rattus norvegicus*)(6), the chicken (*Gallus gallus*)(7) and the domestic dog (*Canis lupus familiaris*)(8). The expectations were high in 2001, when the complete human DNA sequence was available and the work to understand our own genetic code could really be set in motion (9, 10). It turned out that even with this fantastic resource, many questions were still unanswered when there was nothing to compare our own genome to. What are the functions of all genes and their products? What role does the non-coding part of our genome play? What make us unique and what make us similar to all the other mammals? By

comparing the human genome sequence to the genomes of other species, biologically important genomic regions, including regulatory elements, can be identified, as they appear highly conserved between species. Without comparison, these regulatory elements would have appeared irrelevant when in reality they are key factors for creating life and generating phenotypic variation (11). To date, numerous genomes have been decoded and in combination with data about methylation, transcription factor binding and chromatin remodeling been used to create a large database containing thousands of regulatory elements (The Encyclopedia of DNA Elements, ENCODE) in the human genome (12).

Prior to the investigation of how a genetic alteration might influence disease, the mutation has to be identified. Once the mutation is found, the mouse (or the rat) is an excellent animal model to investigate its function. Both mice and rats are advantageous to use as they are bred into different inbred strains, have short generation times, are well-studied and practical to handle. A potential disadvantage is that mutations studied in those models are induced and not spontaneous, hence they do not always mimic the complexity that characterizes a majority of natural disease phenotypes. The laboratory environment is also easy to standardize which gives strength to a rodent experiment but on the other hand it is not reflecting the environmental factors people are exposed to on a daily basis. In the work to connect genomic loci to phenotypes, low levels of genetic variation and a high frequency of the trait of interest is beneficial.

The human genome display high levels of heterogeneity and large numbers of genetic markers and individuals are required to map traits. As most traits with clinical relevance are complex, new approaches have been developed to study human disease. One approach has been to investigate phenotype-genotype relationship using domestic animals as they display an extraordinary phenotypic range as well as a less complex genome structure (13).

Domestic animals

During the domestication process of animals, natural selection has partly been replaced by artificial selection (selective breeding) where the decision of which individual will survive and reproduce lies in the hand of humans. By selecting for favourable phenotypes (such as fertility, size, coat colour or muscle mass), different breeds of domesticated species have emerged that today, at a first sight, have little in common with their wild ancestors. The history of domesticated animals (thousands of generations) has been long enough to allow the evolution of an unusual diversity of phenotypes. From a genetic perspective this implies that domesticated species have captured a wide collection of mutations underlying phenotypic traits (14). At the same time, the heterogeneity within breeds is less extensive as a result of the in-

breeding resulting from selective breeding and reproductive isolation. In addition, many phenotypes have been well monitored for years by studbooks and large pedigrees giving clues of the inheritance pattern of traits, which will help when designing genetic experiments. Mutations of importance for human health have also been mapped by using naturally occurring diseases in domestic animals such as the *grey* mutation in horses connected to melanoma (15), TBC susceptibility in cattle (16), narcolepsy (17) and congenital ichtyosis in dogs (18).

When a favourable trait is selected for and subsequently seen in more individuals, the underlying loci will also look similar. As the selected mutation increases in frequency, neighbouring chromosomal regions will "hitchhike" and form a *selective sweep*. This change in chromosomal structure is a result of genetic variants (alleles) being physically linked and inherited together due to a phenomenon referred to as linkage disequilibrium (LD). Distinctive signatures of selective sweeps are strong LD and extensive genomic blocks inherited together (haplotype blocks). With time, the process of recombination breaks these blocks down and forms new haplotypes. The size of a sweep (often >1Mb) distinguishes it from the reduction in heterozygozity that can be seen as a result of genetic drift. Sweep size depends on the strength of selection as well as recombination and mutation rate (19). Many causative variants have been identified within selective sweeps of domesticated animals such as the grey mutation mentioned in the previous section, the pea comb in chicken and muscle growth in pigs (15, 20, 21).

Dog as a disease model

The domesticated mammal displaying the most phenotypic diversity is undoubtedly the dog (*Canis lupus familaris*)(22), with the existence of more than 400 different pure breeds as well as numerous cross-breeds and feral dogs (23). Geneticists and archaeologist worldwide are among those who have been taking part in the long discussion concerning *when* the dog was domesticated, from which wild ancestor, *where* in the world it happened and especially *why*. Today most have agreed that dog domestication most likely occurred multiple times at separate locations in East Asia sometime during the Pleistocene (at least 15,000 years ago) from one wild ancestor species, the gray wolf (*Canis lupus*) (24-28). As all wolves did not become dogs, all genetic variation in the wild wolf population is not represented in the dog's genome. Consequently, a first genetic bottleneck occurred during the domestication process (**Figure 2**). Being the only large carnivore to become domesticated, the first phenotypes favoured and selected for were probably behaviours that made some wolves more suitable for co-existing with humans. When people went from being hunter-gatherers and settle down in agricultural societies, smaller and more docile dogs were selected for as well as different working abilities such as guarding and herding (29).

The most dramatic changes in the morphology and behaviour of dogs started only some hundreds of years ago during the creation of distinct pure breeds. The extreme diversity seen between dog breeds today is a result of recent selection for physical attributes such as skull shape, size, furnishing, leg length, coat colours etc. Breed standards were introduced together with systemic breeding practices in order to keep breeds pure. Many breeds were formed rapidly by taking advantage of a novel mutation that occurred spontaneously, as the ones underlying brachycephaly (the short face seen in boxer) and chondrodysplasia (the short legs seen in Dachshunds) (30, 31). By selecting these variants, they increased in frequency and after some generations have become the "trademark" of that breed. The reproductive isolation together with back-crossing and the usage of popular sires (that could give >100 of litters in a lifetime) in pure breeds further accentuated breed-defining traits in a population (breed) that with time became more and more homogenous (32). Most breeds have been founded by a small number of individuals and hence, breed creation constitutes a second genetic bottleneck(s) in the domestic dog, as all genetic variation in the pre-breed dog population is not represented in all breeds (**Figure 2**).

The history of domestic dogs has shaped a genome structure that constitutes two main signatures originating from the two genetic bottlenecks. The first one is illustrated by shorter haplotype blocks known to characterize an old genetic bottleneck as the domestication event represent. The level of heterogeneity seen in the total dog population (with ~10kb blocks and 3-5 common ancestral haplotypes) is comparable with the variation of the human genome. Signal number two reflects the recent breed creation and can be detected within each breed where long-range haplotypes of megabase size (0.5-1Mb and 3-6 breed-defining haplotypes) are shared between individuals (8). The second signature has also been affected by the process of selective breeding and explains why a purebred dog appears very similar within breeds.

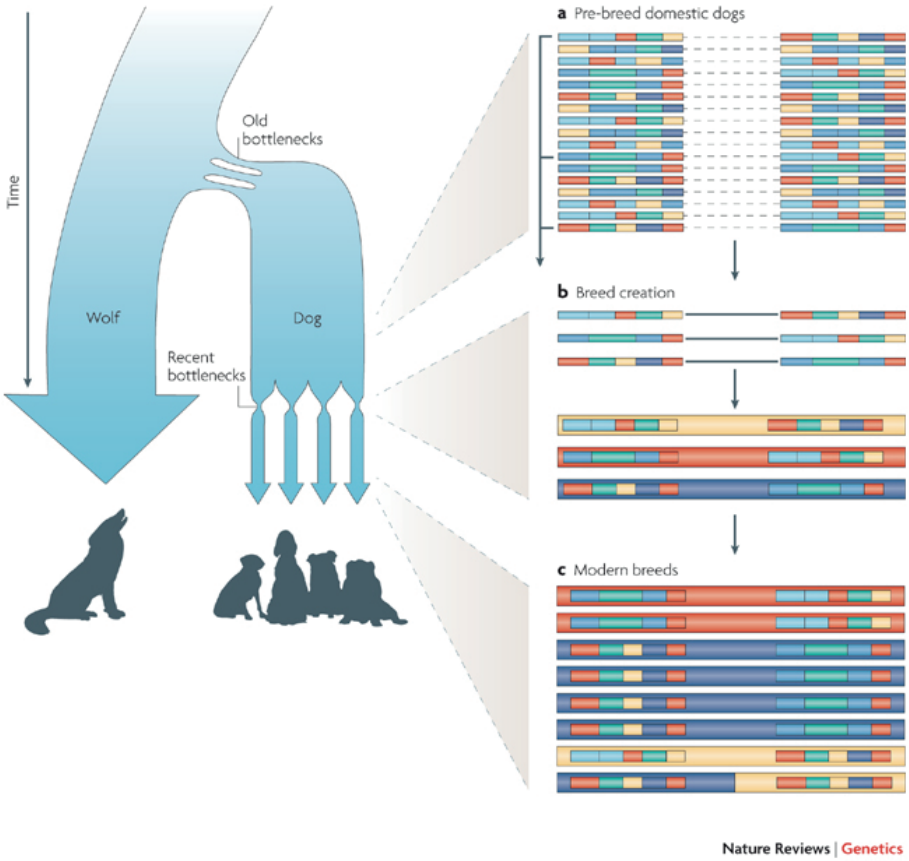

Figure 2. Two genetic bottlenecks have shaped the dog genome. With the assumption that a fraction of the wolf population contributed to the domestication process that started at least ~15,000 years ago, this process created the first genetic bottleneck. From an evolutionary perspective, this bottleneck event is relatively old. The longer time span has allowed the process of recombination to create the short-range haplotype pattern observed in the dog population at the time when modern pure breeds were established **(a).** When only a subset of dogs from the total population was selected as founders of different breeds, a second genetic bottleneck occurred that further limited the genetic variation and thus influenced the genome structure of dogs. Most breeds are younger than 200 years, and therefore the haplotypes represented by the few founders of each new breed are still common in that specific breed **(b)**. Currently, the signatures of these two bottlenecks can be recognized by the combination of long-range haplotypes (seen within breeds) and short-range haplotypes (shared among breeds)**(c).** This genomic structure, especially the long-range haplotypes within a breed makes the dog genome very advantageous when performing genome-wide association studies (Modified from Karlsson & Lindblad-Toh 2008)(33).

