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SNP-based heritability and genetic architecture of cranial cruciate ligament rupture in Labrador Retrievers

Shawna Cook[1,*], **Michael G Conzemius**[2], **Molly E McCue**[3], **Kari J Ekenstedt**[1]

[1]Department of Basic Medical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN

[2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN

[3]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Summary:

Cranial cruciate ligament rupture (CCLR) is one of the leading causes of pelvic limb lameness in dogs. About 6% of Labrador Retrievers suffer from this orthopedic problem. The aim of this study was to determine the heritability of CCLR in this breed using SNP array genotyping data. DNA samples were collected from CCLR affected dogs (n = 190) and unaffected dogs over the age of 8 years (n = 143). All 333 dogs were genotyped directly or imputed up to the ~710k SNPs on the Affymetrix Axiom CanineHD SNP array. Heritability (h^2) of CCLR was calculated using multiple methodologies, including linear mixed models, Bayesian models, and a model that incorporates linkage disequilibrium. The covariates of sex and sterilization status were added to each analysis to assess their impact. Across the algorithms of these models, heritability ranged from 0.550 to 0.886, depending on covariate inclusion. The relatively high heritability for this disease indicates that a substantial genetic component contributes to CCLR in the Labrador Retriever.

Keywords

dog; canine; cranial cruciate ligament rupture; SNP-based heritability

The cruciate ligaments, connecting the femur to the tibia, are called cranial and caudal in quadrupeds; the cranial cruciate ligament (CCL) is analogous to the anterior cruciate ligament (ACL) in humans. Like the ACL, the CCL is prone to rupture. Previous work has shown that ~2.5% of dogs are affected with at least one CCLR, and various factors including

*Correspondence: cook311@purdue.edu; Tel.: +1-765-494-5811.

Conflict of interest

The authors have no conflict of interest to declare.

Availability of Data

Genotype data have been deposited in Dryad, datadryad.org.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplemental Methods Detailed methods describing heritability programs and parameters used in analyses.

breed, sex, age, and sterilization status – including age at sterilization – have some effect on the likelihood of experiencing a CCLR (Witsberger et al., 2008; Simpson et al., 2019). Dog breeds that are considered high-risk for experiencing CCLR mostly consist of large- and giant-breed dogs, such as the Labrador Retriever (LR), Rottweiler, and Newfoundland (Wilke et al., 2006; Witsberger et al., 2008; Baker et al., 2017; Pećin et al., 2017). Conversely, the Greyhound, despite also being a large-breed dog, has a low rate of CCLR, leading to the theory that there is a genetic influence on CCLR predisposition (Witsberger et al., 2008). The prevalence of CCLR in LRs was determined to be 5.79% (Witsberger et al., 2008).

In most cases, canine CCLR is neither a contact injury nor due to acute trauma, leading to various theories: degenerative processes may be occurring within the ligament, there exists an inability to repair damaged tissue at a typical rate, or defects exist within the dog's general conformation (Buote, Fusco, & Radasch, 2009; Muir et al., 2011). Because of the hypothesis that a genetic component contributes to CCLR risk in certain breeds, there have been several attempts to identify the genetic underpinnings of this condition (Clements et al., 2008; Wilke et al., 2009, 2015; Baird et al., 2014a, 2014b; Baker et al., 2017, 2018; Huang et al., 2017).

Heritability is the amount of phenotypic variability of a trait that can be attributed to an individual's genetics, as opposed to the environment the individual is exposed to. Traditionally, heritability was estimated using pedigree-based analyses; however, a "SNP-based heritability" can also be estimated using high-density single nucleotide polymorphism (SNP) genotype data on large populations of unrelated individuals, avoiding the requirement for extensive pedigree information. The utility of SNP-based heritability is shown in previous work examining heritability estimates of complex traits in sheep; when comparing pedigree-based and genotype-based calculations of heritability, the estimates were noticeably similar, with the latter generating smaller standard errors (Bérénos et al., 2014). Modern SNP arrays already contain a surplus number of polymorphic markers to adequately infer relatedness for heritability calculations in populations with unknown pedigrees (Visscher, Hill, & Wray, 2008).

