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Research Article

Genotype Influences Risk of Cranial Cruciate Ligament Disease in The Newfoundland and Labrador retriever Breeds

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Abstract

Cranial cruciate ligament disease (CCLD) is the most common cause of limping in the dog and genetics contributes to etiology in many cases. Newfoundland (n=46) and Labrador retriever (n=333) dogs were evaluated. After exam, DNA was collected, processed, and genotyped using canine high-density genome wide single nucleotide polymorphism (SNP) arrays. A genome-wide association study (GWAS) and efficient mixed model analysis (EMMA) were performed. Significant SNPs were used to build a classification tree using a 5-fold cross validation method and a classification tree was assessed using a receiver operating characteristic (ROC) curve. The objective was to identify genetic markers associated with CCLD in Newfoundland and Labrador retriever dogs and evaluate a diagnostic test that estimates an individual dog's probability of developing CCLD based on genotype.

For Newfoundlands, three SNPs were used in the classification tree to best predict risk of CCLD with the area under the ROC equal to 0.96. For Labrador retrievers, thirteen SNPs were used in the classification tree to best predict risk of CCLD with the area under the ROC equal to 0.88.

Within the Newfoundland and Labrador retriever breeds, genotype, appears to influence the risk of CCLD. A genotype-based classification tree allowed for reasonable estimation of disease risk for an individual dog in this study. These findings should be further validated in additional populations before used as a tool for selection of dogs that have a reduced risk of CCLD.

ABBREVIATIONS

CCLD: cranial cruciate ligament disease; **SNP:** single nucleotide polymorphism; **GWAS:** genome-wide association study; **EMMA:** efficient mixed model analysis **ROC:** receiver operating characteristic

INTRODUCTION

CCLD is a common orthopedic condition in the dog that has enormous economic impact to the veterinary profession [1]. Despite CCLD being a common area of investigation in small animal veterinary medicine, there is no consensus as to its etiology. Dogs affected with CCLD can be of any breed, age, or sex. They can have a variable history reported by the owner, ranging from an acute onset of a severe lameness associated with trauma, to a chronic, subtle lameness with no record of trauma. In addition, physical examination findings can include some or many of the following: evidence of osteoarthritis, joint instability, concurrent meniscal injury, conformational abnormalities, obesity, and/or other orthopedic conditions. This heterogeneity in patients with CCLD suggests a multifactorial etiology.

Although several factors likely influence CCLD manifestation, it has also been suggested that there is an underlying genetic component in some breeds of dogs. Early evidence of genetic predisposition originates from reports that certain breeds of dogs had a high frequency of CCLD while other breeds had a low frequency of CCLD [2-4]. More specific evidence in a study of Newfoundland dogs, reported a prevalence of CCLD at 22%; segregation analysis of that population revealed a possible

recessive mode of inheritance with partial penetrance [5]. Heritability was reported to be 0.27, supporting a genetic predisposition with concurrent environmental influences on disease expression [5]. Subsequent work in the Newfoundland suggested that alleles of 4 microsatellite markers were significantly associated with the CCLD trait [6]. Related work evaluated single nucleotide polymorphism (SNP) within the biological candidate genes *Cartilage Oligomeric Matrix Protein*, *Collagen Type 9 alpha 1*, and *2*, and *Fibrillin 1* but found no association to CCLD status in Newfoundland dogs [7].

Since the dog often has a high level of genetic homogeneity within a breed, it is an ideal species in which to study complex genetic diseases through GWAS [8]. In addition, improvement in high-density SNP arrays allows for better identification of markers associated with complex diseases [9,10]. However, since GWAS associations can lead to spurious findings, it is important to consider drawing conclusions only after analysis of several populations. The objectives of this study were to 1) identify genetic markers that are associated with CCLD in the Newfoundland and Labrador retriever breeds and, 2) establish the validity of a diagnostic test that estimates an individual dog's possibility of developing CCLD based on its genotype. Towards these goals, a GWAS was performed separately in each of two North American populations, the Newfoundland and the Labrador retriever. Every dog had an assigned binary phenotype of unaffected or affected with CCLD. Our null hypothesis was that there are no genetic markers (SNPs), nor any combination of SNP markers, associated with CCLD in our population of Newfoundland or Labrador retriever dogs.