In general, deleterious mutations are eliminated from wild populations by purifying selection and this is also true for their occurrence in most domestic breeds where selective breeding is performed. Dogs, however serve as an

unfortunate exception by survival "thanks" to human interventions and most dog breeds thereby show a very high susceptibility to one or more genetic diseases (**Table 1**). This could also be a result of the short time since the deleterious mutation occurred or that it is a pleiotropic mutation selected for. The combination of high incidence and the breed-specificity of diseases, suggests that relatively few loci contribute to the disease development (8, 33-35). Risk alleles might have increased in frequency when "hitchhiking" with a selected trait if the two are linked due to lack of recombination between the two loci. The selected trait might also be encoded by a gene acting pleiotropically and will affect and change more functions in the body than the one intended (36, 37).

Table 1. The table shows three examples of genetic diseases and the dog breed suffering the highest risk of being affected. Derived from Egenvall 2006 (38), Fall 2007 (39) and Hanson 2012 (40).

Increased risk of genetic diseases in different breeds	
OSTEOSARCOMA	
Irish Wolfhound	19-fold
Great Danes	13-fold
Rottweiler	9-fold
DIABETES MELLITUS	
Australian Terrier	10-fold
Samoyed	7-fold
Border Collie	3-fold
ADDISON'S DISEASE	
Standard poodle	14-fold
Bearded collie	6-fold
Giant schnauzer	4-fold
CARDIOVASCULAR DISEASE	
Irish Wolfhound	15-fold
Cavalier King Charles Spaniel	11-fold
Great Dane	9-fold

In the context of comparative medical genetics, purebred dogs constitute the largest catalogue of naturally occurring genetic diseases in a non-human species. Moreover, diseases in dogs are well monitored and diagnosed by veterinarians and registered at clinics and by insurance companies. More than 360 genetic diseases in dogs have been reported as clinically similar to human disease (29). The wide range of genetic diseases affecting dogs includes different types of cancer, cardiovascular-, neurological-, and inflammatory diseases. Many diseases are also influenced by exogenous factors and the fact that we share our living environment with dogs adds an extra advantage (**Table 2**).

Table 2. The domestic dog serves as a good model for mapping disease traits. Listed below are pros and cons regarding medical as well as genetic aspects.

	Advantages	Disadvantages
Medical aspects	Similar spectrum of disease	
	Similar manifestations of symptoms	Some symptoms might be difficult to identify in dogs as it cannot tell
	Similar treatments	
	Well documented and robust diagnoses	A risk of skewed classification if also based on owners observations
	Spontaneously occurring disease mutations	The exact mutations are not likely to be the same in dogs and humans
	High frequency of disease within breeds	
	Exposed to a similar environment	
	Shorter life span than humans	
	Each breed's risk of disease is tracked by insurance companies	
Genetic aspects	Long-range haplotypes within breeds allows mapping with fewer markers	Haplotypes associated with disease often contain many genes
	Few disease loci with large effect allow mapping with fewer individuals	
	Less divergent from the human genome than murine models	
	Similar gene content (orthologous genes in majority)	
	Extensive pedigree records to estimate inheritance patterns	

The dog's genome and case-control studies

The draft genome sequence of one female Boxer (*Tasha*) was completed and published in 2005 by Lindblad-Toh *et al* and opened up many doors for the dog genetics field. The dog genome constitutes of ~2.4 billion bases spread across 38 autosomal chromosomes and chromosome X. Roughly, dogs have 22,000 genes of which the absolute majority are human orthologues (8). Several generations of SNP arrays have been developed after the completion of the dog genome project, starting with a marker panel consisting of 27K SNPs and improving until the current canine HD Illumina 170K SNP array (19).

Several applications of SNP arrays are possible and include case-control studies, selective sweep mapping, measurement of recombination rates, identification of population structure and ancestry. In case-control studies, traits can be associated to a genomic region by comparing allele frequencies between a group with (cases) and without (controls) the phenotype of interest. As a mutation can be in LD with a genetic marker, the assumption behind the method is that one genetic marker (in this case, a SNP) can give information about the whole haplotype on which it is located. A SNP showing

significantly different allele frequencies between cases and controls is in association with the phenotype that differentiates the two groups. A direct association is unlikely (that the SNP is causative), hence the association signal rather pins down which locus the contributing variant harbors. When typing this many SNPs at a time, the risk of detecting false associations is high as well as obtaining signals that originate from confounding factors (weight, sex, origin etc) between the groups of cases and controls. Multiple testing, usually by applying permutations to the analysis solves the first problem whereas the confounding factors need further consideration. The most typical confounder in case–control studies is *stratification* that comes from population sub-structure or cryptic relatedness. Ideally, efforts to avoid false association signals due to stratification should be considered already at the experimental design stage by sampling unrelated, geographically matched individuals recruited from a broad population. The distributional shift in allele frequencies originating from stratification can be detected by an increased genomic inflation factor, λ.

Compared to humans, LD spans over 40-100 fold longer regions in the dog genome (within breeds) and consequently, fewer markers are needed to capture all haplotypes in dogs (15K compared to 100K in humans). As a few genetic factors with large effect is typical in dogs, fewer individuals are also needed in order to find allelic differences between cases and controls. Karlsson *et al* demonstrated the possibility of mapping a monogenic trait using 15K SNPs and 10+10 individuals (41). During the process of sequencing the dog genome, power calculations were performed that suggested an experimental design of 50+50 for a trait with dominant inheritance and 100+100 for a complex trait. Compared to human studies in which the number of individuals and genetic markers required in a case-control study are ten-fold higher, a case-control study in a dog breed is a much more time- and cost efficient process (8).

Uncontrolled (auto-) inflammation

The first line of defence

Although we are surrounded by potentially disease-causing microorganisms (pathogens), only a fraction of them will actually cause us illness. If a pathogen manages to cross the efficient structural and physiological barriers of the body, the majority is halted by other parts of our first line defence, referred to as the *innate immune system* within hours, or even minutes of infection. Key cells of this sophisticated system are macrophages, monocytes, neutrophils and dendritic cells that constantly patrol our bodies to detect signs of damage or invasion. The sensors they carry are termed *pattern recognition receptors* (PRRs) and are capable of both initiating an immune response as

well as directing the adaptive immunity (42). Depending on which microbe/stress signal is detected by what cell, a cascade of activities is induced in order to coordinate antimicrobial actions and tissue repair.

The group of PRRs is large and includes membrane bound Toll-like receptors (TLRs), C-type lectin receptors and cytoplasmic NOD-like receptors (NLRs) (42-45). As the exogenous structure of most microbes is similar, PRRs will recognize them as *pathogen associated molecular patterns* (PAMPs) and activate an innate immune response. To the group of PAMPs belong common motifs of pathogens such as bacterial carbohydrates (LPS, mannose) and peptides (flagellin), fungal glucans and viral nucleic acid (dsRNA). The immune system not only senses potentially harmful microbes, but also changes of self-structure, such as necrotic tissue damage and molecules released by cells in stress (46). These molecules, undergoing a change of state (concentration or formation) during tissue injury and are termed *danger (or damage) associated molecular patterns* (DAMPs) or alarmins (47, 48). Examples of DAMPs are intracellular heat-shock proteins (HSP) and smaller molecules of hyaluronic acid (HA), a main component of the extracellular matrix, which gets fragmented during tissue damage (49, 50).

A potential outcome of the complex formed by a TLR and a PAMP/DAMP is the activation of the NFκß pathway resulting in an increased production of pro-inflammatory cytokines (such as IL-1ß). Cytokines are proteins that are released by activated macrophages to bind to other cells with receptors for the specific cytokine and change the behaviour of that cell. The genes expressed depend on the molecule that encounters the receptor. More than 80 different cytokines are secreted by infected cells and include the families of Interleukins (IL), Tumor Necrosis Factor (TNF) and interferons (INF). Activated NLRs give a similar downstream effect, but are distinct from the other PRRs as they form multiprotein complexes referred to as the *inflammasomes*, which control the maturation and release of pro-inflammatory cytokines. The activation pattern also depends on which type of immune cell senses the PAMP/DAMP and in which tissue it is present.