Previous work by us and others demonstrates that CCLR risk follows a polygenic model of inheritance of variable penetrance (Nielen, Janss, & Knol, 2001; Wilke et al., 2006, 2009, 2015; Baird et al., 2014a, 2014b; Baker et al., 2017, 2018). The CCLR heritability estimate calculated for LRs using SNP genotype data was 0.493 (Baker et al., 2017), however this study used fewer dogs than the present work. Further, because populations vary, it is ideal to estimate heritability in multiple cohorts to arrive at a more accurate consensus. Therefore, our objective was to more robustly calculate CCLR heritability and to describe the genetic architecture of a cohort of LRs using SNP genotype data.

All dogs in this study were purebred LRs recruited at specialty practices; all owners provided informed consent to participate in the study. Samples were collected with the ethical approval of the University of Minnesota (IACUC #1006A83341 and #0708A14541). Cases were patients undergoing surgery as a treatment for CCLR. Control dogs visited the referral practice for other services (oncology, dermatology, etc.) and were enrolled if they

had no history of pelvic limb lameness, were at least 8 years of age, and their stifles were normal when palpated by a board-certified veterinary surgeon. Sex and sterilization status were recorded, and DNA was extracted from 5-10mL of whole blood from each dog (standard methods, Gentra Puregene Blood Kit, Qiagen). A total of 333 dogs were enrolled, with 190 cases (70 sterilized males, 10 intact males, 100 sterilized females and 10 intact females) and 143 controls (57 sterilized males, 20 intact males, 63 sterilized females and 3 intact females). Sterilization status was missing for two dogs, one male and one female, both in the control group. Although body condition score information was collected for a subset of the dogs in this analysis, we ultimately decided to exclude this parameter in our analyses because we could not guarantee the dog's recorded BCS was identical to its BCS at the time of CCL rupture, and because this value was measured by multiple different individuals, leading us to question the consistency of the applied scale.

Each dog was genotyped on the Illumina CanineHD 170k SNP array, and a subset (n = 48, 24 cases and 24 controls) were also genotyped on the Axiom CanineHD ~710k SNP array. Using the latter group as a reference population, the remaining 285 dogs' genotype data were imputed up to the ~710k level using BEAGLE software (Browning & Browning, 2007), resulting in a total of 333 dogs with genotypes from ~710k SNPs. This imputation methodology has been validated in sheep (O'Brien et al., 2019), horses (McCoy & McCue, 2014; Schaefer et al., 2017), and dogs (Friedenberg & Meurs, 2016; Hayward et al., 2019), even specifically in Labrador Retrievers (Friedrich et al., 2018). Basic quality control filtering was performed in PLINK (Purcell et al., 2007); all dogs and 386,500 SNPs remained for further analyses.

Genetic architecture was estimated from this genotype data with BAYESR (Erbe et al., 2012; Moser, 2014) using the default parameters. CCLR status in this population was explained by 4,538 SNPs, with 33 having a large effect, 301 a moderate effect, and 4,205 a small effect. The model estimated that the SNPs of large effect explained 24.1% of the variance in the population. These results support the hypotheses that this phenotype is polygenic (Moser et al., 2015).

Four software programs were used to calculate heritability using 386,500 SNPs, including GEMMA (Zhou & Stephens, 2012), GCTA (Yang et al., 2011), BLUPF90 (Misztal et al., 2002), and LDAK (Speed et al., 2012). All calculated heritabilities were transformed from the observed scale to the liability scale (Lee et al., 2011). Detailed information on each program and the parameters used in the analyses is provided in Supplemental Methods.

CCLR heritability was moderate-to-high (Table 1) when using all dogs and no covariates. GCTA calculated the smallest heritability, and the PCGC model in LDAK the highest. Including sex and sterilization status as covariates increased the heritability calculated across all programs. The sterilization status of two dogs was unknown; these two dogs were removed from these covariate analyses.