MATERIALS AND METHODS

Study population and diagnostic procedures

The Animal Care and Use Committee at the University of Minnesota approved this study. Informed client consent was required for each dog that participated in the study. American Kennel Club information was gathered when available for dogs selected for the study, but this was not an inclusion criterion due to some samples being obtained from Canada. Instead, owners had to only report that their dog was a purebred Labrador retriever or Newfoundland dog. All dogs were examined by a board certified veterinary surgeon to confirm breed and CCL status via palpation of the stifle joints. For both Newfoundland and Labrador retrievers, dogs categorized as CCLD unaffected were greater than 7 years of age, had no history of rear limb lameness, and had no abnormalities in either knee on orthopedic examination (palpation only). For Newfoundlands, dogs categorized as CCLD affected were less than 2 years of age with unilateral CCLD or under 4 years of age with bilateral CCLD, and they had surgical confirmation of a complete rupture of the CCL(s). For Labrador retrievers, dogs categorized as CCLD affected had surgical confirmation of CCL rupture.

Ten mLs of EDTA stabilized blood was collected. DNA was extracted with a standard protocol[b] and stored at -80°C. DNA was transported to a laboratory for genotyping[c] using a canine high density genome wide SNP array.[a] The SNP array contains 173,662 SNPs, averaging 70 SNPs per Mb that are approximately evenly spaced across the genome.

GWAS and EMMA

GWAS was performed in PLINK [11] and snpMatrix [12]. Quality control tests were performed for the raw data, removing: individuals and SNPs that failed to genotype or had genotyping rates <90%, SNPs with minor allele frequencies <0.05, SNPs with differential case/control missingness having $p \leq 0.01$, and SNPs with Hardy-Weinberg equilibrium $p<0.001$. Chi-square allele association tests, Cochran-Armitage trend tests, and logistic regression tests were conducted to identify the SNPs most significantly associated with a predisposition to, or protection from, CCLD. Correction for multiple testing was performed via phenotype label-swapping permutations (10,000 permutations). Genomic control inflation factor (lambda), which is a measure of population stratification, was calculated for both breeds studied (no stratification when lambda =1) and both populations were stratified (lambda ≠ 1). This population substructure can affect the accuracy of statistical models by falsely inflating significance. EMMA [13] was used to evaluate the genomic data in order to correct for this stratification. EMMA is an association mapping mixed model statistical test that corrects for population structure and relatedness by calculating a kinship matrix (random effects) to be added to the fixed effects (SNPs) logistic regression model. Using the findings from EMMA, SNPs with a $p < 5 \times 10^{-5}$ (for Newfoundlands) or $p < 1 \times 10^{-4}$ (for Labrador retrievers) were selected for diagnostic model assessment.

Diagnostic Model Assessment

After correction for population structure, validated SNPs from the GWAS/EMMA were used to develop a classification tree[d], which is a statistical method that iteratively selects the SNPs that best predict disease status [14]. The classification tree returns the percent chance of CCLD risk for an individual dog based on an assigned variable (i.e. SNP status). The predictive percent at each node/leaf was calculated using an iterative method of the incorporated information from each level of the tree, conservatively assuring non-zero percentages. Statistically, the predictive percent is equivalent to the posterior expected value of a multinomial probability parameter with a conjugate Dirichlet prior. The model was validated using a 5-fold cross validation, which starts by randomly dividing the original population into 5 subsets. Each set/population is used to establish a model that in turn tests the fit of the model on the remaining sets/populations. Thus, fitting a total of 5 models. The model giving the best validation statistic is chosen as the final model. The accuracy of the final model was assessed with the area under ROC curve.

