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Abstract

Symmetrical lupoid onychodystrophy (SLO) is a disease that affects dogs at ages between 3 to 8 years. The dogs start to slough their claws and in a few weeks every claw on each paw is involved. The histological pattern of affected claws resembles other autoimmune diseases. The etiology of SLO is unknown but the symmetrical paw involvement and the histopathological pattern indicates that SLO might be an autoimmune disease.

Alleles and haplotypes of the canine Major Histocompatibility Complex (MHC) class II has been associated with different kinds of autoimmune diseases. MHC class II is in dog referred to the as dog leukocyte antigen (DLA) system: DLA-DRA, DLA-DRB1, DLA-DQA1 and DLA-DQB1. These genes are in high linkage disequilibrium and the DLA-DRB1, DLA-DQA1 and DLA-DQB1 genes are highly polymorphic.

The aim with this study is to evaluate if there is a genetic association between the development of SLO or other claw problems and certain DLA alleles, haplotypes or genotypes.

In Sweden studies have shown that SLO occur at higher frequency in giant schnauzer, schnauzer, bearded collie and Gordon setter. In this study, 110 giant schnauzers and 10 bearded collies were examined. Each dog in this study was categorized in a group according to its claw problem and the sequence of polymorphic genes DLA-DRB1, DLA-DQA1 and DLA-DQB1 were determined. Statistical calculations were made to determine whether some allele, haplotype or genotype give rise to an increased risk for developing SLO or other claw problems.

The results show that in giant schnauzer, haplotype DRB1*01301/DQA1*00301/DQB1*00501 and allele DRB1*01301 are protective against the development of claw problems, with a p-value of 0,001. The haplotype DRB1*00101/DQA1*00101/DQB1*00201 may give rise to an increased risk for developing different kinds of claw problems (frequency of control group 15% versus the case group 26, 9%), although this result is not statistically significant. Statistical analyses indicate that haplotype DRB1*01801/DQA1*00101/DQB1*00201 and allele DQB1*00201 may give rise to a higher risk of developing SLO in bearded collie, however too few individuals were analysed to give statistically significant results.

Table of contents

1. Introduction

The domesticated purebred dog arose from two different canine population bottlenecks. The first bottleneck was created approximately 7.000- 50.000 generations ago when wolfs were domesticated; the second bottleneck is associated with the creation of different breeds, which started approximately 50-100 generations ago. The breeding programmes of the purebred dog, which purpose is to select for different genetic variants, in order to obtain certain morphology, physiology or behaviour, have lead to a reduced polymorphism (i.e. genetic variation) (Lindblad-Toh et al., 2005). Several purebred dog breeds have shown a tendency to develop certain autoimmune diseases. An explanation to this can be the reduced genetic variation caused by the breeding programmes. Studies suggest that there are similar genetic factors between human and dog and also environmental factors, which humans and dogs share, that can contribute to the development of autoimmune diseases (Kennedy et al., 2007).

The membrane bound glycoprotein encoded by Major Histocompatibility Complex (MHC), are located in three tightly linked regions of genes called class I, II and III (Kennedy et al., 2006). MHC class II has been shown to be involved in the development of autoimmune diseases in both human and dogs (Brand et al., 2005) (Kennedy et al. 2006 **68**) (Kennedy et al. 2006 **67**) (Kennedy et al. 2007 **69**). The MHC in dog is referred to the dog leukocyte antigen (DLA) system. MHC class I and II molecules are involved in presenting self and non self antigens to T killer and T helper lymphocytes of the immune system. The genes of MHC class I and II are highly polymorphic, i.e. many different alleles at high frequency in the population, which differ at multiple positions corresponding to the domain encoding the antigen binding pocket. DLA class II in dog include three highly polymorphic genes which are known as DLA-DQB1, DLA-DQA1 and DLA-DRB1 and one monomorphic gene known as DLA-DRA (Kennedy et al., 2006) (Debenham et al., 2005). There are at present 67 DLA-DRB1, 2 DLA-DQA1 and 54 DLA-DQB1 alleles (Kennedy et al., 2006).

In this study we will investigate dogs with different kinds of claw diseases. Some of the dogs have been diagnosed with the disease symmetrical lupoid onychodystrophy (SLO). SLO is a claw disease where otherwise healthy dogs start to slough their claws. It begins with one or two affected claws on a single paw and after a few weeks every claw on each paw is involved (Scott, et al. 1995). The etiology of SLO is unknown; histopathology of the affected claw resembles the histopathology of a number of autoimmune diseases, such as systemic and discoid lupus erythematosus, pemphigus and bullous pemphigoid (Scott, 1982).

The aim with this study is to determine whether certain DLA class II haplotypes, genotypes or alleles can be associated with the development of SLO or other claw problems. Blood samples from giant schnauzer and bearded collies have been examined as epidemiological studies have shown that these breeds are more predisposed to develop SLO (Ferm Katarina, http://hunddna.slu.se/artikelserie/BeardedCollie.pdf). In the future, the information concerning risk or protective DLA class II haplotypes, genotypes or alleles may be used to breed healthier dogs.

2. Literature review

2.1 Immunology

The immune system is built up by both a nonspecific and a specific component. The nonspecific component, the innate immunity, includes different kinds of disease resistance mechanisms, which are

not specific for a certain pathogen. In contrast, the specific components of the immune system, the adaptive immunity, show high degree of specificity to certain antigen/s.

The adaptive immunity can be divided into a humoral immune response and a cellmediated response. In the cellmediated response a T cell binds to peptide antigens presented by a MHC class I molecule expressed on an altered self cell, typically a virus infected cell, and generate an active cytotoxic T lymphocyte response witch will cause cell lysis of the altered virally infected self cell. In the humoral immune response professional antigen-presenting cells such as dendritic cells, macrophages or activated B-cells interact with a foreign antigen, which will activate the B-cell. The antigen will be processed and presented on the B-cells cell membrane combined with a MHC II-molecule. A T-helper cell, that is specific for the antigen, will bind with its T-cell receptor (TCR) to the complex. This binding will activate the T helper cell, which will start to secrete cytokines. The cytokines will stimulate cell division and differentiation of the B-cell to an antigen producing plasma cell and memory cell. The antibodies will bind to the antigen and neutralize it or facilitate its elimination.

The adaptive immune systems function is to eliminate foreign antigens and it is capable to discriminate between self and nonself. The T lymphocytes mature in thymus, when they start to express their antigen binding receptors (TCR) they are either positively or negatively selected. The positive selection takes place in the cortical region of the thymus and permits only survival of those T-cells that recognize self MHC molecules. The T-cell population that survived the positive selection will undergo the negative selection, where the T cells, which interact too strongly with the MHC molecules, or with the MHC molecule together with a self antigen, will be eliminated. Due to this selection process only T cells that recognize the MHC molecules and have an acceptable affinity for the MHC molecule and the MHC molecule plus self antigen, are allowed to mature. Like most biological processes this MHC restriction is incomplete and not all self reactive lymphocytes are deleted during T cells and B cells maturation, which can lead to autoimmunity.

2.1.1 Autoimmunity

Autoimmunity is the failure of the immune system to recognize what is self and what is non self of the organism and the immune system starts to attack the organisms own cells and/or tissue. Thus, the tolerance process described above may have been incomplete or alternatively, established central tolerance may have been broken as a result of a productive infection. The exact mechanisms responsible for developing an autoimmune disease are still unclear. However, the central importance for MHC molecules is well established.

