Risk of canine cranial cruciate ligament rupture is not associated with the major histocompatibility complex

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Summary
Objectives: To investigate the association of the major histocompatibility (MHC) class II allele haplotype frequencies with the diagnosis of cranial cruciate ligament (CCL) rupture in two breeds of dog.
Methods: DNA samples from populations of Labrador Retrievers and Golden Retrievers with CCL rupture and general populations of the same breeds were characterised for three DLA class II loci (DRB1, DQA1 and DQB1)

Results: Although distinct differences in haplotype types, frequencies and homozygosity were observed between the two breeds, no disease specific association could be identified for the development of the CCL rupture within either population.
Clinical significance: The risk for developing CCL rupture was not associated with DLA haplotype group(s) in Labrador Retrievers or Golden Retrievers, thus the hypothesis that there is an autoimmune basis to CCL rupture was not supported.

Introduction
Canine cranial cruciate ligament (CCL) rupture is a naturally-occurring, chronic degenerative condition of the stifle joint resulting from the progressive pathological failure of the canine CCL. The underlying aetiology of CCL rupture is presently unknown, although a number of factors are thought to contribute to the development of CCL rupture, such as genetics, age-related morphological changes in the ligament, activity levels, tibial plateau slope, stenosis of the intercondylar notch, and neuter status (2–11). Historically, an autoimmune-mediated pathogenesis for the development of the rupture had been proposed on the basis of the identification of increased auto-antibodies to collagen type I and type II, immune complexes and rheumatoid factor in the synovial fluid and serum of dogs with CCL rupture, when compared to dogs without CCL rupture (12–15). However, these findings are not specific to CCL rupture alone, and similar observations have also been made with canine osteoarthritis and immune-mediated arthritis of other joints (13–15).

Canine cranial cruciate rupture is regarded as an inflammatory condition. Large numbers of major histocompatibility complex (MHC) Class II dendritic cells and T lymphocytes are identified in the synovial membrane of the CCL deficient stifle, and in the synovial fluid of dogs with CCL rupture (16, 17). Expression profiling of cytokine genes in dogs with CCL rupture suggests that the pattern of cytokine release is very similar to that identified in cases of canine immune mediate polyarthritis (17). Thus, it is highly plausible that autoimmunity may contribute to the marked inflammatory response reported in CCL rupture.

Major histocompatibility complex Class II association (susceptibility) with disease is a hallmark of autoimmunity (18). Major histocompatibility complex Class II susceptibility has been consistently associated with the development of rheumatoid arthritis (HLA-DRB1) in humans, and it has been estimated that MHC accounts for one-third of the genetic component of rheumatoid arthritis risk (19). A similar susceptibility (DLA-DRB1) has been identified for canine immune-mediated joint disease (20). A conserved amino acid motif in the third hypervariable region of DRB1 alleles associated with rheumatoid arthritis has been reported in both dogs and humans (20). A number of other common 某某
autoimmune and inflammatory traits in humans, such as type 1 diabetes mellitus, Crohn’s disease and autoimmune thyroiditis are consistently reported to have associations with specific MHC haplotypes (19, 21). The analogous canine diseases, type 1 diabetes, anal furunculosis and hypothyroidism also have MHC Class II allele associations with disease (22–24).

We hypothesized that cell-mediated autoimmunity plays a role in the development of CCL rupture, and that this will be evidenced by the identification of an MHC Class II association with the development of CCL rupture.

Materials and methods

Populations

Blood samples in ethylenediaminetetraacetic acid anticoagulant were collected from 94 dogs surgically treated for cranial CCL rupture. There were 49 Labrador Retrievers (mean age 4.9 years, range 8 months to 12 years, 10 female, 17 neutered female, 7 male, 15 neutered male) and 45 Golden Retrievers, (mean age 5.7 years, range 9 months to 13 years, 5 female, 26 neutered female, 10 male, 4 neutered male). The diagnosis was based on radiographic evaluation of the stifte joint, clinical examination, and the direct observation of a partial or complete rupture of the CCL at the time of surgical treatment. All samples were obtained from the UK DNA Archive for Companion Animals (25). Control DNA samples were obtained from 136 Labrador Retrievers (mean age 6 years, range 4 months to 14 years, 30 female, 4 neutered female, 24 male, 4 neutered male, 74 sex/age/neuter status not recorded), and 53 Golden Retrievers (mean age 4.4 years, range 3 months to 12 years, 11 female, 27 neutered female, 6 male, 9 neutered male) from a hospital population undergoing routine diagnostic blood tests. The sex, neuter status and age of the dogs were compared by Fisher’s exact tests and students t-test respectively. There were no significant differences between the groups with the exception of neuter status in the Labrador Retriever population (control dogs less likely to be neutered, p <0.0001).

Major histocompatibility complex genotyping for DLA-DRB1, DQA1 and DQB1

DNA was extracted from all blood samples using a commercial silica membrane or standard phenol–chloroform method (26). DNA concentration was measured using a spectrophotometer, and samples were normalised to 20 ng/ml. The dogs were characterised for three DLA class II loci using either sequence-based typing (SBT) or reference strand-mediated conformation analysis (RSCA), using polymerase chain reaction protocols and primers as previously described (23, 27, 28). Cycle sequencing was performed using a cycle sequencing kit, samples were sequenced on a genetic analyzer, and two commercially available software programmes were used to analyze the sequencing data.