Although, the production and release of cytokines are the primary output of innate immunity, other mechanisms such as phagocytosis (engulfment and destruction of the detected pathogen) is induced when a macrophage meets components of the bacterial cell wall and the important recruitment of more immune cells are triggered by chemokines also released by macrophages.

Inflammation

The inflammatory response is a protective vascular connective tissue reaction that serves to isolate and destroy pathogens, eliminate necrotic cells and recruit new cells necessary for healing. Signs of inflammation are redness, swelling, pain and heat. The redness and heat are caused by vasodilatation

(widening of blood vessels) to increase the blood pressure and allow migrating immune cells to the infected site. Swelling of tissue comes with oedema caused by leakage of plasma proteins and fluid into the tissue and some inflammatory mediators cause hyperalgesia (increased sensitivity to pain). In an acute state of inflammation, these signs cease once the stimulus is removed. The release of cytokines is the underlying cause of inflammation. Some of the cytokines are also pyrogens (IL-1α, β, IL-6 and TNFα) and are capable of initiating fever (pyrexia) when reaching the thermoregulatory set point in the hypothalamus through the blood stream. Benefits of fever might be that many important immune reactions speed up at a higher temperature and many pathogens prefer a lower temperature.

Autoinflammatory diseases

A group of recently defined and rare syndromes termed autoinflammatory diseases (AID) are characterized by seemingly unprovoked inflammation and fever with no underlying autoimmune or infectious cause. Many AID are episodic, self-resolving and interspersed with asymptomatic periods, thus they were initially termed *periodic* or *recurrent fever syndromes*. As the clinical spectra and the number of syndromes grew, AID was introduced as a more accurate terminology.

To date, AID covers an ever-extending range of monogenic and multifactorial clinical entities with common symptoms such as fever, arthritis, skin rash and systemic inflammation. Unlike autoimmune diseases, the pathological outcome of AID mainly derives from a dysregulated innate immune response, rather than abnormalities of the adaptive immune response. At the molecular level, the uncontrolled inflammation originates from an improper activation of macrophages by an (most often) unknown endogenous or exogenous trigger (51). A majority of AID is also sub-grouped into IL-1β activating diseases (or inflammasomopathies) based on accumulating evidence of successful anti-IL-1 therapy.

Although a majority of AID presents with flares interspersed by asymptomatic periods, subclinical signs such as persistently elevated levels of pro-inflammatory cytokines and acute-phase proteins can often be detected. This continual nature of inflammation put the patients at risk of developing secondary reactive amyloidosis as a result of acute-phase proteins (such as C-reactive protein and serum amyloid protein A) accumulating in vital organs. As kidneys cannot replace damaged tissue with new cells, the severe outcome of amyloidosis is often kidney failure.

Mutations in several genes encoding key sensors or transducers of the inflammatory response have been implicated in AID (such as pattern recognitions receptors, the inflammasome)(**Table 3**). Despite the great advances in understanding AID at a molecular level, in 75-80% of all AID cases, the underlying mutation(s) is unknown (52). The combination of unavailable

genetic tests and overlapping symptoms for many AID, make diagnostics and treatment challenging.

Different animal models have been used to understand autoinflammation including knockout mice for cryopyrin associated periodic syndrome (CAPS) and gout (*NLRP3*)(53) and knockout mice for several mutations associated with FMF (*MEFV*)(54). Much effort has been put into finding the right model in which an exact AID phenotype is expressed. By studying murine models, understanding of the involved pathways downstream of causative mutations of AID has been greatly improved. However, as all mutations are induced in transgenic mouse, the effect of one single locus might not reflect the complexity of the disease phenotypes.

Table 3. A selection of human AID is listed below, typically observed symptoms, genes that have been connected to disease and the suggested pathways in which inflammation is induced.

Disease	Most common symptoms and disease onset	Involved genes and proposed mechanisms
Familial Mediterranean fever (FMF)	Periodic fever (3-7 days), arthritis, serositis, amyloidosis, onset <20 years	*MEFV,* direct inflammasome mutations that results in IL-1β release
hyper IgD syndrome (HIDS)	Periodic fever (3-7 days), arthritis, skin lesions, amyloidosis, childhood onset	*MKV,* encodes a catalyzing enzyme for hormones needed in caspase-1 activation, which is a part of the inflammasome
cryopyrin-associated periodic syndromes (CAPS)*	Cold induced fevers, meningitis, cochlear inflammation, onset <5 years	*NLRP3,* direct inflammasome mutations, that results in IL-1β release
PAPA syndrome	Pyogenic arthritis, pyoderma granulosum, acne	*PSTPIP1,* disturbs the inhibitory effect of pyrin which is a part of the inflammasome, leads to IL-1β release
gout	Recurrent arthritis (mainly the metatarsal-phalangeal joint)	*Complex acquired,* alternative cleavage of pro-IL-1 β
TNF-associated periodic syndrome (TRAPS)	Periodic fever (1-6 weeks), rash, myalgia, serositis, amyloidosis, variable onset	*TNFRSF1A,* shedding defect and /or protein misfolding
ankylosing spondylitis (AS)	Chronic arthritis of spinal chord	*HLA-B27,* stress response following unfold protein
Blau syndrome	Arthritis, dermatitis, uveitis	*NOD2*, up-regulated NF-κβ activation
Crohn's disease	Intestinal inflammation	*NOD2 (complex),* NF-κβ activation
systemic onset idiopathic juvenile arthritis	Long-lasting arthritis, systemic symptoms, young onset <16 years	*Unknown*
adult onset Still's disease (AOSD)	Long-lasting arthritis, systemic symptoms, adult onset	*Unknown*

* Including MWS=Muckle-Wells syndrome, FCAS=familial cold associated syndrome and NOMID=neonatal onset multisystem inflammatory disease.

A natural canine model for autoinflammation

The dog breed Shar-Pei, is the only animal population in which autoinflammation is reported to occur spontaneously at any rate. A high proportion of dogs belonging to this breed are affected by AID that closely resemble human disease with cardinal signs such as flares with fevers, swollen joints (arthritis), skin rash, signs of systemic inflammation (abdominal pain, hunched back) and a high incidence of secondary amyloidosis. Interestingly, the joints most affected by arthritis in Shar-Pei are the hocks, which correspond anatomically to human ankles- the main joint affected in FMF **(Table 4**).

In addition to similar clinical signs, the autoinflammatory nature of the Shar-Pei condition is implied by the fact that many Shar-Pei experiences therapeutic benefit from IL-1β inhibitors, suggesting that a dysregulation of innate immunity underlies the symptoms in this canine model. The disease in Shar-Pei is habitually termed *Familial Shar-Pei fever (FSF),* and to the dog owners the cardinal sign is periodic fevers. In addition, a link is believed to exist between what they refer to as swollen-hock syndrome (the arthritis described above) and amyloidosis.

Like in human AID, affected Shar-Pei dogs also show subclinical signs in terms of a constant elevation of IL-6, another pyrogenic cytokine (55). IL-6 is also an inducer of acute phase proteins, which are precursors of the amyloid type AA proteins that accumulates extracellularly in secondary amyloidosis (56). The heterogeneous group of disorders termed amyloidosis is distinguished by depositions in extracellular space by insoluble proteins termed amyloid. Three types of amyloidosis have been described; immunoglobulin-associated (primary), reactive (secondary) and senile (heredofamilial). The reactive amyloidosis observed in Shar-Pei is built up of the acute phase protein *amyloid A*, released predominantly by the liver during inflammation. Although amyloidosis in Shar-Pei is systemic, the most reported cause of early death is kidney failure (57), and more rarely hepatic failure (58).

Despite the diverse origin, most amyloid proteins share structural and chemical properties detectable by staining with Congo red and thioflavin T (59). Amyloidosis in Shar-Pei is also fairly different from what is observed in other breeds where most amyloid depositions in kidney are seen in the cortex part while in Shar-Pei, the medulla seems to be more or equally affected (57). The average age of onset is also significantly lower in Shar-Pei compared to other dog breeds (57, 60).

While the periodic fevers and amyloidosis are the most severe and frequent health problems in the Shar-Pei breed, it is unfortunately far from the only one. Shar-Pei dogs also show a high incidence of mast cell disease (cancer), dermatitis, inflammatory bowel disease, allergies, otitis, glaucoma, entropion, lymphangitis, Immunoglobulin A deficiency, cobalamin deficiency, hypothyroidism and vasculitis (**Figure 3**).