RESULTS AND DISCUSSION

Descriptive results

Newfoundlands: For Newfoundland dogs, DNA from 46 dogs (24 unaffected controls and 22 CCLD affected) was studied. The mean age of the 24 (11 male, 13 female) unaffected control dogs at time of inclusion was 8.9 years (range 7.0 to 12.6 years). The mean age of the 22 (9 male, 13 female) CCLD affected dogs was 2.13 years (range 0.8 to 3.9 years).

Labrador retrievers: For Labrador retrievers, DNA from 333 dogs (190 affected and 143 unaffected dogs) was available. The affected group included 80 males and 110 females, with a

mean age at time of inclusion in the study of 6.2 years (range 0.9 to 15.3). The average age at the time of CCLD diagnosis in affected dogs was 4.4 years (range 0.4 to 12.5). The unaffected group included 77 males and 66 females, with a mean age of 10.2 years (range 7.5 to 14.1 years).

GWAS and EMMA: The Newfoundland and Labrador retriever populations were highly stratified (lambda=1.783 and 1.6, respectively), so EMMA was used to evaluate the genomic data (Figure 1). Following population correction, lambda=1.04 for the Newfoundlands and lambda=1.01 for the Labrador retrievers. Using the findings from EMMA, 19 SNPs were identified for evaluation in the diagnostic model for the Newfoundlands and 13 SNPs were identified for the Labrador retrievers (Table 1).

Diagnostic Model Assessment: Using the classification tree, risk of CCLD was not related to sex status in either breed. The area under the ROC for the Newfoundland dogs was 56.35% (r^2=0.013) and the area under the ROC for the Labrador retrievers was 55.84% (r^2=0.01).

Newfoundland Dogs: The validated classification tree utilized three SNPs (Figure 2). The predictive percent (risk) of an individual dog in the study cohort of having CCLD (based on genotype of the three SNPs), ranged from 3.13 to 97.09%. The area under the ROC curve (represents the probability of accurately selecting between an affected and unaffected subject) for this classification tree was 95.5%.

Labrador Dogs

The validated classification tree utilized thirteen SNPs (Table 2). The predictive percent (risk) of an individual dog in the study cohort of having CCLD (based on genotype of the thirteen SNPs), ranged from 2.96 to 98.57%. The area under the ROC curve for this classification tree was 88.4%.

GWAS has become a common technique to identify SNPs associated with risk of disease. They have previously been applied for investigation of diseases in individual dog breeds including systemic lupus erythematosus in the Nova Scotia duck tolling retriever [9], hip dysplasia in the Bernese mountain dog [15], and renal disease in soft-coated wheaten terriers [16]. Canine hip dysplasia is a complex disease with likely parallels to CCLD,