Autoimmune diseases can be divided into two general categories, the organspecific and the systemic. The organspecific is characterized by immune responses directed against antigens specific to a single organ or gland in the organism. This will either directly damage the cells of the organ or indirect by the humoral or cellmediated immune response. Antibodies produced by the humoral immune response may also over stimulate or under stimulate the normal function of the organ. A systemic disease is when the immune response is directed toward a variety of organs in the organism. This disease reflects a general defect of the immune system, which results in a widespread tissue damage (Goldsby R. A, Kindt T. J and Osborne B. A, 2001).

2.2 The Major Histocompatibility Complex (MHC)

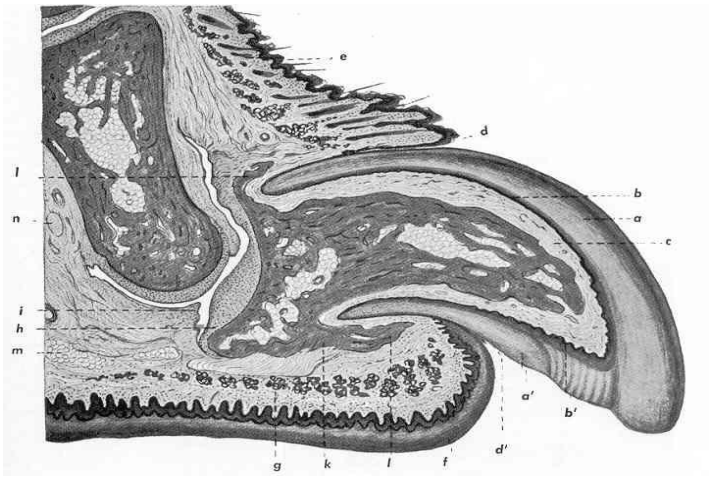
MHC molecules are expressed by three tightly linked clusters of genes, called class I, II and III. The MHC contains several class I and class II genes that encode membrane bound glycoproteins. MHC class I

molecules are expressed on virtually all nucleated cells, with the exception of neurons and certain gonad cells and germline cells. In contrast, MHC class II molecules are expressed by professional antigen presenting cells (B-cells, macrophages, dendritic cells and activated T-cells) in the immune system. The genes in the MHC in dogs are called the dog leukocyte antigen (DLA). The function of MHC class I and II is to present self and non self antigens to the immune system. Three genes within the DLA class II are highly polymorphic i.e. presence of multiple alleles at a given locus and are known as DLA-DRB1, DLA-DQA1 and DLA- DQB1 and one gene known as DLA-DRA, appears to be monomorphic (Kennedy et al., 2006) (Debenham et al., 2005). There are at present 67 DLA-DRB1, 2 DLA-DQA1 and 54 DLA-DQB1 alleles (Kennedy et al., 2006).

Different haplotypes and alleles of DQA1, DQB1 and DRB1 genes have been shown to either protect or predispose for many different autoimmune diseases (Brand et al., 2005). In dog, several DLA class II alleles have been associated with different autoimmune diseases such as hypothyroidism (Wilbe et al. submitted) (Kennedy et al. 2006 **67**), immune-mediated anemia (Kennedy et al. 2006 **68**), diabetes mellitus (Kennedy et al. 2007 69). In humans, the MHC is referred to the human leukocyte antigen (HLA) system. The HLA class II are highly polymorphic and different class II molecules have been shown to be major risk factors in the development of several autoimmune diseases (Brand et al., 2005). Studies have shown that a conserved amino acid sequence in the hyper variable region present in some DRB1 alleles II in both human and dog is involved in higher susceptibility to develop rheumatoid arthritis (Ollier et al., 2001), association has also been found between canine and human diabetes mellitus (DQA1 Arg 52 in human and DQA1 Arg 55 canine). This indicates that there is a shared pathology and genetic susceptibility to develop certain autoimmune diseases in human and dogs (Kennedy et al. 2007).

2.3 The Claw

The claw is a specialized structure in the dog. Its function is to work as a prehensile and locomotion organ but also as a protection of underlying tissue. The claw is compressed laterally and can be divided into the lateral and medial walls, the sole (ventral) and the dorsal ridge of the coronary band. The distal phalanx has a dorsal process called the ungual crest; dermis is continuous with this dorsal process and continues distally as the periosteum of the distal phalanx. Dermis has many fine papillae that extend into the lamellae of the epidermis. The epidermis of the claw is supported by the dermis. The highest growth activity of the epidermal basal layer is found in the coronary and dorsal ridge areas. The epidermis of the claw sole has distinct granular and clear layers, compared to the epidermis of the rest of the claw, which is composed of a thick horny layer of flat cornified epidermal cells, fused into a horny plate with an absent stratum granulosum.



Picture 1. Midsagital section of claw: a: epidermis, c: dermis, l: ungual crest (Scott, Miller 1992)

In human medicine, many systemic diseases have been associated with nail deformations. Examples of such diseases are psoriasis, epidermolysis bullosa, alopecia areata etc. Diseases associated with the claw in dogs are trauma, demodicosis, infections, pemphigus, viral infection, systemic lupus erythematosus, pemphigoid, vascular insufficiency, seborrheic dermatitis, acromegaly and senility.

2.3.1 Disorders of the claw

Paronychia: is an inflammation in the soft tissue around the claw. The inflammation is usually caused by bacteria (i.e. bacterial paronychia), but it is also caused by dermatophytes or yeast (Muller et al., 1989). When only a single claw is affected, it is likely due to a trauma (e.g. bite wound, crush injury, torn claw) (Scott, Miller 1992), but if multiple claws on multiple paws are involved it is likely due to an underlying systemic disease such as pemphigus, bullous pemphigoid, systemic lupus erythematosus, lymphosarcoma, generalized demodicosis or diabetes mellitus.

Onychomycosis: fungal infection usually caused by *Trichophyton mentagrophytes.*

Onychorrhexis (brittle nails): when the nail breaks from the free edge, this occurs spontaneously in some dogs. When only a single claw is involved it is usually due to a trauma, but when several nails are involved on more than one paw the cause is unknown.

Onychomadesis: the hard keratin layer separate, this causes loss (slough) of the nail. This can be caused by trauma, infection, vascular disorders or autoimmune disorders such as pemphigus and systemic lupus erythematosus.

Miscellaneous: when the nails grow very rapidly and are abnormally thick, as in canine acromegaly and zinc responsive/deficient disorders (Muller et al., 1989).

2.3.2 Symmetrical lupoid onychodystrophy (SLO)

Symmetrical lupoid onychodystrophy is a disease that affects every claw on all paws in canines (Scott et al., 1995). Symmetrical paw involvement (i.e. all four paws) indicates a systemic disorder such as immune-mediated, endocrine, nutrial and/or immunosuppressive disease (Scott & Miller, 1992). The disease typically appears at an age of 3-8 years and male dogs have shown to be predisposed (Bohnhorst J. Ö et al., 2001). SLO have been reported to occur in a higher prevalence in German shepherds (Scott et al., 1995). In Sweden, studies have shown that SLO occur in a higher frequency in giant schnauzer, schnauzer, bearded collie and Gordon setter (Ferm Katarina, http://hunddna.slu.se/artikelserie/BeardedCollie.pdf).

The disease is characterized by separation at the claw bed and sloughing of the claws (i.e. onychomadesis), sometimes a brown line at the claw bed is noticed, and this is likely due to hemorrhage prior to sloughing. Usually the owners discover one affected claw on one or two paws and within 2-9 weeks every claw on all four paws are affected. Lameness and pain is seen in half of the cases, whereas the other half does not seem to be aware of their disease. The regrowth of the affected claws are characterized by short, soft, dry and very fragile misshapen (i.e. onychodystrophy) claws. Dogs with symmetrical lupoid onychodystrophy are otherwise in good health and do not show any other signs of other diseases (Scott et al., 1995).