The effect of cryptic relatedness on the heritability of CCLR in LRs was tested in GCTA (Table 2) using the weighted genetic relationship matrix (wGRM) created in LDAK. A variety of cutoffs were used, with the more stringent calculations tending to yield decreased

heritabilities and increased standard errors (due to smaller sample numbers). Our most stringent relatedness cutoff (0.15) removed roughly 100 dogs from the analysis, which is likely excessive. The number of cases and controls for each cutoff is provided in Supplemental Table 1.

Because heritability increased at the 0.2 cutoff, we assessed the reliability of these calculations by bootstrapping the estimates 100 times, randomly removing 10% of the population each time (Wickham et al., 2019). Resampling iterations were performed in GCTA using the wGRM for the 0.15, 0.2, and 0.25 cutoffs (Fig S1). These results were essentially identical to those previously obtained (Table 2), demonstrating that once cryptic relatedness is adequately controlled, the estimate of heritability is stable.

A previous study of CCLR in LRs used both SNP data and pedigree data to calculate heritabilities of 0.538 and 0.521, respectively (Baker et al., 2017). We have reprised this work, now using a larger sample size (333 dogs vs 237 dogs) and more SNPs (over 300,000 vs less than 100,000). Overall, we observed a slight increase in the heritability prediction compared to the previous work (Baker et al., 2017). Other differences between our work and the former include: 1) the previous study made no mention of the use of covariates for the heritability estimate, whereas we used both sex and sterilization status as covariates in our heritability estimates; and 2) the previous study noted that their data could not be used to estimate heritability in a restricted maximum likelihood (REML) model, whereas this calculation was successfully performed in the present study. Pedigree information was not widely available in the present study, making it impossible to calculate heritability via the traditional pedigree method. Rather, the higher-density SNP data accurately predicted the degree of relatedness between dogs using the genetic relationship matrices created in both GCTA and GEMMA. Our current work builds on that previously published, and the slight differences in estimated heritability likely reflect differences in not only dog and SNP numbers, but the computational approaches, our inclusion of covariates, and the different population cohorts.

In the current work, heritability estimates were not remarkably different between programs. After converting heritabilities from the observed scale to the liability scale, all of the programs calculated similar estimates of heritability. However, the LDAK PCGC model estimated a larger heritability compared to all other programs; LDAK allows mirroring the effect of linkage disequilibrium (LD) decay, and, although this is not typically necessary in human studies (Speed et al., 2012), canids are known to have much larger regions of LD than humans (Sutter, 2004), making it reasonable to employ here.

To get a more accurate heritability estimate, the correction of confounding variables should be included in the calculation (Visscher et al., 2008). We observed small increases in heritability when correcting for (removing) the environmental phenotypic contributions of both sex and sterilization status. Future heritability calculations would likely benefit from the inclusion of additional covariates, such as age at injury, age at time of sterilization, and other environmental influences such as body condition score, the latter of which would ideally be recorded consistently by one individual.

In order to validate our SNP imputation, we repeated heritability calculations using the same programs and covariates, with our original, Illumina CanineHD 170k SNP (non-imputed) data (Supplemental Table 2). A total of 103,642 SNPs remained after quality control, and results were very consistent with those reported above, indicating results obtained with the imputed dataset are reliable.

Finally, it should be noted that REML estimation in case-control studies may be biased to underestimate heritability, by missing common variants with effects too small to allow identification (Golan, Lander, & Rosset, 2014); this suggests our REML-based heritability estimates are downwardly biased. The PCGC regression is probably the least biased approach in the present work, and captures more of the heritability due to common variants (Golan et al., 2014).

In conclusion, we calculated SNP-based heritability estimates for CCLR in LRs, ranging from 0.550 to 0.886 across all calculation methods. The importance of including LD in the model and correcting for sex and sterilization status is reflected in our results. The genetic architecture we observed implies that many SNPs tag genomic areas that contribute to the risk of CCLR, with few variants contributing a large effect size and many variants contributing a small effect size to CCLR. Although heritability estimates do not point us directly to which genes are involved in CCLR, they do lead to the conclusion that CCLR is moderately heritable in LRs. Future studies should now focus on determining susceptibility loci and building genetic risk models for CCLR in this high-risk breed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Heritability of CCLR calculated in multiple programs. For detailed methods, see main text and supplemental methods.