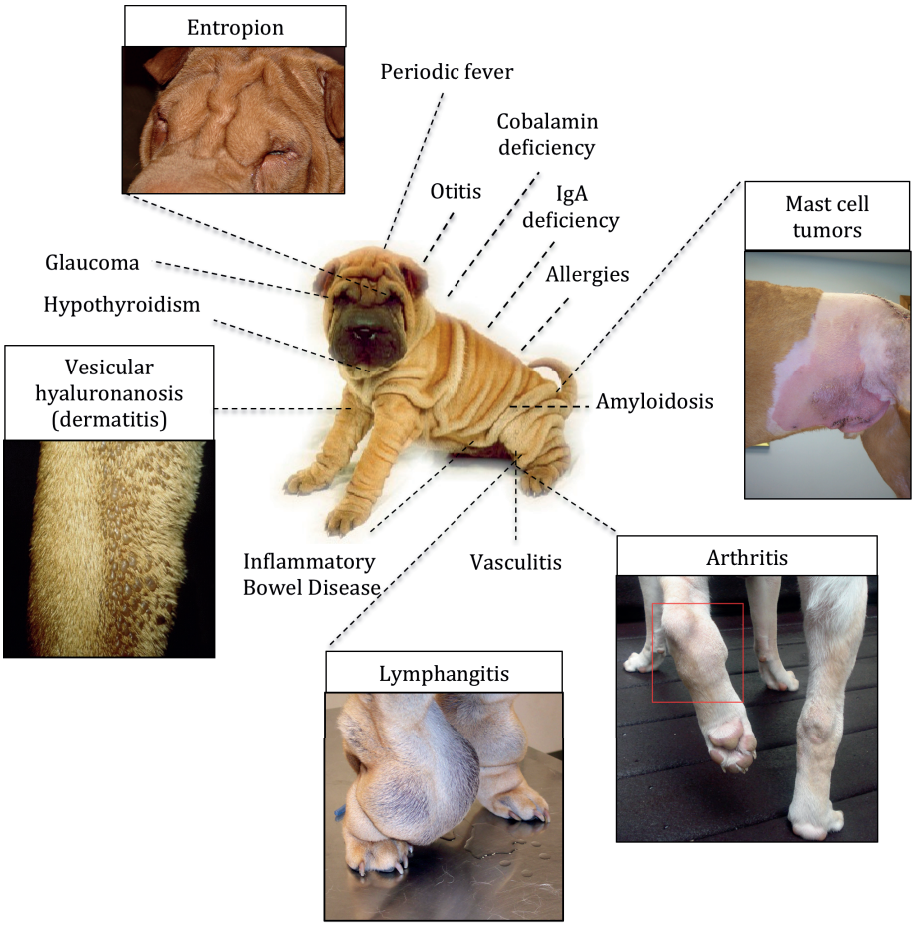

Figure 3. The Shar-Pei breed is predisposed to many pathological conditions.

Table 4. A comparison of the most common human AID (Familial Mediterranean fever, FMF) and the canine AID (habitually called Familial Shar-Pei fever, FSF)

Familial Shar-Pei fever (FSF)	Familial Mediterranean fever (FMF)
12-36h attacks of high fever	12-72h attacks of high fever
Arthritis, the hock joint most affected	Arthritis, the ankle most affected
Skin rash	Skin rash
Abdominal pain	Abdominal pain
Back pain (?), walk with a hunched back	Back pain
Asymptomatic between attacks	Asymptomatic between attacks
Typically an early onset	Early onset
40% die premature following amyloidosis	Sometimes develop amyloidosis
Benefit from colchicine and IL-1β inhibitors	Benefit from colchicine and IL-1β inhibitors

For thousands of years, Shar-Pei dogs have been companions of the Chinese people, used for guarding, hunting and in some regions also for fighting. In the beginning of the communist era, the breeding and ownership of pet dogs was discouraged by the high taxes imposed. The Chinese dog population declined dramatically and the Shar-Pei breed was close to extinction when a handful of individuals were exported to the US in the late 1960s.

The distinctive appearance of Shar-Pei made them extremely popular in the US (and later also in Europe) and the population originating from these few imported individuals exploded and is still growing at a high rate. The breed-defining trait of Shar-Pei is their thickened and folded (wrinkled) skin, a trait that is expressed in variable degrees. Back in China, many of the dogs are of the *traditional* type that are born with a folded skin but by age outgrow the most accentuated phenotype. The adult traditional individuals keep smaller skin folds on their forehead and by the whither.

Shar-Pei dogs entered the western world with the characteristic folded skin but ever since, the trait has been a target of strong artificial selection. Nowadays, a Shar-Pei from the western population keeps the wrinkled and folded skin into adulthood and looks strikingly different to the traditional breed type and is called the *meatmouth* type (**Figure 4**). The skin folds have habitually been termed cuteanous mucinosis that, apart from the obvious appearance, sometimes also displays with “blisters” on the skin.

Figure 4. Shar-Pei breed-types

The wrinkled skin phenotype in Shar-Pei (hyaluronanosis) is represented in a wide phenotypic spectrum and ranges from the smooth traditional type (right hand) to the meatmouth type (the other three) with heavy skin folds and padded muzzle.

Aim of the thesis

The overall aim of this thesis was to identify new genes involved in the process of autoinflammation in a canine genetic model.

The specific aims were:

- To characterize the autoinflammatory disease phenotype in Shar-Pei
- To identify and characterize the mutation(s) underlying autoinflammation in Shar-Pei
- To investigate the relevance of gene (s) identified in dogs and their mechanisms in humans with similar pathology

Present Investigations

Paper I: Mapping of two breed-specific features in the Shar-Pei dog

Background and study design

In an epidemiological survey from 1992, a large proportion (23%) of Shar-Pei dogs from the US population were reported to suffer from periodic fevers of unexplained origin. Although fever was presented as the cardinal sign it was frequently accompanied with swollen joints (53%) and strongly associated with renal amyloidosis and premature death (55). In the perspective of animal health it became evident that there was an urgent need for a deeper understanding of these conditions and genetic tools to counsel breeding.

The authors of the study also recognized the potential of Shar-Pei as a natural model for Familiar Mediterranean fever (FMF), which was the best-described human periodic fever syndrome at the time with clinical manifestations very similar to the symptoms reported in Shar-Pei. Following the resemblance with FMF, the autoinflammatory disease in Shar-Pei was termed Familial Shar-Pei fever (FSF).

In 2006, a candidate gene study was set up by *Puppo et al* (unpublished work) in order to screen the genes in Shar-Pei homologues of the gene implicated in FMF (*MEFV*) as well as the other genes implicated in human periodic fever syndromes; TRAPS (*TNFRSF1*), HIDS (*MKV*) and CAPS (*CIAS1/NLRP3*). No mutations were identified in the coding regions of any of these genes.

Shar-Pei fever

The clinical similarity to human AID together with the absence of mutations in known AID genes, suggests that Shar-Pei could be a useful animal model to explore alterations of new pathways that result in uncontrolled inflammation. To date, many more AID have been characterized and new genes and mutations identified, but still the list of patients without a molecular diagnosis is growing (75-80% (52)). When the canine SNP arrays became available, the natural approach to map FSF was a case-control genome-wide association study by using samples collected from privately owned pet Shar-Pei dogs.

In the initial attempt to map FSF, individuals were classified into affected (cases, n=50) and unaffected (controls, n=50) by unexplained fever according to the owner's observations and genotyped using an 18K SNP array. With the initial classification system, no signals of association were observed. The sample set was evaluated again, this time by using a stricter classification criteria based on more information about each dog, including medical records. A Shar-Pei was now considered a case if it had experienced recurrent fever attacks with an early onset (< 1 year of age) accompanied with swollen hocks (arthritis)(cases, n=39). In the healthy control group were only individuals older than 5 years, with no signs of persistent inflammation and with no first-degree relatives with unexplained fevers included (controls, n=17).

The wrinkled skin

In parallel we were interested in mapping the wrinkled skin phenotype by using the same dataset. This trait is also associated with health problems in Shar-Pei dogs, as the heavy skin folds on the forehead (probably together with weakened structure of the eye-lids) put them at risk of developing *entropion* when the eyelashes roll inwards and damages the cornea. This is especially a problem during puppyhood when the wrinkled phenotype is more accentuated and an intervention by suturing the skin fold ("eye tacking") is frequently needed to protect the eyes of the puppies.

As the wrinkled skin is a breed-specific trait strongly selected for, the genomic region in which the causative mutation resides is likely to be part of a unique selective sweep. Therefore, we scanned the genome for signatures of selection and reduced heterozygosity in the Shar-Pei breed by comparing the genotypes from the whole genome scan to dogs (n=230) representing 24 other breeds. Sweep signatures were detected by scanning all genotypes in sliding windows of 10 SNPs (≈1 Mb) from which the relative heterozygosity was calculated for Shar-Pei and other breeds separately.

Results and discussion

Mapping the region

The strongest signature of a selective sweep encompasses a 3.7 Mb stretch on chromosome 13, where Shar-Pei have a 10-fold reduction in heterozygosity compared to the other breeds. The size of the region indicates that a favourable genetic variant on chromosome 13 has swept along neighbouring loci and thereby changed the allele frequencies. In the case-control analysis, for the periodic fever, a genome-wide significant signal was also identified on chromosome 13.

When combining the results from both analyses it was evident that the two signals co-located on chromosome 13 and SNPs were interspersed so

that they were either a part of the sweep (close to or completely monomorphic) or the association signal. Two peaks flanking the sweep region actually made up the association signal, and so it was difficult to conclude where the strongest signal of association to FSF was really located.