Surgical amputation of the third phalanx and histological examination of the claw bed is required to confirm diagnosis (Scott & Miller, 1992). Histopathology of affected claws present infiltrate of mononuclear cells, apoptosis of the epidermal basal cells and hydropic degeneration of the epidermal basal cells. The histological changes in dermis are characterized by edema, hemorrhage and fibrosis. The histopathological lesions are usually seen along the coronary band on the dorsal aspect of the claw. Typically the inflammatory infiltrate forms a parallel band to the basement membrane, this is called lichenoid pattern. Pigmentary incontinence is also often observed in the dermis (Scott et al., 1995). The inflammatory infiltrate is predominantly represented by B cells and T cells and macrophages are only present in a small number (Mueller et al., 2004). This type of inflammatory response where hydrophic degeneration and a lichenoid pattern is seen is usually associated with autoimmune disorders such as systemic and discoid lupus erythematosus, pemphigus and bullous pemphigoid (Scott, 1982). Although the histological changes resemble those of systemic lupus erythematosus, no other signs of systemic lupus erythematosus is seen as hemograms, urine analysis antinuclear antibody titers (ANA) appears to be normal in dogs affected by SLO compared to dogs affected by systemic lupus erythematosus (Mueller et al., 2000).

During the last decade there has been an increased incidence of Gordon setters affected by SLO, in Norway. Pedigrees from 56 of these cases could be traced back to a common ancestor. During the same period, Gordon setters at an age of 1-2 years, that abruptly started to shed their black hair without normal regrowth, was observed. A study made in Norway suggests that SLO and black hair follicular dysplacia in Gordon setters may be pathogenetically related. The cutaneous affections is similar to those described in the human disease alopecia areata, which is an autoimmune disease, where 10-44% of the patients have nail involvements such as longitudinal ridging and thickening to friability and shedding (Bohnhorst J. Ö et al., 2001).

The etiology of this condition is unknown. Diet and prior drug exposure can't be excluded as disease causing factors (Scott et al., 1995). Treatments with omega-3 and omega-6 fatty acids have shown to have a positive effect for clinical improvement (Bergvall, 1997), yet treatment with trental (pentoxyphyllin) and tetracycline or doxyckline in combination with niacinamid have also been successful (Öhlen, Bergvall, 1999) (Mueller, 2003). If the affected dog doesn't respond to the treatment, the next choice of treatment would be immunosuppressive drugs such as glucocorticoids (Mueller, 2003). In a long term observation study, where one crossbred pointer dog affected of SLO were treated with mechanical treatment such as cutting and filing of the nails , the trimming appeared to minimize the chances of further traumatic damage to the already affected nails (Verde & Basurco 2000).

3. Material and method

3.1 Study material

Blood samples from 110 giant schnauzers and 10 bearded collies with different kinds of claw problems were collected. Of these 120 samples, 31 giant schnauzer samples and 10 bearded collies samples were analysed in this study, also included in this study were 79 giant schnauzers samples that were already analysed by Maria Wilbe in a previous study (Wilbe et al., submitted).

3.2 Diagnostic criteria

Dogs within this study were classified according to their claw problems. The classifications were based on a questionnaire that each owner had completed, except from 14 giant schnauzer owners that could not

be reached. These dogs had different claw problems and were grouped as unclassified claw problems, UCP. The classification of the claw problems were made according to following criteria:
E: claw fracture one claw one time
EK: claw fracture less or no more than two times, with deviant claw quality, eventually secondary caused claw infections
ÅK: returning claw fractures three times or more, with deviant claw quality, eventually secondary caused claw infections
ÅI: returning primary claw infections on many claws, with no visible claw injury
SLO: symmetrical lupoid onychodystrophy
PEC or other tumor: squamous cells carcinoma or other tumor
UCP: unclassified claw problems

3.3 Extraction of DNA

Genomic DNA was extracted from 200µL EDTA blood using QIAGEN DNA mini kit (QIAmp DNA Mini and Blood Mini Handbook 11/2007). The concentrations of the DNA were measured with nano-drop spectrophotometer.

3.4 PCR amplification

The genomic DNA was diluted with distilled water to a final concentration between 15-25ng/µL. For each dog the DNA sequences containing DLA-DRB1, DLA-DQA1 and DLA-DQB1 were amplified. Different primers were used to obtain amplification specific DLA fragments, see Table 1. T7 tailed primers were used to label the PCR products. 2µL DNA were mixed with a 18µL reaction mix containing: distilled water, 1 x PCR buffer, $MgCl_2$ at a final concentration of 1,5mM, forward primer at a final concentration of 10µM, reverse primer at a final concentration of 10µM, dNTP at a final concentration of 20mM and ampliTaq at a final concentration of 5U/µL. A touchdown PCR protocol was used for all amplifications to avoid amplification of nonspecific sequences. The PCR had a start temperature of 95° for 15 min, followed by 14 cycles of 30s at 95°C , 1 min annealing at 62°C (DRB1), 54°C (DQA1), 73°C (DQB1) reduced by 0,5°C for each cycle and 1 min elongation at 72°C. Thereafter, 20 cycles at 95°C for 30s followed by 1 min at 55°C (DRB1), 47°C (DQA1), 60°C (DQB1), followed by 72°C for 1 min and at last 10 min at 72°C. 5µL of the PCR product were run on a 2% agarose gel, to determine whether the PCR reaction was successful.

Table 1. Sequence for T7 and primers used for amplification of DLA- DRB1, DQA1 and DQB1.

Primer	Primer sequence	Size
DLA-DRB1 F	CCGTCCCCACAGCACATTC	303bp
DLA-DRB1 R	**T7**-TGTGTCACACACCTCAGCACCA	
DLA-DQA1 F	TAAGGTTCTTTTCTCCCTCT	345bp
DLA-DQA1 R	**T7-**GGACAGATTCAGTGAAGAGA	
DLA-DQB1 F	**T7**-CTCACTGGCCCGGCTGTCTC	300bp
DLA-DQB1 R	CACCTCGCCGCTGCAACGTG	
T7	TAATACGACTCACTATAGGG	

3.5 Purifying the PCR product and nucleotide sequence analysis

The products from the PCR reactions were purified. For this, an ExoSAP reaction mix was used. 2 µL of the ExoSAP reaction mix were put into each wells on the PCR plate and the incubation time for ExoSAP was: 37°C for 1h and 85°C for 15min.

To dilute the PCR product, 70 µL distilled water was added to each well. 3 µL of the PCR product were transferred to a new PCR plate and 3 µL T7 at a concentration of 5M were added to each well. As all PCR products were labelled with T7, which was used as a sequence primer. 12 µL distilled H_2O were added to each well for further dilution. The PCR plates were sent to Uppsala Genome Center for sequencing.

3.6 Data analysis

Match Tools and Match Tools Navigator (Applied Biosystems), were used to analyse the sequence data. The sequences were first analyzed in Match Tools Navigator where the sequences were compared to a consensus sequence. Each polymorphic site was manually corrected. After the sequences were analyzed in Match Tools Navigator the sequences were compared in Match Tool to an already existing library consisting of different known alleles. The program then matched the sequence to the closest matching allele.

3.7 Statistical analysis

All samples were classified into controls or cases. The cases and controls were categorized into groups according to the presence of a certain haplotype, genotype or DQB1/DQA1/DRB1 allele. The total numbers of cases versus controls in every group were calculated. The frequencies were calculated for each group. The cases were also categorized according to classification and presence of a certain haplotype. The total number in each group was calculated and frequencies were calculated (see Table 2-13).