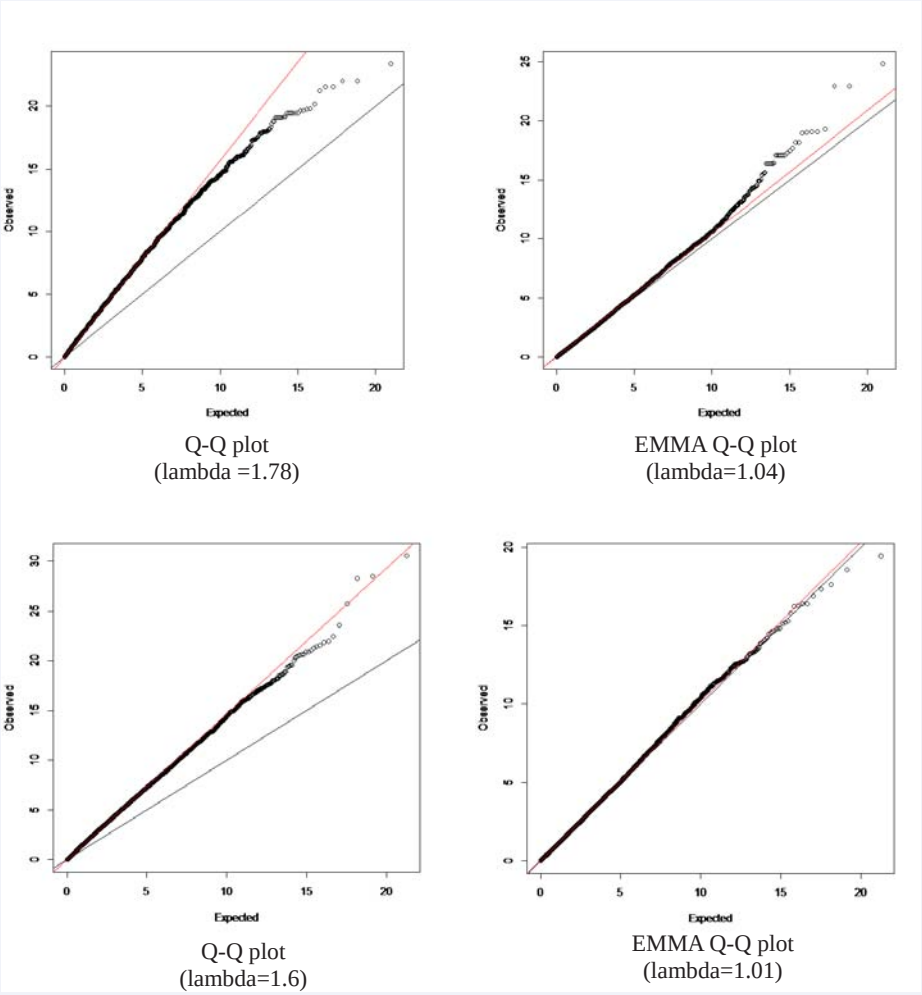

Figure 1 Population stratification for the Newfoundland (top) and Labrador retriever (bottom) dogs before (left) and after (right) EMMA correction. All axes are –log corrected p-values with observed p-values represented by circles; the red line demonstrating observed p-values and the black line demonstrating expected p-values.

Table 1: The identification and location of SNPs after EMMA testing that were used for the diagnostic model. SNPs included in the final classification tree to identify risk of CCLD are identified with an asterisk (*). P-values reported are those calculated after EMMA correction.

Newfoundlands			
CHR	SNP	BP	p-value
1	BICF2G630708028*	3717421	1.12E-05
19	BICF2G63043428	24378714	2.91E-05
19	BICF2P198171	38861102	1.23E-05
19	BICF2P1011301	39106168	3.19E-05
19	BICF2S22919640	39136305	1.23E-05
19	BICF2P609664	39378169	2.62E-05
19	TIGRP2P266544_rs8768950	40534504	6.21E-07
19	BICF2S2304227*	41733351	2.00E-05
19	BICF2S23013498	41758956	2.00E-05
30	BICF2P1350505*	5732655	1.29E-05
30	BICF2S23521050	5821467	3.60E-05
30	BICF2P990879	5854318	3.60E-05
30	BICF2P300167	5953320	3.60E-05
30	BICF2P402177	6003205	3.60E-05
30	BICF2S23317052	6133473	3.60E-05
30	BICF2P26849	6317227	3.60E-05
30	BICF2S23622498	7585210	1.70E-06
30	BICF2G630411303	7607798	1.70E-06
31	BICF2S22919272	15492628	1.30E-05
Labrador Retrievers			
CHR	**SNP**	**BP**	**p-value**
4	BICF2S2316808*	32288857	4.01E-05
4	BICF2P29608*	62699303	3.51E-05
4	BICF2S2307254*	62711827	2.29E-05
4	BICF2S23023642*	62721397	7.54E-06
11	BICF2S23033910*	28018338	6.80E-05
11	BICF2P1084064*	73369833	8.32E-05
11	BICF2P651659*	73394890	4.13E-05
11	BICF2G630306909*	73591010	5.01E-05
17	BICF2G630206855*	40796323	1.71E-05
17	BICF2P876617*	47300227	5.52E-05
21	BICF2G630652640*	19570912	6.84E-05
23	BICF2P135171*	50291215	9.28E-05
25	TIGRP2P326326_rs8928566*	22394156	9.61E-05