Within the region of co-locating signals, the gene Hyaluronic Acid Synthase 2 (*HAS2*) is present, which represents a great candidate gene for the skin phenotype, as hyaluronic acid (HA) is a main component of mucin that is known to build up the skin folds in Shar-Pei.

Mutation identification

In order to find the exact mutations for the two phenotypes, targeted resequencing was performed in traditional and meatmouth type of Shar-Pei as well as in three control breeds. As the technology at the time only allowed sequencing of 1.5 Mb, the first priority was to capture *HAS2* and the closest surrounding region. Of all possible polymorphisms identified from the sequencing, we further examined the ones located in conserved regions. Only two duplications were unique for Shar-Pei, whilst the other polymorphisms also occurred in other breeds and consequently were not in concordance with the target breed-specific phenotypes.

In fact, the duplications were first detected by looking at sequence coverage. In a 16.1 kb stretch, a 4-5 fold higher coverage was seen for the two meatmouth Shar-Pei compared to control dogs and a similar pattern was seen in an overlapping 14.8 kb region for the two traditional Shar-Pei. The two duplications were named *meatmouth* and *traditional* depending on in which Shar-Pei type they were identified. Further examination of the duplications revealed that the duplications could vary in copy number in different Shar-Pei individuals.

Biological relevance

To estimate how many duplications each individual carry, two different copy number assays were designed and the copy number was estimated as the relative fold enrichment between an amplicon within the duplication and another in a house-keeping gene known to be present as a single copy. Again, the breed-specificity was confirmed for both mutations as it did not occur in any of the 24 other dog breeds but was present in all Shar-Pei tested. Shar-Pei of the traditional type only carried the traditional duplication whilst the meatmouth type dog appeared to have both of the two duplications.

To evaluate the functional importance of the duplications, we measured the expression of *HAS2* in cultured dermal fibroblast of Shar-Pei (n=6). Although the number of individuals examined was small, we could see an increased *HAS2* expression in Shar-Pei with a higher copy number of the meatmouth duplication, suggesting that the mutation harbours a regulatory element that controls *HAS2* activity.

In the last step, the clinical history of all Shar-Pei individuals was added to the analysis. The biological significance of the (meatmouth) duplication was even clearer in this analysis as a higher copy number is also significantly associated with the risk of experiencing recurrent fever attacks. No such correlation was seen between FSF and the traditional duplication.

We identified one pleiotropic mutation to correlate with two breed-specific phenotypes in Shar-Pei of which one has been strongly selected for (wrinkled skin), while the other has been targeted unintentionally (AID). The key molecule for both traits emerged to be hyaluronic acid (HA), a multifunctional glycosaminoglycan whose specific role is size- and location dependent. High molecular weight (HMW) HA displays a great variety of biological roles including being a main component of the connective tissue matrix of the skin, involved in cell migration and differentiation and as a lubricator in the synovial fluid. For this reason, a connection between a mutation controlling *HAS2* expression and the wrinkled skin phenotype in Shar-Pei is biologically attractive.

As the deposition in the Shar-Pei skin, now is linked to a certain molecule, we proposed the terminology *hyaluronanosis* to replace the more vague (cuteanous) mucinosis, inspired also by a similar condition reported in humans when a child was born with high levels of circulating HA and heavily thickened and folded skin (61).

Hyaluronic acid (HA) and inflammation

In the inflammatory response, HA plays a dual size-dependent role. HMW-HA is anti-inflammatory and is recruited to sites of inflammation as an immune suppressor and new building blocks for the healing process. Low molecular weight HA (LMW-HA) on the contrary is recognized as a danger signal (a DAMP) by the key sensors of the innate immunity (PRRs). Fragmented HA is a fundamental warning of tissue damage that will induce inflammation by two distinct but co-operating routes; (i) by binding to TLR2 or 4, LMW-HA activates the nuclear factor (NF) κß pathway with an increased expression of IL-1ß mRNA as a result and (ii) by binding to CD44, LMW-HA is endocytosed and further degraded by hyaluronidase 2 in the cytoplasm into a fragment size recognized by another receptor, NLRP3, that will form the multiprotein complex referred to as the inflammasome. The inflammasome is responsible for the transformation of pro-IL1ß into the mature form that is secreted from the cell (**Figure 4**).

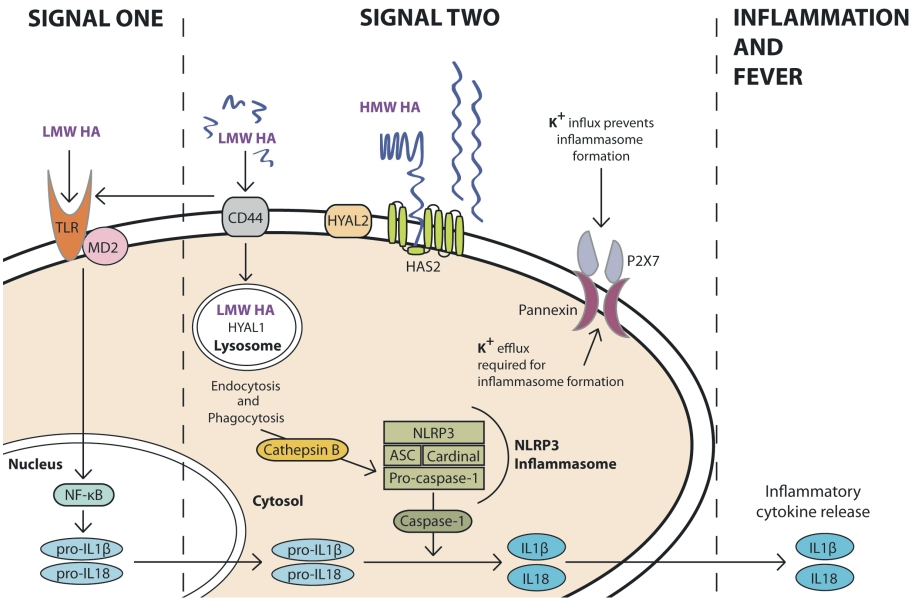

Figure 5. LMW-HA as danger signals induces release of pyrogenic cytokines.

When triggering inflammation, HA is signalling through two distinct but co-working routes. In the first one, HA gets recognized as a danger signal (DAMP) by TLR2, 4 and activates the NF-κβ pathway and the increased production of pro-IL1β and 18 to the cytosol. In the second route, HA binds to CD44, become internalized and further degraded by HYAL2. When reaching this fragment size, HA is capable of trigger the formation of the inflammasome, which role is to cleave the pro-IL-1β into its active forms, which will get released by the cell. Figure kindly provided by Dr J.R.S. Meadows who adapted it from http://www.invivogen.com.

The elevated IL-6 levels reported in Shar-Pei (55) could have followed the IL-1 release from these co-operating pathways. IL-6 has been proposed as a "marker" of the more fluctuating IL-1 that was not measured in the referred study. Many Shar-Pei benefit from treatment with IL-1β inhibitors, which further supports IL-1β to be the principal cytokine in Shar-Pei AID.

The reason why pro-inflammatory HA is circulating in Shar-Pei remains unclear. Possibly the large amounts of HMW-HA produced will get fragmented through pathogenic HYALs or by reactive oxygen species (ROS) (62) released during cellular stress.

The mutation identified in Shar-Pei fits in very well in the autoinflammatory pathways, although it is located upstream of mutations described earlier in humans that result in similar symptoms (such as mutations in NLRP3 causing CAPS). Here, the actual trigger sensed by the innate immune system is altered instead of the key players.

Paper II: Introducing SPAID: an autoinflammatory disease complex inadvertently under selection in Shar-Pei

Background and study design

During the process of classifying Familial Shar-Pei fever (FSF) in paper I, it became evident that many more signs of persistent inflammation affect the breed. We hypothesized that the autoinflammatory disease in Shar-Pei presents with a much wider clinical spectrum and these additional signs may be associated with the same chromosome 13 locus. To increase the phenotypic resolution, > 500 additional Shar-Pei were sampled and classified according to all detectable signs of recurrent or persistent inflammation.

The rigorous classification process involved owner completed health questionnaires (specially designed for this purpose), medical journals for each Shar-Pei and when possible, discourse with the attending veterinarian. By dividing all different inflammatory signs into subsets, the aim was to map them separately in a case control-design. A higher genomic resolution was also expected by using a denser SNP array with170K markers compared to the 18K panel used in paper I.

Rigorous phenotypic classification

In the health questionnaire, the owner of each Shar-Pei was asked explicitly if the dog had experienced fever and/or arthritis and if so, what was the age of onset, frequency- and length of episodes, how high was the fever, how quickly did the episodes resolve and was the dog treated to break the fever. In addition, a declaration of other signs of persistent or recurrent inflammation was asked for including otitis (ears), dermatitis (skin), Inflammatory Bowel Disease (gastrointestinal), vasculitis (blood vessels), lymphangitis (lymphatic channels) and amyloidosis (or signs of impoverished kidney function). Other less obvious signs of systemic inflammation were addressed by asking if the dog had experienced days or periods when it was reluctant to move, seemed to have abdominal pain or walked stiffly with a hunched back. The medical histories of the closest relatives were also collected, as well as information about treatment or abnormal laboratory results (such as high proteinuria that could suggest amyloidosis). Other health issues were evaluated as well and included; entropion, glaucoma, lens luxation, allergies, hypothyroidism, cancer, mast cell disease, heart problems and neurological disease.