Statistical analysis was performed using a 2x2 contingency table. Calculations and results are presented in a table when the presence or absence of certain haplotype, genotype or DLA allele differentiated more than or 10 % between the case group and the control group. The number of cases where a specific DLA haplotype, genotype or allele were present and absent were calculated and compared with the number of controls with the same DLA haplotype, genotype or allele. Odds ratio, Relative risk and p-values were calculated. Statistical analyses were calculated using Vassar Stats (http://faculty.vassar.edu/lowry/VassarStats.html)

Relative risk was used to determine the risk for developing the disease if carrying a certain haplotype, genotype or DLA allele. The relative risk describe how much greater risk the exposed group have for developing the disease compared to the non exposed group. The relative risk can be calculated from following formula:
RR = [a/ (a+b)] / [c/(c+d)]
A relative risk > 1 is equal to an increased risk of developing disease if a certain haplotype, genotype or DLA allele is present and a relative risk < 1 is equal to a decreased risk for developing the disease if a certain haplotype, genotype, DLA allele is present, also called protective factor.

Odds ratio is calculated from odds. The odds of an outcome can be described as the probability that an outcome does occur divided with the probability that the outcome does not occur. The odds of an outcome in the exposed group and the odds of an outcome in the unexposed group can be described as:
$odds_{exp}$ = [a/ (a+b)] / [b/ (a+b)] = a/b

odds $_{unexp}$ = [c/(c+d)] / [d/(c+d)] = c/d

The odds ratio defines the ratio between the odds, and can be calculated:
OR= (a/b)/ (c/d) = ad/bc
(http://radiology.rsna.org/content/230/1/12)

The definition of p-value is the probability to obtain a result that differ as much as or the same as the result we would expect if the null hypothesis was true. P-value was used to decide if the association was statistical significant i.e. did not arose by chance. At a p-value < 0, 05 is the null hypothesis is rejected and the result show statistical significance.

X^2- test were to see that the result were statistically significant and did not arose by chance. For the X^2-test, 95% confidence interval and Yates values, which is corrected for continuity, were used.

4. Results

Classification, haplotype and genotype for each dog within this study are shown in supplementary Table 1.

4.1 Data analysis

4.1.1 Haplotype frequency

In this study, ten different haplotypes were found in the giant schnauzer breed. Three haplotypes (DRB1*00101/DQA1*00101/DQB1*00201, DRB1*00601/DQA1*00401/DQB1*01303 and (DRB1*01301/DQA1*00301/DQB1*00501) were common, with a frequency of > 15%, whereas seven haplotypes were less common with a frequency of <15%. The frequency of haplotype DRB1*00101/DQA1*00101/DQB1*00201 was higher in the case group compared to the control group (control group 15% versus the case group 26, 9%). The frequency of haplotype DRB1*01301/DQA1*00101/DQB1*00201 (case group 5% versus control group 15%) and haplotype DRB1*01301/DQA1*00301/DQB1*00501 (case group 13, 1% versus control group 25%) were higher in the control group compared to the case group (see Table 2).

Five different haplotypes were found in bearded collie. Two haplotypes were more common (DRB1*01801/DQA1*00101/DQB1*00802 and DRB1*01801/DQA1*00101/DQB1*00201) with a frequency of > 15%, whereas three haplotypes were less common with a frequency of <15%. Two haplotypes occurred in a higher frequency in the case group compared to the control group, haplotype DRB1*01801/DQA1*00101/DQB1*00802 (case group 40% versus control group 20%) and haplotype DRB1*1801/DQA1*00101/DQB1*00201 (case group 60% versus control group 40%) (see Table 3).

The haplotype frequencies according to classification are shown in Table 10. Group X consists of group EK, ÅK, ÅI and SLO. Haplotype DRB1*00101/DQA1*00101/DQB1*00201 is more frequent occurring in dogs with SLO (50%) and dogs classified in the X group (28%) compared to the control group (15%). Haplotype DRB1*01201/DQA1*00101/DQB1*00201 is more common in dogs classified in the ÅI group (60%) compared to the control group (11, 7%). Haplotype DRB1*02301/DQA1*00301/DQB1*00501 is more frequent occurring in the PEC/tumor and ÅK group, both has a frequency of 16, 7% compared to the control group with a frequency of 5%.

Table 2. Haplotype frequency for giant schnauzer in the total population and classified as controls or cases.

Haplotype DRB1/DQA1/DQB1	Case and control (%) (220)	Case (%) (160)	Control (%) (60)
00101/00101/00201	23,6% (52)	**26,9%** (43)	**15%** (9)
00601/00401/01303	20, 9% (46)	21,3% (34)	20% (12)
00901/00101/008011	1,82 % (4)	0,63% (1)	5% (3)
01201/00101/00201	13,6% (30)	14,4% (23)	11,7% (7)
01301/00101/00201	7,73% (17)	**5,0 %** (8)	**15%** (9)
01301/00301/00501	16,4% (36)	**13,1%** (21)	**25%** (15)
02301/00301/00501	9,55% (21)	11,3% (18)	5% (3)
01501/00601/00301	1,82% (4)	1,88% (3)	1,67% (1)
01501/00601/02201	4,09% (9)	5,0% (8)	1,67% (1)
02001/00401/01303	0,45% (1)	0,63% (1)	0

Table 3. Haplotype frequency for bearded collies in the total population and classified as controls or cases.

Haplotype DRB1/DQA1/DQB1	Case and controls (%) (20)	Case (%) (10)	Controls (%) (10)
01801/00101/00802	30% (6)	**40%** (4)	**20%** (2)
01801/00101/00201	50% (10)	**60%** (6)	**40%** (4)
01501/00601/00301	5% (1)	0	10% (1)
00201/00901/00101	5% (1)	0	10% (1)
01501/00601/02301	10% (2)	0	20% (2)

Table 4. Haplotype frequencies in the total population and according to classification in giant schnauzer

Haplotype DRB1/DQA1/DQB1	Case & controls % (220)	Control % (60)	E % (8)	EK % (44)	ÅK % (42)	ÅI % (10)	SLO% (14)	PEC/tumour % (24)	UCP% (28)	X % (100)
00101/00101/00201	23,6% (52)	**15%** (9)	25% (2)	31,8% (14)	19% (8)	10% (1)	**50%** (7)	20,8% (5)	28,6% (8)	**28%** (28)
00601/00401/01303	20,9% (46)	20% (12)	0,13% (1)	29,5% (13)	14.3% (6)	10% (1)	14,3% (2)	37,5% (9)	25% (7)	17% (17)

00901/00101/08011	1,82 % (4)	5% (3)	0,13% (1)	0	0	0	0	0	0	0
01201/00101/00201	13,6% (30)	**11,7%** (7)	0,13% (1)	13,6% (6)	14,3% (6)	**60%** (6)	7,14% (1)	12,5% (3)	3,57% (1)	18% (18)
01301/00101/00201	7,73% (17)	15% (9)	0	2,27% (1)	4,76% (2)	10% (1)	7,14% (1)	8,33% (2)	3,57% (1)	5% (5)
01301/00301/00501	16,4% (36)	25% (15)	0	13,6% (6)	17,5% (7)	0	14,3% (2)	0	21,4% (6)	15% (15)
02301/00301/00501	9,54% (21)	**5%** (3)	0,13% (1)	4,55% (2)	**16,7%** (7)	10% (1)	0	**16,7%** (4)	17,9% (5)	8% (8)
01501/00601/00301	1,82% (4)	1,67% (1)	0,13% (1)	2,27% (1)	0	0	0	4,17% (1)	0	1% (1)
01501/00601/02201	4,09% (9)	1,67% (1)	0,13% (1)	2,27% (1)	11,9% (5)	0	7,14% (1)	0	0	7% (7)
02001/00401/01303	0,45% (1)	0	0	0	2,38% (1)	0	0	0	0	1% (1)

4.1.2 Allele frequency

In giant schnauzer, eight different DRB1 alleles were found. Three alleles were more common (DRB1*00101, DRB1*00601 and DRB1*01301) with a frequency of > 15%, whereas five were less common with a frequency of <15%. The allele DRB1*00101 occurred in a higher frequency in the case group compared to the control group (control group 15% versus case group 26, 9%). The frequency of allele DRB1*1301 were higher in the control group compared to the case group (control group 40% versus case group 18, 1%) (see Table 5).