Abbreviations: CHR: chromosome; SNP: single nucleotide polymorphism; BP: base pair.

where both genetics and environmental pressures influence phenotype and these pressures vary across different breeds. A report that canine hip dysplasia is predictable by genotyping [17] supports our suggestion that risk of a complex orthopedic disease in the dog can be identified by genotyping. Most recently, a GWAS was used to identify genomic regions associated with CCLD in the Newfoundland [18,19]. Similar to our findings, these investigations have identified more than one SNP associated with risk of disease. GWAS findings used to create a diagnostic panel to estimate risk of disease should be validated by study of multiple populations or confirmed by identification of positional disease liability genes. In this study, we used statistical methods that establish a diagnostic panel of SNPs from a randomly selected population and then tests validity in the remaining population. The statistical method used, performs multiple iterations of randomly selected a population from the study group to create a test and validating against the remaining study group until the test with the greatest ROC is identified.

Results from a GWAS of a complex disease are improved when a large number of dogs are studied within a single breed and comparisons are made between population extremes (distinct phenotype classifications). We evaluated population extremes (particularly in the Newfoundlands) and a large sample size (particularly in the Labrador retriever) in this study. These methods provided enough statistical power for us to detect SNPs associated with disease risk in a complex disease in two separately analyzed breeds of dog. In addition, we restricted inclusion criteria by only including disease-affected dogs that were young and had their phenotype surgically confirmed, and by only including disease-unaffected dogs that were older [20] and were examined by a surgical specialist to verify normal stifles. Although these inclusion criteria, and the size of the study population, allowed for an estimate of disease risk for each dog one could argue that the population is not representative for these breeds of dogs across North America and further corroborative findings should be performed.

Breed has a large influence on CCLD frequency [3,4]. Within the Newfoundland and Labrador retriever breeds, we found that genotypes at multiple SNPs can be associated with CCLD frequency. Depending upon genotype, the possibility of an individual dog in our study population of having CCLD ranged from 3.13% to 97.09% or 4.21% to 97.31% for Newfoundlands and Labradors retrievers, respectively. Having a better understanding of risk assessment within these two breeds of dogs could 1) provide a tool for dog breeders to apply selection pressure in their breeding programs, 2) provide information for potential pet owners so they could choose a pet that has a reduced possibility of developing CCLD or, 3) lead to therapeutic intervention that might prevent phenotypic expression of the disease when a dog has exceptionally high risk of CCLD.

Identification of definitive genetic and environmental factors that define CCLD phenotype remains elusive. Investigating potential candidate genes in the regions of interest we reported may yield some explanation of the biological pathways involved in CCLD. However, identifying which candidate genes to study may be challenging given that the etiopathogenesis of CCLD could be related to a myriad of neuromuscular [18] or musculoskeletal causes. Interestingly, alleles of some SNPs we identified appeared to predispose a dog to CCLD, while alleles of other SNPs reduced the possibility of CCLD. This suggests that identification of any single molecular pathway or phenotypic trait that appears to be associated with CCLD status would incompletely describe disease association. Another strategy to investigate complex genetic diseases in the dog was demonstrated by Mateescu et al.