As there is no quantitative measure of the wrinkled and thickened skin in Shar-Pei, owners were asked to state to which breed type they considered their dog belonged (traditional-, bonemouth- or meatmouth type). The picture shown as **Figure 3** of the introduction was used for guidance and in addition, the owner was encouraged to attach a photo of their Shar-Pei.

Amyloidosis

We were also puzzled by the strong but inconsistent link between FSF and reactive amyloidosis occurring secondary to persistent inflammation in Shar-Pei. Neither the number nor the strength of FSF events seems to correlate with the risk of acquiring amyloid depositions. Also, Shar-Pei with no medical history of FSF seemed to be predisposed to amyloidosis. We speculated that the incidence of amyloidosis could be affected by additional (modifying) loci and therefore no inheritance pattern or straight link to FSF would be observed. In order to perform a separate case-control study also for this phenotype, we sampled >40 Shar-Pei individuals that were diagnosed post mortem with amyloidosis. A discrete control group was also established with Shar-Pei proven free of amyloid depositions using biopsy.

The outcome of the phenotyping process was five subsets of various inflammatory phenotypes in sufficient numbers for case-control analyses and three control groups with differently defined criteria. In addition, the two extremes of Shar-Pei breed type (heavily and less wrinkled) were considered as cases and controls in one analysis.

Classification with one sign of inflammation was not always exclusive and one individual can belong to several case subsets. The sample set finally genotyped consisted of 255 Shar-Pei, five-fold more than in paper 1.

Subsets of Shar-Pei, cases

- Fever (n=129)
- Arthritis (n=108)
- Dermatitis (n=46)
- Amyloidosis (n=38)
- Otitis (n=26)

Subsets of Shar-Pei, controls

- Strict control group (n=24): Shar-Pei older than 7 years, with no signs of inflammation and with no first-degree relatives displaying inflammatory signs
- Relaxed control group (n=36): Shar-Pei older than 4 years with no signs of inflammation.
- Amyloidosis controls (n=14): Shar-Pei with no signs of inflammation and biopsy-proven proven free of amyloidosis.

Subsets of Shar-Pei, breed-types (intermediate types excluded):

- Bonemouth (n=33): less wrinkled Shar-Pei
- Meatmouth (n=123): heavily winkled Shar-Pei

Results and discussion

In the first study, the wrinkled skin locus was mapped to a region on chromosome 13 by using selective sweep mapping. A long genomic stretch of reduced heterozygosity surrounding *HAS2,* was identified in Shar-Pei. With the new phenotyping and a denser SNP map, we were able to confirm this region with the approach of using the two Shar-Pei breed-types, in a case-control study. By calculating genomic fixation (F_{ST}) we could see the peak of differentiation was located in this same region, just downstream of *HAS2*.

Five signs of inflammation, one genomic region

All five inflammatory signs were successfully mapped separately to the sweep region on chromosome 13 using the same strict control group for each subset of cases. The signals of association were based in two regions (23.5Mb and 27Mb) flanking the sweep signal. Breed type and dermatitis showed a stronger association to the 23.5Mb region while fever, arthritis and amyloidosis to 27Mb. The two peaks were not in high LD, but the observation of significant genomic association at the 23.5 region containing *HAS2* suggests that this locus is still involved in fever, arthritis and amyloidosis, either directly or by hitchhiking.

When scrutinize the genome-wide significant SNPs for all subset we found that there was a clear overlap. By using the most associated SNPs for the groups, a disease haplotype was defined that occurred in 70 % of all Shar-Pei with inflammatory signs. As the range of clinical signs appear to be connected, we propose the new terminology for autoinflammation in the breed; Shar-Pei autoinflammatory disease (SPAID) including (to date) fever, arthritis, dermatitis, otitis as well as a high risk of secondary amyloidosis. The dermatitis seen in Shar-Pei is a result of specific dermatological changes of blisters and bubbles of HA on the skin surface; we propose the terminology vascular hyaluronanosis to describe that type of dermatitis in Shar-Pei.

All phenotypes except for amyloidosis have two association signals flanking the sweep but the most associated SNPs differed slightly. In addition to the amyloidosis peak on chromosome 13 (at 27Mb), we identified a new (close to significant) signal of association on chromosome 14, by using a stricter control group containing only Shar-Pei individuals confirmed to be unaffected by amyloidosis. The signal constitutes of two peaks with several biologically attractive genes including *IL-6*, which is a pyrogenic cytokine known to trigger amyloid A, and is reported to be elevated in Shar-Pei with AID. This finding probably reflects the inconsistent link between severity of autoinflammation and amyloidosis, as it suggests a multifactorial mode for this specific condition modified by other genomic loci. A candidate gene expression study of 16 genes within the chromosome 14 peak as well as *HAS2*, was performed in kidney tissue from Shar-Pei affected and unaffected by amy-

loidosis. Four genes, previously known to influence renal health and inflammation, showed significantly higher renal expression in SharPei affected by amyloidosis.

Although *HAS2* overexpression must to be considered as a strong risk factor, other genes located in the larger region on chromosome 13 are likely to contribute to the complexity of SPAID. We speculate that the whole chromosomal region is hitchhiking with the selected locus although different haplotypes might give rise to various combinations of inflammatory signs. In addition, the new association signal for amyloidosis on chromosome 14 together with a higher expression pattern in affected Shar-Pei in this region might reveal important modifiers for the severe outcome of autoinflammation that amyloidosis represent.

Paper III: Investigating the role of HA in human AID

Background and study design

From our studies of SPAID in the canine model, the key molecule emerged to be hyaluronic acid (HA). In Shar-Pei, the gene *HAS2* is overexpressed in dermal fibroblasts (and perhaps more cell-types) and HA is accumulating in the upper dermis of the skin. Through drainage to the circulatory system, the overproduction of HA can also be detected in sera from Shar-Pei dogs and therefore represent a useful marker for pathological changes in HA metabolism.

Also in human inflammatory disorders circulating HA has been reported to be elevated (63-68). In addition, a reduced average size of HA has been observed in synovial fluid of both rheumatoid- and osteoarthritis (69), and the underlying mechanism was suggested to be the increased expression of the HA fragmentation enzyme, hyaluronidase 2 (70). Together with our findings in canine SPAID, this suggests LMW-HA to initiate or foster sterile inflammation.

In this study, serum HA was used as a biomarker to investigate its potential involvement in human AID. Given that the biological role of HA is size-dependent, we aimed to estimate the presence of circulating high and low molecular weight HA by using two different Enzyme-Linked Immunosorbent Assays (ELISAs). The assays are based on different methodologies, one is a so called competitive and the other a non-competitive ("sandwich") ELISA. With the competitive method also LWM-HA (from 6.4kDa and upwards) is reported whereas with the non-competitive ELISA, the smallest HA molecules are not captured (reports HA from 27kDa and upwards)(71). We hypothesized that subjects with normal serum HA levels measured by

the less size-sensitive assay, might report high levels with the LMW-HA sensitive assay if LMW-HA occurs predominantly.

Subjects representing various types of AID were included in the study, some with a molecular diagnosis (a causative mutation identified) and others for which the diagnosis was only based on clinical features and where the underlying genetics is still unknown. In this study Familial Mediterranean fever (FMF), TNF receptor associated periodic syndrome (TRAPS) and cryopyrin associated periodic syndrome (CAPS), hyper IgD syndrome (HIDS) belonged to the cohort of *genetically explained AID*. The second cohort of *AID with unknown etiology* included adult onset Still's disease (AOSD), systemic juvenile idiopathic arthritis (sJIA), gout, ankylosing spondylitits with unidentified AID and amyloidosis (AS/UAID). As the groups of AOSD and sJIA were more extensive regarding size as well as clinical data, these were in focus of this study.

AOSD and sJIA

AOSD is a systemic inflammatory disorder of unknown genetic origin presented with long lasting arthritis (>6 weeks) and spiking fevers. Patients with AOSD can present with one single episode, recurrent episodes or a chronic inflammatory reaction (72). The systemic nature is manifested by multiple accompanying symptoms such as skin rash, myalgia, pharyngitis, lymphadenopathy, hepatosplenomegaly, pleuritis, pneumonitis, pericarditis, and hepatomegaly (73, 74). A high level of ferritin has been reported in AOSD patients and is used as a diagnostic tool together with a panel of clinical features (75, 76). High ferritin is a marker of macrophage activation (77), which in turn is triggered by LMW-HA (78). The activity of AOSD can be assessed by a score system (a modified Pouchot's score) counting twelve commonly seen symptoms including high ferritin (79, 80).