Four different DQA1 alleles were found. One allele was less common (DQA1*00601), with a frequency of < 15%, whereas the other three alleles were more common with a frequency of >15%. There was no DQA1 allele that occurred in a higher/lower frequency (difference of more than 10%) in the case group compared to the control group (see Table 6). Six different DQB1 alleles were found. Three alleles were more common (DQB1*00201, DQB1*01303 and DQB1*00501) with a frequency of >15%, whereas three alleles were less common, with a frequency of <15%. There was no DQB1 allele that occurred in a higher/lower frequency (difference of more than 10%) in the case group compared to the control group (see Table 7)

In bearded collie, three DRB1 alleles were found. Allele DRB1*1801 was very common (80%) compared to the other two alleles DRB1*00201 (5%) and DRB1*01501 (15%). Three DQA1 alleles were found. Allele DQA1*00101 was very common (80%) compared to the other two alleles DQA1*00901 (5%) and DQA1*00601 (15%). Both the DRB1*01801 allele and DQA1*00101 allele were present at a frequency of 100% in the case group compared to 60% in the control group (see Table 8 and 9). Five different DQB1 alleles were found, two alleles (DQB1*00201 and DQB1*00802) were more common with a frequency >15%, the other three were less common with a frequency of <15%. Both DQB1 alleles DQB1*00201 and DQB1*00802 were more common among the cases compared to the controls (see Table 10).

Table 5. Frequency of DRB1 alleles for total population, cases and controls in giant schnauzer.

Allele DRB1	Case and control % (220)	Control % (60)	Case % (160)
00101	23,6% (52)	**15%** (9)	**26,9%** (43)
00601	20,9% (46)	20% (12)	21,3% (34)
01301	24,1% (53)	**40%** (24)	**18,1%** (29)
00901	1,82% (4)	5% (3)	0,625% (1)
01201	13,6% (30)	11,7% (7)	14,4% (23)
02301	9,54% (21)	5% (3)	11,3% (18)
01501	5,91% (13)	3,33% (2)	6,88% (11)
02001	0,45% (1)	0	0,625% (1)

Table 6. Frequency of DQA1 alleles for total population, cases and controls in giant schnauzer.

Allele DQA1	Control and case % (220)	Control % (60)	Case % (160)
00101	46,8% (103)	46,7% (28)	46,9% (75)
00401	21,4% (47)	20% (12)	21,9% (35)
00301	25,9% (57)	30% (18)	24,4% (39)
00601	5,91% (13)	3,33% (2)	6,87% (11)

Table 7. Frequency of DQB1 alleles for total population, cases and controls in giant schnauzer.

Allele DQB1	Control and case % (220)	Control % (60)	Case % (160)
00201	45% (99)	41,7% (25)	46,3% (74)
01303	21,4% (47)	20% (12)	21,9% (35)
00501	25,9% (57)	30% (18)	24,4% (39)
08011	1,82% (4)	5% (3)	0,625% (1)
00301	1,82% (4)	1,67% (1)	1,88% (3)

02201	4,09% (9)	1,67% (1)	0

Table 8. Frequency of DRB1 alleles for total population, cases and controls in bearded collie.

Allele DRB1	Control and case % (20)	Control % 10st	Case % (10)
01801	80% (16)	**60%** (6)	**100%** (10)
00201	5% (1)	10% (1)	0
01501	15% (3)	30% (3)	0

Table 9. Frequency of DQA1 alleles for total population, cases and controls in bearded collie.

Allele DQA1	Control and case % (20)	Control % (10)	Case % (10)
00101	80% (16)	**60%** (6)	**100%** (10)
00901	5% (1)	10% (1)	0
00601	15% (3)	30% (3)	0

Table 10. Frequency of DQB1 alleles for total population, cases and controls in bearded collie.

Allele DQB1	Control and case % (20)	Control % (10)	Case % (10)
00201	50% (10)	**40%** (4)	**60%** (6)
00802	30% (6)	**20%** (2)	**40%** (4)
02301	10% (2)	20% (2)	0
00301	5% (1)	10% (1)	0
00101	5% (1)	10% (1)	0

4.1.3 Genotype frequency

In giant schnauzer, thirty genotypes were found. Every genotype occurred in a frequency less than 15% and there was no significant difference between the frequency in the case group and the control group (see supplementary Table 2).

In bearded collie, four different genotypes were found. The genotype DRB1*01801/DQA1*00101/DQB1*00802-DRB2*01801/DQA2*00101/DQB2*00202 occurred in a higher

frequency (80%) in the case group (all classified in the SLO group), compared to the control group (40%) (see supplementary Table 3).

4.2 Statistical analyses

Odds ratio, relative risk and p-values were calculated for those results where the frequency between the case and control group differentiated more than 10%, to see whether those results indicate an increased risk or a protective role for developing claw problems and to determine if results were statistically significant.

In giant schnauzer, statistical analyses of haplotype DRB1*00101/DQA1*00101/DQB1*00201 showed a relative risk of 1,79 an odds ratio of 2,08 and a p-value of 0,095. The allele DRB1*00101 had a relative risk of 1,79 an odds ratio of 2,08 and a p-value of 0,095. Statistical analyses of haplotype DRB1*01301/DQA1*00301/DQB1*00501 showed a relative risk of 0,525 and odds ratio of 0,453 and a p-value of 0,055. The allele DRB1*01301 had a relative risk of 0,453 an odds ratio of 0,332 and a p-value of 0,0014 (Table 11).

Table 11. Statistical analyses of haplotypes and alleles in giant schnauzer were the frequency between the case and control group differentiated more than 10%.

	Case (No)	Total Case (No)	Control (No)	Total Control (No)	RR	OR	p-value
Haplotype DRB1*00101/DQA1*00101/DQB1*00201	43	160	9	60	1,79	2,08	0,095
Haplotype DRB1*01301/DQA1*00301/DQB1*00501	21	160	15	60	0,525	0,453	0,055
Haplotype DRB1*01301/DQA1*00101/DQB1*00201	8	160	9	60	0,333	0,298	N.A
Allele DRB1*00101	43	160	9	60	1,79	2,08	0,095
Allele DRB1*01301	29	160	24	60	0,453	0,332	0,001

Statistical analyses of the X group, which consists of cases classified in the EK, ÅK ÅI and SLO group, showed that the haplotype DRB1*00101/DQA1*00101/DQB1*00201 had a relative risk of 1,87 an odds ratio of 2,20 and a p-value of 0,090 (see Table 12).

Table 12. Statistical analyses of haplotypes in giant schnauzer were the frequency between the classified case and control group differentiated more than 10%.

	Case (No)	Total Case (No)	Control (No)	Total Control (No)	RR	OR	p- value
Haplotype DRB1*00101/DQA1*00101/DQB1*00201	7 SLO	14 SLO	9	60	3,33	5,67	N.A
Haplotype DRB1*00101/DQA1*00101/DQB1*00201	28 X	100 X	9	60	1,87	2,20	0,090
Haplotype DRB1*01201/DQA1*00101/DQB100201	6 ÅI	10 ÅI	7	60	5,14	11,4	N.A
Haplotype	7 ÅK	42 ÅK	3	60	3,33	3,8	N.A

DRB1*02301/DQA1*00301/DQB1*00501

Statistical analyses in bearded collie showed that haplotype DRB1*01801/DQA1*00101/DQB1*00201 had a relative risk of 1,5 an odds ratio of 2,25 and a p-value of 0,65. Allele DQB1*00201 had a relative risk of 1,5 an odds ratio of 2,25 and a p-value of 0,65 (see Table 13).