Program	Heritability all dogs (n=333)	Heritability with sex as covariate (n=333)	Heritability with sterilization status as covariate (n=331)	Heritability with sex and sterilization status as covariates (n=331)
GCTA ± SE	0.640 ± 0.160 ($P = 2.27 \times 10^{-7}$)	0.654 ± 0.161 ($P = 2.27 \times 10^{-7}$)	0.671 ± 0.163 ($P = 2.15 \times 10^{-7}$)	0.674 ± 0.164 ($P = 2.67 \times 10^{-7}$)
GCTA with wGRM ± SE	0.669 ± 0.158 ($P = 1.57 \times 10^{-7}$)	0.682 ± 0.158 ($P = 1.48 \times 10^{-7}$)	0.705 ± 0.161 ($P = 1.38 \times 10^{-7}$)	0.707 ± 0.161 ($P = 1.62 \times 10^{-7}$)
GEMMA ± SE	0.645 ± 0.151	0.656 ± 0.154	0.673 ± 0.157	0.675 ± 0.159
BLUPF90 ± SD	0.658 ± 0.140	0.660 ± 0.138	0.670 ± 0.137	0.677 ± 0.136
LDAK (REML) with wGRM ± SD	0.669 ± 0.158 ($P = 1.57 \times 10^{-7}$)	0.682 ± 0.158 ($P = 1.48 \times 10^{-7}$)	0.691 ± 0.159 ($P = 1.06 \times 10^{-7}$)	0.694 ± 0.160 ($P = 1.21 \times 10^{-7}$)
LDAK (PCGC) with wGRM ± SD	0.846 ± 0.346 ($P = 3.32 \times 10^{-23}$)	0.875 ± 0.355 ($P = 5.10 \times 10^{-24}$)	0.867 ± 0.347 ($P = 1.30 \times 10^{-24}$)	0.886 ± 0.355 ($P = 7.25 \times 10^{-25}$)

Table 2.

Estimates of heritability calculated in GCTA using the wGRM with removal of cryptically related dogs.

Cryptic relatedness cutoff	Standard heritability	Heritability with sex as covariate	Sample size	Heritability with sterilization status as covariate	Heritability with sex and sterilization status as covariates	Sample size
0.15	0.606 ± 0.279 ($P = 2.39 \times 10^{-3}$)	0.574 ± 0.271 ($P = 1.40 \times 10^{-3}$)	233	0.583 ± 0.284 ($P = 2.79 \times 10^{-3}$)	0.555 ± 0.275 ($P = 1.66 \times 10^{-3}$)	230
0.2	0.689 ± 0.247 ($P = 6.53 \times 10^{-4}$)	0.670 ± 0.249 ($P = 7.54 \times 10^{-4}$)	267	0.667 ± 0.255 ($P = 1.66 \times 10^{-3}$)	0.647 ± 0.256 ($P = 1.75 \times 10^{-3}$)	265
0.25	0.562 ± 0.224 ($P = 1.17 \times 10^{-3}$)	0.553 ± 0.224 ($P = 9.89 \times 10^{-4}$)	293	0.573 ± 0.232 ($P = 1.99 \times 10^{-3}$)	0.550 ± 0.231 ($P = 1.83 \times 10^{-3}$)	291
0.45	0.613 ± 0.185 ($P = 9.01 \times 10^{-6}$)	0.627 ± 0.186 ($P = 6.74 \times 10^{-6}$)	320	0.639 ± 0.189 ($P = 8.75 \times 10^{-6}$)	0.642 ± 0.190 ($P = 8.17 \times 10^{-6}$)	318
0.5	0.629 ± 0.158 ($P = 2.76 \times 10^{-6}$)	0.639 ± 0.176 ($P = 2.37 \times 10^{-6}$)	324	0.659 ± 0.179 ($P = 2.84 \times 10^{-6}$)	0.658 ± 0.180 ($P = 2.93 \times 10^{-6}$)	322

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