Systemic onset juvenile arthritis (sJIA) in principle shows with the same clinical signs in patients younger than sixteen years old. No underlying mutations have been connected to AOSD or sJIA, although associations to the HLA complex implied AOSD to be of an autoimmune nature (81, 82). However, recent studies have suggested the two diseases to rather belong to the group of AID as the disease is mediated by IL-1β, patients response well to anti-IL-1-therapy and many of the clinical features resembles several others in group of AID (83, 84).

Results and discussion

HA levels were higher in the cohort of subjects with genetically unexplained disease compared to those with a known mutation. The difference was significant when HA was reported with the less size-sensitive assay (reporting HA 27kDa and upwards).

The disease groups within the two cohorts were dissected separately and the proportion of subjects with elevated HA reported by the different assays was compared. It was evident that the proportion of subjects with high HA detected by the LMW-HA sensitive assay was larger in seven out of the nine disease groups, compared to when reported by the less size-sensitive assay. This might suggest that the larger proportion of HA in these subjects are of the pro-inflammatory size, capable of initiating or foster inflammation.

In both AOSD and sJIA, elevated HA (reported by the LMW-HA sensitive assay) was correlated with the activity (acute/in remission) and severity of disease (number of accompanying symptoms in AOSD and the number of joints affected by arthritis in sJIA). The correlation between high HA and polyarthritis has been described before (67) although it is interesting that we only detect the correlation by using the assay capturing also LMW-HA. In AOSD, no correlation to high HA has been associated to severity in terms of number of symptoms prior to this study. HA was also correlated with high ferritin, the major laboratory marker for AOSD (see above). This chain of events form a potential mechanism whereby HA might trigger disease in AOSD.

General discussion and future perspectives

In paper I, a novel CNV was identified in Shar-Pei by using the combined approaches of selective sweep mapping and a case-control study. The study design turned out successful which is logical considering the proposed mechanism of disease alleles that *hitchhike* with selected variants. In Shar-Pei, both the selected variant and the disease causing mutation appear to be one pleiotropic genetic factor. The CNV identified in Shar-Pei adds to the list of structural variants that has been identified in regulatory regions of domestic animals (15, 20, 85). Interestingly, CNVs found in the human genome is often neutral whereas in domestic animals many appear to have large effect on phenotypic variation.

We also show that an increased copy number of the CNV is associated with a higher *HAS2* expression. Moreover, the absolute link between increased mRNA and an amplified HA production in skin of Shar-Pei has been established by others (86). The role of HA overproduction and its link to inflammation in the canine model, needs to be further characterized. HA has an extremely fast turnover rate following the constant process of synthesis (by HAS genes) and degradation (by HYALs or environmental insults). The balance between the health promoting HMW-HA and pro-inflammatory LMW-HA must be altered in Shar-Pei prior to flares of inflammation. It could be that HA is produced in a periodic nature as well: builds up over time and reaches a level when the size ratios shift due to an imbalance of fragmentation and the clearance process of small HA fragments. This would create an extracellular environment where LMW-HA dominates. When the level of small HA fragments finally is reduced the asymptomatic period starts. Another possible scenario is that HA synthesis and/or fragmentation is triggered by one or several external factors. For example, treatment with corticosteroids is known to reduce the "wrinkledness" of a Shar-Pei temporarily (especially the padded muzzle shrinks). This is the outcome of corticosteroids shutting down *HAS2* expression (87) and should also result in an environment with a skewed ratio of low and high MW-HA. Although it is difficult to evaluate stress in the canine model, it appears as if many Shar-Pei dogs experience some sort of trauma (infection, wound etc.) or stressful change in their life in connection to the first flare of inflammation. In the case of a trauma, a damaged ECM as well as microbial infection secreting pathological HYALs, and the relation to small HA fragments is straightfor-

ward. In the case of stress, other hormones naturally released during stress could perhaps also alter HA metabolism similar to corticosteroids.

A regulatory element appears to be located within the duplicated sequence. Functional studies in terms of enhancer assays are in process in order to evaluate the action of the duplication on *HAS2* expression. The CNV could also be in LD with the causative mutation and represent a risk haplotype rather than the actual mutation. The excess of HA in Shar-Pei skin is creating the "wrinkledness" which is the trademark of the breed and has been the target of artificial selection. This phenotype shows a great variation from the smooth (traditional type) to slightly wrinkled (bonemouth type) to individuals with a heavily thickened and folded skin and padded muzzle (meatmouth type). Interestingly, we detected two overlapping duplications, of which we could only connect the one found exclusively in the meatmouth type, to disease risk and *HAS2* expression. Of the true traditional Shar-Pei individuals in our sample set, none have the duplication associated to disease and *HAS2* expression and they also did not show any signs of AID.

In paper II, the genetic origin of the wide spectrum of inflammatory signs in Shar-Pei was addressed in separate case-control studies. Although, all inflammatory signs could be mapped separately to the same chromosomal region surrounding the selective sweep, the location of the most associated SNPs appeared to be different among the phenotypic groups. Given the strong correlation between higher copy number and each inflammatory sign, the over expression of *HAS2* must be considered the major risk factor of SPAID. However, a deeper dissection of the whole region by large-scale sequencing is necessary to identify (or rule out) other possible mutations that might work in concert with the *HAS2* regulatory CNV. The pattern indeed appears more complicated although different combinations of contributing risk factors is attractive in order to explain the complexity of SPAID.

In the case of amyloidosis, the persistent inflammation (signal at chromosome 13) is a plausible and realistic strong genetic risk factor. However, the variation in incidence of amyloidosis could be explained by additional loci (chromosome 14) that might influence each individual's capacity to resolve inflammation. Sequencing and gene expression studies are ongoing in order to better understand the role of the chromosome 14 locus in amyloidosis. Shar-Pei dogs are reported to have a unique pattern of amyloid depositions predominantly (or equally) in the kidney medulla compared to other breeds where amyloid is only seen in the kidney cortex. In the kidney medulla, HA is normally abundant and determine the renal ability to concentrate urine (88, 89). The up-regulation of HA might have a role also in these specific extracellular depositions and thereby affect the amyloidosis incidence.

Throughout all case-control studies, the most significant results have been achieved by using the strictest classifications. This is especially true for healthy controls, where the rigorous classification resulted in exclusion of the majority of individuals that were classified as healthy by their owners. Phenotyping errors like this reflect one downside of working with pet dogs as a disease model and much effort must be placed into characterization of disease for each individual to define inclusion and exclusion criteria for the disease phenotype. Ideally, depending of careful clinical diagnosis performed by veterinarians.

Three new terms have been introduced through the work behind this thesis. Firstly, *SPAID* to describe the wide spectrum of persistent inflammatory signs seen in Shar-Pei. All signs included in *SPAID* (so far) must be considered as autoinflammatory as they are correlated with a molecule (HA) that function as a danger signal (a DAMP) that triggers the inflammatory response. In addition, SPAID resembles human AID that also presents with multiple inflammatory signs. Many Shar-Pei with *SPAID* are also experience therapeutic benefit from IL-1β inhibitors, which suggests a cytokine-driven inflammation and a dysregulation of the innate immune response. The second term is *hyaluronanosis* to describe the breed-specific skin phenotype that earlier was termed (cuteanous) mucinosis. We propose *hyaluronanosis* to be more accurate considering the knowledge of increased HA in Shar-Pei achieved by us and other research groups (86, 90, 91). The third new term is *vesicular hyaluronanosis* to be used in order to describe the specific dermatological changes observed in Shar-Pei. These changes originates in islets or "bubbles" of HA on the skin surface that easily ruptures and cause inflammation and secondary infections. In paper 2, this condition is expressed simply as dermatitis in the phenotypic outcome.

In paper III, the knowledge regarding the role of HA in canine AID was translated into human disease. The correlation between increased HA and some inflammatory diseases has been described earlier but here we suggest the signal to predominantly constitute the pro-inflammatory molecular size of HA. All correlations detected between clinical signs and high HA (in AOSD and sJIA) were detected exclusively by using the LMW-HA sensitive assay. Although we cannot prove HA to initiate or fostering the inflammatory response, it is interesting that a potential mediator of inflammation is increased in the circulatory system of human subjects with AID. In order to prove that the circulating HA detected is actually capable of initiating inflammation, further experiments is required such as treating macrophages with serum to determine whether pro-inflammatory cytokines are released. HA levels were also significantly higher in subjects suffering from AID of an unknown etiology compared to those with genetically explained disease. The vast majority of all AID subjects have no molecular diagnosis and the

underlying genetics is completely unknown. Pathological changes in HA correspond well with the picture of what is already known about AID. In Shar-Pei, the genetic cause is located upstream of mutations previously recognized to alter inflammasome function and IL-1 release. All genes involved in HA metabolism are in the process to being sequenced in larger cohorts of various human AID. By using Shar-Pei as a model for human AID, we have hopefully extended the perspective of pathways involved in autoinflammation that might benefit both Shar-Pei dogs and humans suffering from AID.