Table 13. Statistical analyses of haplotypes, genotypes and alleles in bearded collie were the frequency between the case and control group differentiated more than 10%.

	Case (No)	Total Case (No)	Control (No)	Total Control (No)	RR	OR	p-value
Haplotype DRB1*01801/DQA1*00101/DQB1*00802	4 SLO	10 SLO	2	10	2	2,67	N.A
Haplotype DRB1*01801/DQA1*00101/DQB1*00201	6 SLO	10 SLO	4	10	1,5	2,25	0,65
Genotype DRB1*01801/DQA1*00101/DQB11*00802-DRB1*01801/DQA1*00101/DQB1*00201	4	5	2	5	2	6	N.A
Allele DRB1*01801	10	10	6	10	1,67	infinity	N.A
Allele DQA1*00101	10	10	6	10	1,67	infinity	N.A
Allele DQB1*00201	6	10	4	10	1,5	2,25	0,65
Allele DQB1*00802	4	10	2	10	2	2,67	N.A

5. Discussion and Conclusions

The aim with this study was to establish the nucleotide sequence of the highly polymorph DLA loci DLA-DRB1, DLA-DQA1 and DLA-DQB1 and to determine whether certain haplotypes, genotypes or DLA- alleles can be associated with a higher risk of development of symmetrical lupoid onychodystrophy.

In giant schnauzer the haplotypes DRB1*01301/DQA1*00101/DQB1*00201 and DRB1*01301/DQA1*00301/DQB1*00501 occurred in a higher frequency in the control group compared to the case group. Statistical analyses indicate that haplotype DRB1*01301/DQA1*00301/DQB1*00501 may be protective against the development of claw problems, although this result is not statistically significant (p-value=0,055). Allele DRB1*01301 occurred in a higher frequency in the control group (40%) compared to the case group (18,1%) with a relative risk of 0,453, odds ratio of 0,332 and a p-value of 0,001, these results show that allele DRB1*01301 is a protective allele against development of claw problems and importantly these results are statistically significant. In a recent study, a protective DLA-DRB1/DQA1/DQB1 haplotype (DRB1*01301/DQA1*00301/DQB1*00501) for developing canine lymphocytic thyroiditis in giant schnauzer have been identified (Wilbe et al. submitted). The protective haplotype found in this study haplotype DRB1*01301/DQA1*00301/DQB1*00501 agrees with that result. In both studies giant schnauzers where examined and allele DRB1*01301 and haplotype DRB1*01301/DQA1*00301/DQB1*00501 were shown to be protective against both claw problems and hypothyroidism. An explanation to this may be that canine lymphocytic thyroiditis is known to sometimes predispose development claw infections and slough of the claw as a result, thus may dogs with a protective DLA haplotype or allele against development of lymphocytic thyroiditis also be protective against development of claw problems.

In giant schnauzer, haplotype DRB1*00101/DQA1*00101/DQB1*00201 occurred in a higher frequency in the case group (26,9%) compared to the control group (15%). The allele DRB1*00101 also occurred in a higher frequency in the case group (26,9%) compared to the control group (15%). This indicated that the haplotype DRB1*00101/DQA1*00101/DQB1*00201 and allele DRB1*00101 may increase the risk for developing claw problems, however since the p-value is not <0,05 those results are not statistically significant. Since neither the allele DQA1*00101 nor the allele DQB1*00201 had a higher frequency in the case group compared to the control group it is more likely that it is the allele DRB1*00101 that may give rise to an increased risk for developing claw problems, than the haplotype DRB1*00101/DQA1*00101/DQB1*00201.
The haplotype DRB1*00101/DQA1*00101/DQB1*00201 occurred in 50% of the SLO cases compared to 15% of the control group. In the X group, which consists of cases classified in the EK, ÅK ÅI and SLO group, statistical analyses indicates that the haplotype DRB1*00101/DQA1*00101/DQB1*00201 may be a risk haplotype, although this result is not statistically significant (p-value=0,095 and OR=2,08). DRB1*00101 or haplotype DRB1*00101/DQA1*00101/DQB1*00201 may be one of the risk factors for development of different claw problems and SLO. There may be a majority of unknown risk factors that are involved in the development of SLO, but these results imply that the DRB1*00101 or haplotype DRB1*00101/DQA1*00101/DQB1*00201 may be one of them.

In bearded collie, the haplotypes DRB1*01801/DQA1*00101/DQB1*00802 and DRB1*01801/DQA1*00101/DQB1*00202 and the genotype DRB1*01801/DQA1*00101/DQB1*00802-DRB2*01801/DQA1*00101/DQB2*00202 group occur in a higher frequency in the case group compared to the control group. Statistical analyses indicate that haplotype DRB1*01801/DQA1*00101/DQB1*00201 and allele DQB1*00201 may give rise to a higher risk of developing SLO in bearded collie, however those result show no statistical significance (p-value=0,65). In an unpublished study concerning genetic association between DLA class II and SLO in Gordon setter, the same predisposing haplotype (DRB1*01801/DQA1*00101/DQB1*00802) that was found in this study has been found with statistically significant results (Personal message Maria Wilbe). This finding strengthen the suspicion that dogs with haplotype DRB1*01801/DQA1*00101/DQB1*00802 is predisposed to develop SLO.

Amino acid substitutions caused by DLA polymorphism located in the MHC molecules three hyper variable regions (HVR) cause structural changes in the MHC binding pockets as the amino acids have different charge and size. The structural changes in the HVR may influence peptides ability to bind to the MHC molecule but also the T cell recognition. MHC associated autoimmunity could be due to activation of peripheral T cells that have escaped the negative selection process in thymus and have to high affinity to self antigens, or those that have to low affinity for self antigens but cross react to external stimuli such as viruses and bacteria which imitate self antigens. The stability of the MHC molecule bound to the antigen may also be a part in autoimmunity, if the MHC molecule is very unstable when bound to antigen there is a risk that T cells will escape the negative selection process in thymus since the interaction between the MHC molecule and the peptide is to short, and T cells with to high affinity for self antigens will not be detected and eliminated (Brand et al., 2005).

The result obtained in this study support the supposition that SLO may be an autoimmune disease. As both protective and predisposing DLA class II alleles and haplotypes, have been found. The reasons that some of the result is not statistically significant may be that some of the result arose by chance or it may be a result of too few analysed samples, further studies with more individuals may give an answer to this. The information about risk and protective DLA-alleles, haplotypes and genotypes can be used in

future breeding practice in order to obtain healthier dogs, but also give more understanding about SLO and other autoimmune diseases.

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Supplementary Table 1. Sample ID, classification, haplotype and genotype for each dog in this study.