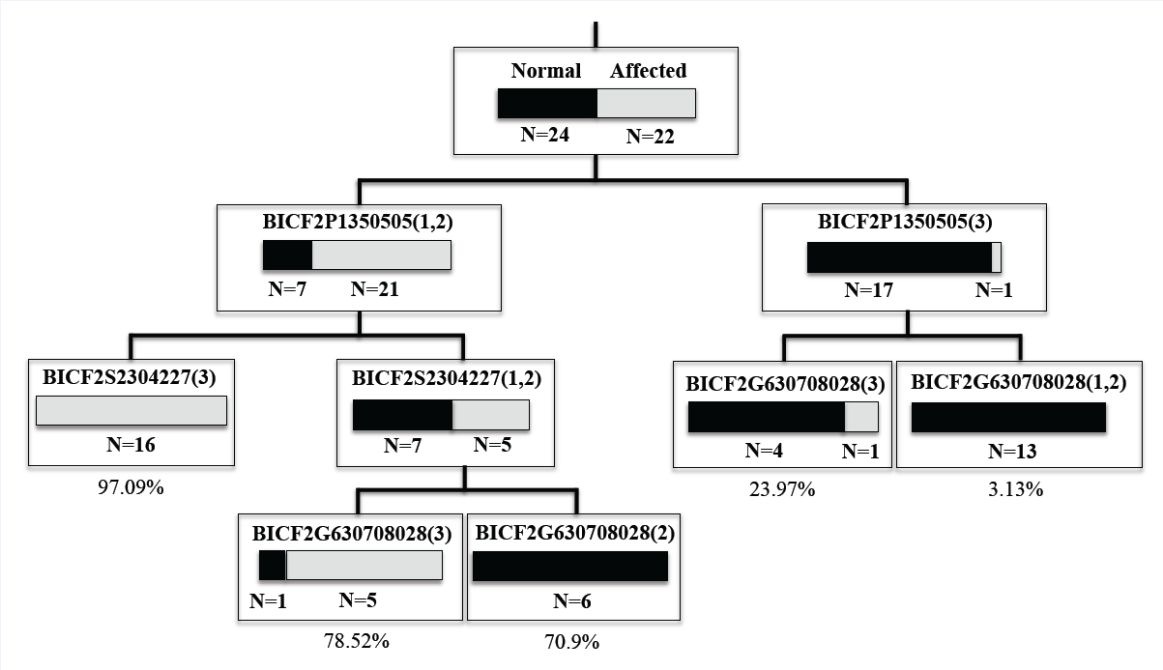

Figure 1 The validated classification tree for Newfoundlands utilizing SNPs: BICF2P1350505, BICF2S2304227, and BICF2G630708028. Following each SNP the number in parentheses designates genotype. For BICF2P1350505 (1=TT, 2=GT, 3=GG); BICF2S2304227 (1=GG, 2=GT, 3=TT); and BICF2G630708028 (1=CC, 2=CT, 3=TT). The possibility of a dog within the study cohort being CCLD affected is reported at the end of each branch of the tree. For example, if a dog had the genotype BICF2P1350505 (1 or 2) and BICF2S2304227 (3) there was a 97.09% chance that it was an affected dog.

Table 2: The validated classification tree for Labradors utilizing the 13 SNPs reported in Table 1. The left column reports the possibility (%) of a dog within the study cohort being CCLD affected. The right column reports each SNP (genotype) reported from the classification tree. Following each SNP the number in parentheses designates genotype. For BICF2S2316808 (1=AA, 2=AG, 3=GG); BICF2P29608 (1=GG, 2=AG, 3=AA); BICF2S2307254 (1=AA, 2=AG, 3=GG); BICF2S23023642 (1=GG, 2=GT, 3=TT); BICF2S23033910 (1=AA, 2=AG, 3=GG); BICF2P1084064 (1=GG, 2=GA, 3=AA); BICF2P651659 (1=TT, 2=CT, 3=CC); BICF2G630306909 (1=GG, 2=GC, 3=CC); BICF2G630206855 (1=TT, 2=TC, 3=CC); BICF2P876617 (1=TT, 2= AT, 3=AA); BICF2G630652640 (1=GG, 2=GC, 3=CC); BICF2P135171 (1=CC, 2=CT, 3=TT); TIGRP2P326326_rs8928566 (1=TT, 2=TC, 3=CC).