Haplotype assignment and statistical methods

Three-locus, DLA-DRB1/DQA1/DQB1, haplotypes were identified by following a sequential analytical process, as previously described (23). First, all dogs that were homozygous at all three loci were selected, and from these, several different DLA-DRB1/DQA1/DQB1 haplotype combinations were identified. Dogs that were homozygous at only two loci were then selected. From these dogs, many of the previous haplotypes were confirmed, and also, several further haplotypes were identified. The remaining dogs were examined using the haplotype data already identified, and haplotypes were assigned to each of these dogs. From these dogs, further possible haplotypes were identified. Odds ratio’s, 95% confidence intervals and Chi square p-values were identified for the haplotype totals and the number of dogs with a particular haplotype.

Study Power

The statistical power of the study was calculated using a web-based programme (PS: Power and Sample Size Calculation) (29). Assuming the haplotype ratio in the control population was 20%, the study was powered to detect risk haplotypes in the Labrador Retriever and Golden Retriever populations with an odds ratio of 2.09 and 2.46 respectively with 80% power. Similarly, the study was powered to detect protective haplotypes in the same breeds with an odds ratio of 0.36 and 0.27 respectively.

Results

All case and control dogs had DLA alleles and haplotypes assigned to, and the frequencies of DLA-DRB1/DQA1/DQB1 haplotypes recorded in each of the two breeds; the data for the two breeds considered together are presented in Table 1. Haplotype diversity was greater in the Labrador Retrievers, with 20 haplotypes being identified, when compared to the Golden Retrievers, in which nine different haplotypes were identified. Seven of the haplotypes were recorded in both breeds.

There was not any significant association identified between the individual DLA haplotypes reported and the risk of CCL rupture within either of the breeds (Table 1). When the data were analysed to compare the number of dogs with a given haplotype (regardless of the hetero- or homozygosity of the haplotype) no significant associations were identified between the individual DLA haplotypes and CCL rupture (data not shown). The overall MHC Class II homozygosity rate irrespective of haplotype type was calculated for each CCL rupture population (29% and 33% in Labrador Retrievers and Golden Retrievers respectively) and was not significantly different to that of the control (19% and 38% respectively).

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Discussion

An MHC Class II haplotype association with CCL rupture was not identified for Labrador Retrievers or Golden Retrievers in this study. Major histocompatibility complex Class II susceptibilities have previously been reported for canine autoimmune joint diseases. None of the DLA-DRB1 alleles (*002, *008, *009, *011) associated with the risk for canine rheumatoid arthritis or the DRB1*00601/DQA1*00501/ DQB1*02001 haplotype associated with a canine SLE-related disease were associated with CCL rupture in this study, although the frequencies of these haplotypes in the breeds evaluated in this study were low (20, 31). An association between MHC Class II homozygosity independent of the allele type and the risk of developing hypoadrenocorticism has been reported in the Nova Scotia Duck Tolling Retriever, but this was not observed with CCL rupture in this study (30).

The two haplotypes most commonly identified in Labrador Retrievers were DRB1*015/DQA1*00601/DQB1*02301 and DRB1*01201/DQA1*00401/DQB1*013017 (44% and 55% of dogs with CCL rupture and 40% and 56% of the control dogs), and the most common haplotype in Golden Retrievers was DRB1*01201/DQA1*00401/- DQB1*01303 (67% and 51% respectively). Interestingly the DLA-DRB1*015/- DQA1*00601/DQB1*02301 haplotype has been associated with other canine diseases (22, 30). The DLA-DRB1*00101 allele is strongly associated with the risk of developing anal furunculosis in German Shepherd dogs and the risk of hypothyroidism in the Doberman, Rhodesian Ridgeback and English Setter breeds (23, 24). Commonality between disease risk haplotypes and widely differing autoimmune phenotypes is well recognised in humans, where, for example diseases such as rheumatoid arthritis share a number of MHC gene alleles with type 1 diabetes and Crohn’s disease respectively (19).

A greater diversity of MHC Class II haplotypes was identified in Labrador Retrievers when compared to Golden Retrievers. Previous studies of single nucleotide polymorphism diversity between different canine breeds have reported that the lowest
level of linkage disequilibrium exists in Labrador Retrievers, and that when compared to other breeds, the highest level of haplotype sharing is identified between Golden Retrievers and Labrador Retrievers (32). Undoubtedly, the creation of the Golden Retriever breed in the 1850s from a combination of breeds which included the Labrador Retriever resulted in the relative relatedness of the two breeds, which is still evident today in both their physical appearance and the sharing of common genotypes such as we identified at the DLA loci.

The use of a large control population with accurate phenotyping would have been preferable to the use of samples obtained from a general hospital population, and may have precluded the identification of other small but significant risks within the confines of the power of this study. Obtaining breed matched controls for CCL rupture is challenging, as it can develop at any time during life; consequently a control population undergoing blood sampling for unrelated reasons was used, accepting that the reported prevalence of CCL rupture in Labrador Retrievers and Golden Retrievers is not insignificant (3.8% and 1.4% respectively) (3). Clearly this further reduced the chances of obtaining positive associations with relatively small study populations.

In conclusion, this study could not identify a change in risk for developing CCL rupture with a DLA haplotype group in Labrador Retrievers or Golden Retrievers but further studies are required to document whether the rejection of the hypothesis can be replicated in larger studies with greater control phenotype stringency.

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Conflicts of interest
None declared.

References