Sample ID	Breed	Status	DRB1 Allele 1	DQA1 Allele 1	DQB1 Allele 1	DRB1 Allele 2	DQA1 Allele 2	DQB1 Allele 2	Haplotye	Genotype
2.45.130	GS	ÅI	01301	00101	00201	01201	00101	00201	4, 2	**24**
2.45.140	GS	ÅI	01201	00101	00201	01201	00101	00201	2, 2	**26**
2.45.196	GS	ÅI	01201	00101	00201	01201	00101	00201	2, 2	**26**
2.45.32	GS	ÅK	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.113	GS	ÅK	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.13	GS	ÅK	00601	00401	01303	01201	00101	00201	3, 2	**13**
2.45.399	GS	ÅK	00601	00401	01303	01501	00601	02201	3, 8	**15**
2.45.488	GS	ÅK	01301	00301	00501	01501	00601	02201	5, 8	**16**
2.45.91	GS	ÅK	01301	00301	00501	01501	00601	02201	5, 8	**16**
2.45.239	GS	ÅK	01301	00301	00501	01501	00601	02201	5, 8	**16**
2.45.78	GS	ÅK	01301	00301	00501	01201	00101	00201	5, 2	**18**
2.45.47	GS	ÅK	01301	00101	00201	01301	00101	00201	4, 4	**23**
2.45.491	GS	ÅK	01201	00101	00201	02301	00301	00501	2, 6	**27**
2.45.158	GS	ÅK	01201	00101	00201	02301	00301	00501	2, 6	**27**
2.45.163	GS	ÅK	01201	00101	00201	02301	00301	00501	2, 6	**27**
2.45.452	GS	ÅK	02301	00301	00501	01501	00601	02201	6, 8	**28**
2.45.274	GS	ÅK	00601	00401	01303	02001	0401	01303	3, 9	**32**
2.45.231	GS	ÅK	00101	00101	00201	01301	00301	00501	1, 5	**4**
2.45.378	GS	ÅK	00101	00101	00201	02301	00301	00501	1, 6	**5**
2.45.82	GS	ÅK	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.180	GS	ÅK	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.331	GS	ÅK (ÅI)	00601	00401	01303	02301	00301	00501	3, 6	**14**
2.45.119	GS	ÅK (ÅI)	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.254	GS	control	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.1	GS	control	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.66	GS	control	00601	00401	01303	00901	00101	08011	3, 7	**10**
2.45.48	GS	control	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.92	GS	control	01301	00301	00501	01501	00601	02201	5, 8	**16**
2.45.63	GS	control	01301	00301	00501	01301	00101	00201	5, 4	**17**
2.45.87	GS	control	01301	00301	00501	01301	00101	00201	5, 4	**17**
2.45.219	GS	control	01301	00301	00501	01201	00101	00201	5, 2	**18**
2.45.171	GS	control	01301	00301	00501	01301	00301	00501	5, 5	**19**
2.45.221	GS	control	01301	00301	00501	01301	00301	00501	5, 5	**19**
2.45.79	GS	control	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.36	GS	control	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.161	GS	control	01301	00301	00501	01501	00601	00301	5, 10	**20**
2.45.89	GS	control	0901	00101	08011	00901	00101	08011	7, 7	**22**
2.45.70	GS	control	01301	00101	00201	01301	00101	00201	4, 4	**23**
2.45.64	GS	control	01201	00101	00201	01201	00101	00201	2, 2	**26**
2.45.51	GS	control	01201	00101	00201	02301	00301	00501	2, 6	**27**
2.45.54	GS	control	02301	00301	00501	02301	00301	00501	6,6	**29**
2.45.245	GS	control	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.232	GS	control	00101	00101	00201	01301	00301	00501	1, 5	**4**
2.45.33	GS	control	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.80	GS	control	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.102	GS	control	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.93	GS	control	00601	00401	01303	01301	00301	00501	3, 5	**8**

2.45.99	GS	control	00601	00401	01303	01301	00101	00201	3, 4	**9**
2.45.177	GS	control	00601	00401	01303	01301	00101	00201	3, 4	**9**
2.45.53	GS	control	00601	00401	01303	01301	00101	00201	3, 4	**9**
2.45.61	GS	control	00601	00401	01303	01301	00101	00201	3, 4	**9**
2.45.77	GS	control	00601	00401	01303	01301	00101	00201	3, 4	**9**
2.45.109	GS	control	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.75	GS	E	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.367	GS	E	00601	00401	01303	00901	00101	08011	3, 7	**10**
2.45.189	GS	E	01201	00101	00201	02301	00301	00501	2, 6	**27**
2.45.379	GS	E	01501	00601	00301	01501	00601	02201	10, 8	**31**
2.45.267	GS	EK	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.351/396	GS	EK	00601	00401	01303	00601	00401	01303	3, 3	**12**
2.45.458	GS	EK	01301	00301	00501	01201	00101	00201	5, 2	**18**
2.45.401	GS	EK	01301	00301	00501	01201	00101	00201	5, 2	**18**
2.45.490	GS	EK	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.49	GS	EK	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.84	GS	EK	01201	00101	00201	01201	00101	00201	2, 2	**26**
2.45.487	GS	EK	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.350	GS	EK	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.18	GS	EK	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.207	GS	EK	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.24	GS	EK	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.529	GS	EK	01501	00601	00301	01501	00601	02201	10, 8	**31**
2.45.455	GS	EK	00101	00101	00201	01301	00301	00501	1, 5	**4**
2.45.101	GS	EK	00101	00101	00201	01301	00301	501	1, 5	**4**
2.45.334	GS	EK	00101	00101	00201	02301	00301	00501	1,6	**5**
2.45.5	GS	EK	00101	00101	00201	02301	00301	00501	1, 6	**5**
2.45.440	GS	EK	00101	00101	00201	01301	00101	00201	1, 4	**6**
2.45.513	GS	EK	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.329	GS	EK	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.489	GS	PEC	00601	00401	01303	00601	00401	01303	3, 3	**12**
2.45.530	GS	PEC	00601	00401	01303	01201	00101	00201	3, 2	**13**
2.45.527	GS	PEC	00601	00401	01303	02301	00301	00501	3, 6	**14**
2.45.191	GS	PEC	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.433	GS	PEC	01301	00101	00201	02301	00301	00501	4, 6	**25**
2.45.56	GS	PEC	01201	00101	00201	02301	00301	00501	2, 6	**27**
2.45.421	GS	PEC	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.407	GS	PEC	00101	00101	00201	01501	00601	00301	1, 10	**7**
2.45.159	GS	PEC (ÅK)	00101	00101	00201	02301	00301	00501	1, 6	**5**
2.45.7	GS	PEC (EK)	00601	00401	01303	00601	00401	01303	3, 3	**12**
2.45.336	GS	PEC/tomour	00101	00101	00201	01301	00101	00201	1, 4	**6**
2.45.344	GS	PEC/tomour (EK)	00601	00401	01303	00601	00401	01303	3, 3	**12**
2.45.528	GS	SLO	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.519	GS	SLO	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.524	GS	SLO	00601	00401	01303	00601	00401	01303	3, 3	**12**
2.45.328	GS	SLO	01301	00301	00501	01501	00601	02201	5, 8	**16**
2.45.411	GS	SLO	00101	00101	00201	01201	00101	00201	1, 2	**2**
2.45.39	GS	SLO	00101	00101	00201	01301	00301	00501	1, 5	**4**
2.45.504	GS	SLO	00101	00101	00201	01301	00101	00201	1, 4	**6**
2.45.457	GS	UCP	00601	00401	01303	02301	00301	00501	3, 6	**14**