CCLD affected	SNP (Genotype) in order reported from classification tree.
98.57	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (3), BICF2S2316808 (1,2)
97.86	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (3), BICF2G630652640 (1,2), BICF2G630306909 (1,3)
97.31	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2S2316808 (1)
96.99	BICF2S23023642 (2,3), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2S2316808 (2), BICF2P651659 (2), BICF2P876617 (1,2), BICF2S2307254 (2)
95.98	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (2), BICF2P135171 (3)
95.27	BICF2S23023642 (2,3), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2S2316808 (2), BICF2P651659 (2), BICF2P876617 (3), BICF2G630306909 (2), BICF2G630652640 (1,2)
94.84	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (3), BICF2G630652640 (1,2), BICF2G630306909 (2), BICF2P876617 (1,2)
94.67	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (3), BICF2S2316808 (3), BICF2P876617 (2)
94.16	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2P29608 (3), BICF2G630652640 (2)
85.21	BICF2S23023642 (2,3), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2S2316808 (2), BICF2P651659 (2), BICF2P876617 (1,2), BICF2S2307254 (3)
81.14	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (3), BICF2S2316808 (3), BICF2P876617 (3)
81.05	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (3), BICF2G630652640 (3), BICF2P876617 (1,2), BICF2G630306909 (2)
79.34	BICF2S23023642 (1), BICF2P135171 (3), TIGRP2P326326_rs8928566 (3), BICF2P29608 (1)

77.89	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (3), BICF2G630652640 (3), BICF2P876617 (1,2), BICF2G630306909 (1)
74.1	BICF2S23023642 (2,3), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2S2316808 (2), BICF2P651659 (2), BICF2P876617 (3), BICF2G630306909 (2), BICF2G630652640 (3)
70.38	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (2), BICF2S2307254 (3)
69.36	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (1,2), BICF2P135171 (3), BICF2P1084064 (2), BICF2P29608 (1,2)
65.54	BICF2S23023642 (1), BICF2P135171 (3), TIGRP2P326326_rs8928566 (3), BICF2P29608 (2)
64.59	BICF2S23023642 (1), BICF2S2316808 (1,2), BICF2P135171 (3), TIGRP2P326326_rs8928566 (2), BICF2G630306909 (1,3)
61.63	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (2), BICF2S23033910 (3), BICF2S2307254 (3)
61.51	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (3), BICF2G630652640 (1,2), BICF2G630306909 (2), BICF2P876617 (3)
56.59	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (3), BICF2G630652640 (3), BICF2P876617 (3),
50.7	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (1,2), BICF2P135171 (3), BICF2P1084064 (2), BICF2P29608 (3)
49.48	BICF2S23023642 (1), BICF2S2316808 (3), BICF2G630306909 (1,2), BICF2S23033910 (3), BICF2P651659 (3)
46.19	BICF2S23023642 (2,3), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2S2316808 (2), BICF2P651659 (1)
45.38	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (3), BICF2P29608 (1,2), BICF2G630206855 (2), BICF2S2307254 (2)
44.15	BICF2S23023642 (2,3), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2S2316808 (2), BICF2P651659 (2), BICF2P876617 (3), BICF2G630306909 (1,3)
35.75	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (1,2), BICF2P135171 (1,2), BICF2P29608 (3)
35.61	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2P29608 (3), BICF2G630652640 (1,3), BICF2G630306909 (2)
31.77	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (1,2), BICF2P135171 (3), BICF2P1084064 (1)
29.56	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (2), BICF2S23033910 (3), BICF2S2307254 (2)
24.81	BICF2S23023642 (1), BICF2S2316808 (1,2), BICF2P135171 (1,2), BICF2P876617 (2)
23.61	BICF2S23023642 (2,3), BICF2P651659 (3), BICF2S23033910 (2), BICF2P135171 (1,2)
22.39	BICF2S23023642 (1), BICF2S2316808 (3), BICF2G630306909 (1,2), BICF2S23033910 (3), BICF2P651659 (2)
22.3	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (3), BICF2P29608 (3), BICF2G630652640 (1,3), BICF2G630306909 (3)
22.29	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (3), BICF2G630206855 (2), BICF2S23033910 (2)
19.01	BICF2S23023642 (1), BICF2S2316808 (1,2), BICF2P135171 (3), TIGRP2P326326_rs8928566 (2), BICF2G630306909 (2)
6.84	BICF2S23023642 (1), BICF2S2316808 (3), BICF2G630306909 (1,2), BICF2S23033910 (1,2)
5.43	BICF2S23023642 (1), BICF2S2316808 (1,2), BICF2P135171 (1,2), BICF2P876617 (3)
4.21	BICF2S23023642 (1), BICF2S2316808 (3), BICF2G630306909 (3)
2.96	BICF2S23023642 (2,3), BICF2P651659 (1,2), TIGRP2P326326_rs8928566 (1,2), BICF2P135171 (1,2), BICF2P29608 (1,2)