Populärvetenskaplig sammanfattning

En av de stora utmaningarna inom medicinsk genetik är att finna de gener och genetiska förändringar (mutationer) som ger ökad risk för ärftliga sjukdomar. När kunskap finns om den exakta mutationen och hur den förändrar kroppens funktioner, kan bättre metoder för diagnostik, effektivare behandlingar och mediciner tas fram. När man identifierat den gen (eller de gener) som orsakar en sjukdom så säger man att sjukdomen kartläggs och det görs genom att använda olika molekylärgenetiska metoder och ofta lämpliga sjukdomsmodeller. Domesticerade djur har visat sig vara särskilt lämpade för att finna samband mellan det vi ser (fenotyp), t.ex. en sjukdom, och den genetiska bakgrunden (genotyp) för densamma. Genom att favorisera vissa egenskaper hos domesticerade djur (t.ex. mjölkproduktion hos kor eller pälsfärg hos en hund) så har vi adderat en artificiell selektion då vi människor väljer ut individer för avel. Detta leder till att mutationer som påverkar fenotypiska egenskaper anrikats hos domesticerade djur samtidigt som den genetiska variationen minskat eftersom endast ett fåtal individer bidrar till genpoolen i nästa generation. Kombinationen av en hög frekvens av ”synliga” mutationer och en låg genetisk variation inom raser av domesticerade djur, gör dem till utmärkta genetiska modeller för att förstå sambandet mellan en fenotyp och de bakomliggande genetiska faktorerna. I genetiska studier är detta mycket fördelaktiga eftersom genetisk variation utgör ett ”bakgrundsbrus” som gör det svårare att hitta biologiskt viktiga signaler.

Hundens roll i jakten på sjukdomsgener

Tamhunden är det domesticerade däggdjur som står för mest fenotypisk variation. Fler än 400 olika hundraser representerar idag den enorma diversitet av egenskaper vi ser hos hundar, både gällande utseende och beteende. Ofta har man dragit fördel av, och avlat vidare på, en ny genetisk variant som uppstått spontant (en mutation) som korta ben hos tax eller kort skalle hos boxer. Med tiden har dessa egenskaper fixerats och blivit själva varumärket för en ras. Begreppet hundras är relativt nytt då de allra flesta har bildats de senaste 100-200 åren. Innan dess fanns olika typer av hundar men det var först när stamböcker och rasstandarder introducerades som aveln blev strikt begränsad till individer inom samma ras. Oftast har en ny ras grundlagts av ett fåtal individer som representerat en egenskap som vi människor velat

bevara. Detta har lett till en mycket begränsad ursprunglig genpool och i kombination med strikta avelsregler, som satts upp av rasklubbar för att bevara de rastypiska egenskaperna, har det resulterat i att alla individer inom en ras genetiskt (och fenotypiskt) är lika varandra.

Hundar drabbas liksom vi av olika typer av cancer, hjärt- och kärlsjukdomar, allergier, autoimmuna sjukdomar och neurologiska sjukdomar. Dock i högre frekvens än oss och olika sjukdomar är koncentrerade till en eller flera raser. Sjukdomsframkallande mutationer försvinner oftast ur en population p.g.a. naturlig selektion. Hos hundar stämmer inte alltid detta då vissa sjukdomsalstrande mutationer har ansamlats hos vissa hundraser. Sammantaget gör detta hunden till en utomordentlig genetisk modell för sjukdomar hos människa. Kunskapen om vilka genetiska varianter som orsakar sjukdom hos hund hjälper inte bara oss människor utan kan förbättra också hundars hälsa genom. Genom att utveckla genetiska test kan hunduppfödare vägledas i avelsarbetet för att undvika anlagsbärare och minska sjukdomsfrekvensen i den drabbade rasen.

Ett överbeskyddande immunförsvar

I de forskningsstudier som presenteras i den här avhandlingen står den kinesiska Shar-Pei hunden i fokus. Denna ras är mest känd för sin unikt rynkiga hud som är just den egenskap som avlats på och har således blivit rastypisk. Unikt också för Shar-Pei rasen är att många hundar drabbas av oförklarliga attacker av inflammation och feber. Liknade symtom på ”spontan” inflammation förekommer hos människa och kallas för autoinflammatoriska sjukdomar. Hos både människa och hund uppträder korta attacker av feber och symptom på inflammation av leder (artriter), hudutslag och muskelvärk.

Immunförsvaret har i uppgift att skydda oss mot infektion av mikroorganismer (som parasiter, bakterier och virus) och vår första försvarslinje utgörs av det så kallade medfödda immunförsvaret. Denna del av alla komplexa försvarsmekanismer, utgörs av celler som ständigt patrullerar vår kropp för att upptäcka tecken på invasion. När ett sådant tecken uppfattas så försöker cellerna först elimineras hotet direkt. Om det inte lyckas sätts fler processer igång såsom inflammation, feber och aktivering av fler immunologiska celler som kan hjälpa till i bekämpningen. Det medfödda immunförvaret har också hushållningsuppgifter i kroppen som att reparera vävnadsskada och städa bort döda celler.

Hos en person med en autoinflammatorisk sjukdom fungerar inte denna livsviktiga mekanism tillräckligt precist och immunförsvaret överreagerar på stimuli som inte utgör ett egentligt hot. En allvarlig konsekvens av autoinflammation är ett sjukdomstillstånd som kallas amyloidos, vilket är ett resultat av att immunmolekyler ansamlats i kroppens vävnader och orsakar organsvikt. De genetiska mekanismer som ligger bakom autoinflammation hos

människa är okända i en majoritet av alla sjukdomsfall. Syftet med denna avhandling var att utnyttja ett spontant förekommande sjukdomstillstånd hos Shar-Pei hundar som sjukdomsmodell för att identifiera nya genetiska faktorer som resulterar i spontan inflammation också hos människor.

Nya forskningsresultat

I den första studien som presenteras i avhandlingen identifierades den mutation som orsakar autoinflammation hos Shar-Pei hundar. Genen som förändrats producerar en molekyl som kallas hyaluronsyra och mutationen är starkt kopplad till att abnorma mängder av hyaluronsyra bildas hos Shar-Pei. Mutationen som hittades förklarar inte bara en egenskap i rasen, utan två - både autoinflammation och den rastypiska rynkigheten.

Hyaluronsyra har många funktioner i kroppen däribland som ett naturligt ”utfyllnadsmaterial” i huden, vilket förklarar kopplingen till rynkigheten hos Shar-Pei hundar. Men hyaluronsyra har också en roll i det medfödda immunförsvaret. När molekylen sönderdelas i mindre delar så påminner den immunförsvaret om en vävnadsskada och en inflammationsrespons aktiveras för att åtgärda skadan. Hos en Shar-Pei hund som har onormalt stora mänger hyaluronsyra skickas denna signal om vävnadsskada till immunförvarets celler utan att en egentlig skada har uppstått. Inflammationen sägs uppstå ”spontant” och benämns därför som autoinflammation. Genom att avla på den rynkiga huden hos Shar-Pei så har oavsiktligt även risken för autoinflammation anrikats i rasen.

I avhandlingens andra studie kunde fler symtom på inflammation med samma genetiska ursprung kopplas till sjukdomsbilden hos Shar-Pei och en ny terminologi för sjukdomen introducerades; SPAID =*Shar-Pei Autoinflammatory Disease.* Till diagnosen SPAID hör nu attacker av feber, inflammation av leder (artrit), öron (otit) och hud (dermatit) samt ökad risk för att drabbas av amyloidos och organsvikt. I en separat genetisk studie kunde vi finna ytterligare gener som kan vara inblandade i risken att även drabbas av amyloidos. Eftersom risken för amyloidos är något oförutsägbar och inte verkar kopplad till antalet inflammationsattacker, så är det troligt att ytterligare gener styr just detta sjukdomsförlopp.

I den tredje och sista studien översattes den nya kunskapen från forskningen om SPAID till människa. Koncentrationen av hyaluronsyra mättes i serum från människor med autoinflammatoriska sjukdomar för att få ett mått på eventuella förändringar i metabolismen av hyaluronsyra. Genom att jämföra de patienter vars sjukdom redan blivit genetiskt förklarad med sjukdomsfall där den genetiska faktorn var okänd, kunde en signifikant skillnad upptäckas mellan grupperna. Patienter vars sjukdom var genetiskt ”okänd” hade en

högre koncentration av hyaluronsyra än de som redan är genetiskt ”känd”. Detta indikerar att förändringar i metabolismen av hyaluronsyra kan ligga bakom några av de oförklarade fallen av autoinflammation hos människa. Högre halter av hyaluronsyra uppmättes också i patienter med ett akut sjukdomstillstånd och var korrelerat med hur allvarlig sjukdomen var.

Sammanfattningsvis har forskningen rörande Shar-Pei hundarnas rynkiga hud och dess immunmedierade symptom, identifierat ytterligare an gen och en biologisk signalväg för autoinflammation. Den genetiska förändringen ligger bakom såväl den rastypiska rynkiga huden och autoinflammation. Därför att selektion för rynkig hud oavsiktligt anrikat även risken att drabbas av autoinflammation, vilket förklarar varför sjukdomen är så vanlig i rasen. Initiala studier indikerar att samma signalväg som innefattar metabolismen av hyaluronsyra mycket väl kan vara relevant för att klargöra hittills oförklarliga fall av autoinflammation som förekommer hos människor med liknande sjukdomstillstånd.

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