2.45.249	GS	UCP	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.108	GS	UCP	01301	00301	00501	01201	00101	00201	5, 2	**18**
2.45.2	GS	UCP	00101	00101	00201	01301	00301	00501	1, 5	**4**
2.45.104	GS	UCP	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.11	GS	UCP	00101	00101	00201	00101	00101	00201	1, 1	**1**
2.45.144	GS	UCP	00601	00401	01303	00601	00401	01303	3, 3	**12**
2.45.97	GS	UCP	01301	00301	00501	02301	00301	00501	5, 6	**21**
2.45.17	GS	UCP	00601	00401	01303	01301	00301	00501	3, 5	**8**
2.45.508	GS	UCP	00601	00401	01303	02301	00301	00501	3, 6	**14**
2.45.466	GS	UCP	01301	00101	00201	02301	00301	00501	4, 6	**25**
2.45.226	GS	UCP	01301	00301	00501	02301	00301	00501	5, 6	**21**
'2.45.40	GS	UCP	00101	00101	00201	00601	00401	01303	1, 3	**3**
2.45.60	GS	UCP	00601	00401	01303	01301	00301	00501	3, 5	**8**
1.6.4	BC	control	01801	00101	00201	01801	00101	00802	1, 2	**32**
1.6.7	BC	control	01801	00101	00201	01801	00101	00802	1, 2	**32**
1.6.2	BC	control	01501	00601	00301	01501	00601	02301	5, 4	**33**
1.6.3	BC	control	00201	00901	00101	01501	00601	02301	3, 4	**34**
1.6.6	BC	control	01801	00101	00201	01801	00101	00201	1, 1	**35**
1.6.1	BC	SLO	01801	00101	00201	01801	00101	00802	1, 2	**32**
1.6.10	BC	SLO	01801	00101	00201	01801	00101	00802	1, 2	**32**
1.6.8	BC	SLO	01801	00101	00201	01801	00101	00802	1, 2	**32**
1.6.9	BC	SLO	01801	00101	00201	01801	00101	00802	1, 2	**32**
1.6.5	BC	SLO	01801	00101	00201	01801	00101	00201	1, 1	**35**

Haplotypes GS

	Allele	Allele	Allele
Code	**DRB1**	**DQA1**	**DQB1**
1	00101	00101	00201
2	01201	00101	00201
3	00601	00401	01303
4	01301	00101	00201
5	01301	00301	00501
6	02301	00301	00501
7	00901	00101	08011
8	01501	00601	002201
9	02001	0401	01303
10	01501	00601	00301

Haplotypes BC

	Allele	Allele	Allele
Code	**DRB1**	**DQA1**	**DQB1**
1	01801	00101	00802
2	01801	00101	00201
3	00201	00901	00101
4	01501	00601	02301
5	01501	00601	00301

Genotype GS

Code	**DRB1**	**DQA1**	**DQB1**	**DRB2**	**DQA2**	**DQB2**
1	00101	00101	00201	00101	00101	00201

2	00101	00101	00201	01201	00101	00201
3	00101	00101	00201	00601	00401	01303
4	00101	00101	00201	01301	00301	00501
5	00101	00101	00201	02301	00301	00501
6	101	101	00201	01301	101	00201
7	101	101	00201	01501	00601	00301
8	601	401	1303	1301	301	501
9	601	401	1303	1301	101	201
10	00601	00401	01303	00901	101	008011
12	601	401	1303	601	401	1303
13	00601	00401	01303	01201	101	00201
14	601	401	1303	2301	301	501
15	00601	00401	01303	01501	00601	02201
16	1301	301	501	1501	601	2201
17	1301	301	501	1301	00101	201
18	1301	301	501	1201	00101	201
19	1301	301	501	1301	301	501
20	1301	301	501	1501	601	301
21	01301	00301	00501	02301	00301	00501
22	901	00101	8011	901	00101	8011
23	1301	00101	201	1301	00101	201
24	1301	101	201	1201	101	201
25	01301	101	00201	02301	00301	00501
26	1201	101	201	1201	101	201
27	1201	101	201	2301	301	501
28	02301	00301	00501	01501	00601	02201
29	2301	301	501	2301	301	501
31	01501	00601	00301	01501	00601	002201
32	601	401	1303	2001	401	1303

Genotype BC

Code	DRB1	DQA1	DQB1	DRB2	DQA2	DQB2
32	01801	101	00201	01801	101	00802
33	01501	00601	00301	01501	00601	02301
34	00201	00901	101	01501	00601	02301
35	01801	101	00201	01801	101	00201

Supplementary Table 2. Genotype frequency and genotype code for giant schnauzer in the total population and classified as controls or cases.

Genotype DRB1/DQA1/DQB1*DRB2/DQA2/DQB2	Genotype code	Case and control % (110)	Control % (30)	Case % (80)
00101/00101/00201*00101/00101/00201	1	10% (11)	6,67% (2)	11,3% (9)
00101/00101/00201*01201/00101/00201	2	7,27% (8)	10% (3)	6,25% (5)
00101/00101/00201*00601/00401/01303	3	7,27% (8)	3,33% (1)	8,75% (7)

00101/00101/00201*01301/00301/00501	4	5,45% (6)	3,33% (1)	6,25% (5)
00101/00101/00201*02301/00301/00501	5	3,64% (4)	0	5% (4)
00101/00101/00201*01301/00101/00201	6	2,73% (3)	0	3,75% (3)
00101/00101/00201*01501/00601/00301	7	0,91% (1)	0	1,25% (1)
00601/00401/01303*01301/00301/00501	8	10% (11)	12,9%(5)	7,5% (6)
00601/00401/01303*01301/00101/00201	9	4,55% (5)	16,7%(5)	0
00601/00401/01303*00901/00101/08011	10	1,82% (2)	3,33%(1)	1,25% (1)
00601/00401/01303*00601/00401/01303	12	5,45% (6)	0	7,5% (6)
00601/00401/01303*01201/00101/00201	13	1,82% (2)	0	2,5% (2)
00601/00401/01303*02301/00301/00501	14	3,64% (4)	0	5% (4)
00601/00401/01303*01501/00601/02201	15	0,91% (1)	3,33% (1)	0
01301/00301/00501*01501/00601/02201	16	4,55% (5)	3,33% (1)	5% (4)
01301/00301/00501*01301/00101/00201	17	1,82% (2)	3,33% (1)	1,25% (1)
01301/00301/00501*01201/00101/00201	18	4,55% (5)	3,33% (1)	5% (4)
01301/00301/00501*01301/00301/00501	19	1,82% (2)	6,67% (2)	0
01301/00301/00501*01501/00601/00301	20	0,91% (1)	3,33% (1)	0
01301/00301/00501*02301/00301/00501	21	1,82% (2)	0	2,5% (2)
00901/00101/08011*00901/00101/08011	22	0,91% (1)	3,33% (1)	0
01301/00101/00201*01301/00101/00201	23	1,82% (2)	3,33% (1)	1,25% (1)
01301/00101/00201*01201/00101/00201	24	0,91% (1)	0	1,25% (1)
01301/00101/00201*02301/00301/00501	25	1,82% (2)	0	2,5% (2)
01201/00101/00201*01201/00101/00201	26	3,64% (4)	3,33% (1)	3,75% (3)
01201/00101/00201*02301/00301/00501	27	5,45% (6)	3,33% (1)	6,25% (5)
01201/00101/00201*01501/00601/02201	28	0,91% (1)	0	1,25% (1)
02301/00301/00501*02301/00301/00501	29	0,91% (1)	3,33% (1)	0

01501/00601/00301*01501/00601/02201	31	1,82% (2)	0	2,5% (2)
00601/00401/01303*2001/00401/01303	32	0,91%(1)	0	1,25% (1)

Supplementary Table 3. Genotype frequency and genotype code for bearded collies in the total population and classified as controls or cases.

Genotype DRB1/DQA1/DQB1*DRB2/DQA2/DQB2	**Genotype code**	**Case and control % (10)**	**Control % (5)**	**Case % (5)**
01801/00101/00802*01801/00101/00201	32	60% (6)	**40%** (2)	**80%** (4)
01501/00601/00301*01501/00601/02301	33	10% (1)	20% (1)	0
00201/00901/00101*01501/00601/02301	34	10% (1)	20% (1)	0
01801/00101/00201*01801/00101/00201	35	20% (2)	20% (1)	20% (1)