Abbreviations: CCLD: cranial cruciate ligament disease; **SNP:** single nucleotide polymorphism.

when they performed linkage analysis on an experimental canine pedigree between Labrador retrievers with hip dysplasia and unaffected Greyhounds [21].

It is not surprising that different SNPs were found to be associated with CCLD between the two breeds studied. Since the methodology to study these breeds were nearly identical, the differences could be attributed to different genetic heterogeneity or environmental contributions to phenotypic expression between the two breeds. In the present study, we identified different SNPs in the Newfoundland breed than those that were previously identified in a microsatellite-based study [6] and to those identified to a population in the United Kingdom [18,19]. This can be explained by the dramatic expansion of available markers on the canine high-density genome wide SNP arrays, the population extremes that were used as inclusion criteria to define diseased and normal dogs in this more recent study, and heterogeneity between populations in different countries. However, these different findings should also lead to skepticism that the results from any single study are definitive.

From a comparative standpoint, there have been several recent studies that have investigated anterior cruciate ligament (ACL) injuries in humans to determine if there is an underlying genetic contribution to its development. A familial predisposition to ACL tears was demonstrated in a case control study, which found that patients with an ACL injury were twice as likely to have had a family member with an ACL injury compared to the control patients that were selected based on not having had an ACL injury [22]. In addition, two studies have evaluated a mutation (G1023T; rs1800012) in collagen type 1 alpha 1, the most abundant collagen in the CCL, for a possible association

to ACL injury [23,24]. Patients that had the rare homozygous thymine (TT) genotype seemed to be protected from developing an ACL injury. In another study, female patients with an ACL injury were 2.4 times more like to have the homozygous adenine (AA) mutation (rs970547, S3058G) found in collagen type 12 alpha 1 [25]. These studies support that there are genetic mechanisms involved in ACL injuries in humans and that specific genotypes may be used to predict if an athlete is more or less likely to develop an injury. This suggests that, should genetic contributions to canine cruciate rupture be identified, specific genotypes may likewise be used in a predictive capacity.

CONCLUSION

This study took a genome-wide association approach to identify regions in two breeds of dogs to identify significant SNPs that might be associated with CCLD. Normal and abnormal dogs were phenotypically distinct, which assisted in SNPs associated with disease. Although these findings must be confirmed, results from a genotype-based statistical test and a receiver operating curve suggest that within the Newfoundland and Labrador retriever breeds, genotype can be used to estimate risk for CCLD development for an individual dog.

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FOOTNOTES

[a] Canine High Density® Bead Chips, Illumina, Inc., San Diego, CA

[b] QiaAmp DNA Blood Maxi Kit, Qiagen, Inc., Valencia, CA

[c] Neogen Corporation, Lincoln, NE

[d] JMP 10.0.0, JMP, Cary, NC

CONFLICT OF INTEREST

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