

Inheritance of rupture of the cranial cruciate ligament in Newfoundlands

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Objective—To determine prevalence, level of inbreeding, heritability, and mode of inheritance for rupture of the cranial cruciate ligament (RCCL) in Newfoundlands.

Design—Retrospective and recruitment study.

Animals—574 client-owned Newfoundlands.

Procedure—Medical records from January 1, 1996, to December 31, 2002, were evaluated for prevalence of RCCL. A pedigree was constructed by use of recruited Newfoundlands with RCCL status based on results of veterinary examination; level of inbreeding, heritability, and mode of inheritance were calculated.

Results—Hospital prevalence for RCCL was 22%; dogs in the pedigree from the recruitment study had a mean level of inbreeding of 1.19×10^{-4} , heritability of 0.27, and a possible recessive mode of inheritance with 51% penetrance for RCCL.

Conclusions and Clinical Relevance—Identification of a genetic basis for RCCL in Newfoundlands provided evidence that investigators can now focus on developing methods to identify carriers to reduce the prevalence of RCCL. (*J Am Vet Med Assoc* 2006;228:61–64)

Rupture of the CCL is the leading cause of lameness in dogs and affects nearly 20% of dogs evaluated at university hospitals for lameness.¹ Dogs with RCCL develop stifle joint osteoarthritis and lameness from the instability and inflammation that occur as a result of the damage to the CCL. Although many dogs develop RCCL from a single high-load traumatic event, it is also common for dogs with RCCL to have a history of only mild trauma.²

In addition, statistical analysis suggests that some breeds of dogs are predisposed to RCCL, whereas oth-

ers appear protected. Whitehair et al³ were the first to report that Newfoundlands, along with Rottweilers and Staffordshire Terriers, had the highest prevalence of RCCL. This finding was later supported when Duval et al⁴ concluded that the Newfoundland breed had an increased risk for RCCL with an odds ratio of 6.65. In contrast, the odds ratio was low for Golden Retrievers (0.48), Doberman Pinschers (0.33), and German Shepherd Dogs (0.25).

These findings, in addition to a history of low-load trauma (the effect of daily mechanical wear²) that causes RCCL, strongly support the notion that RCCL has a heritable component in some dog breeds.⁵ We hypothesized that there is a genetic basis for RCCL in dogs. The purposes of the study reported here were to determine the prevalence of RCCL in Newfoundlands evaluated at the ISU-CVM and determine the mean level of inbreeding, heritability, and mode of inheritance for naturally occurring RCCL in a sampled population of Newfoundlands.

Materials and Methods

Data collection—Two populations of Newfoundlands were studied. First, to determine prevalence of RCCL in a hospital population, medical records for all Newfoundlands evaluated at the ISU-CVM from 1996 to 2002 were evaluated for a diagnosis of RCCL. A diagnosis of RCCL was made only if RCCL was confirmed at the time of surgery. Second, to determine the level of inbreeding, heritability, and mode of inheritance for RCCL, a large-scale recruitment study was undertaken. The recruitment study targeted owners and breeders of Newfoundlands that were identified by the National Newfoundland Registry and Web sites and referred by other Newfoundland breeders and ISU-CVM clients. The study protocol was approved by the ISU Committee on Animal Care, and written consent was obtained by all owners of study participants. In addition, one of the study veterinarians (VLW) attended national Newfoundland shows to solicit study participants. All Newfoundlands were eligible for study enrollment if the owners were aware of its medical history, a 5-generation pedigree was available for the dog, and the dog was examined by a veterinarian. In an effort to collect random data, no specific Newfoundland population was targeted and no collected data were discarded. In addition, attempts were made to collect data from across the country so that specific families of Newfoundlands were not overrepresented.

The medical history of all dogs participating in the study was collected from the owners, and if possible and relevant,

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CCL	Cranial cruciate ligament
RCCL	Rupture of the CCL
ISU-CVM	Iowa State University College of Veterinary Medicine
MRI	Magnetic resonance imaging

the date of CCL injury was ascertained. Cheek swabs and, in some dogs, blood samples were obtained, from which DNA was extracted for future analyses. Five-generation pedigrees were collected from healthy and RCCL-affected dogs. All Newfoundlands identified in the recruitment study were examined by a study veterinarian (VLW) and classified as RCCL affected on the basis of signs of pain during hyperextension of the stifle joint, stifle joint effusion, decreased range of motion, positive cranial drawer sign, or cranial tibial thrust.⁶ In addition, dogs that had a history of lameness (as reported by the owner), radiographic evidence of stifle effusion, osteoarthritis, and a diagnosis of RCCL based on either physical examination or surgical confirmation (as reported by the dog's veterinarian) were considered RCCL affected.⁶ Because the exact time of onset of lameness or diagnosis was not always available for investigation, mean age at the time of diagnosis was estimated by subtracting either the known date of RCCL diagnosis or the dog's age at the time the information was reported for this study from the dog's date of birth.

Statistical analysis—The ISU-CVM hospital prevalence rate was calculated on the basis of the number of dogs affected with RCCL divided by total number of Newfoundlands evaluated during that period. The Newfoundland pedigree generated from the recruitment study was used to determine mean level of inbreeding, heritability, and mode of inheritance for RCCL. Inbreeding coefficients of individual dogs were calculated from a standard relationship matrix based on the pedigree that was constructed.^{7,8,ab} Heritability was determined by use of standard statistical software.^{9c} The RCCL status was categorized as a binary trait because the dogs were considered either unaffected or affected. Heritability was computed by use of a single-trait model analysis of RCCL status via the restricted maximum likelihood method. The following model was used:

$$\text{RCCL status} = \mu + a + s + e$$

where μ is the overall mean, a is the individual animal effect (random), s is the sex effect (fixed effect: male or female), and e is the random error. The effects a and e had a mean of zero and variances σ_a^2 and σ_e^2 , respectively. To estimate heritability, repeat analyses were performed that evaluated different prior heritability estimates as starting values until the maximum likelihood value (ie, the measurement value for which data best fit the model for heritability) was determined.

A different analysis was carried out by use of the method of Kinghorn¹⁰ to consider the possibility of a single major gene. If there are mutant (m) and wild-type ($+$) alleles, the mode of inheritance can be described as the percentage penetrance for each of the 3 genotypes ($++$, $+m$, and mm). For example, a simple recessive mode of inheritance has penetrance values, or percentage expression of the trait, of 0, 0, and 100 for homozygous, RCCL-unaffected animals; heterozygous carrier animals; and homozygous, RCCL-affected animals, respectively. The method makes an initial assumption for allele frequency and penetrance values and uses complex segregation analysis of the RCCL data and the pedigree to calculate the probabilities of being $++$, $+m$, or mm for each dog. These probabilities are used to make updated estimates of the frequency of allele m and the 3 penetrance values. These updated estimates depend to some extent on the starting values, so the process is iterated until there is little change between iterations in the estimates. The Kinghorn method assumes that the single locus is the only locus affecting the trait concerned, so conclusions must be made with caution.

Results

Hospital prevalence rate—One hundred sixty-three Newfoundlands (36 castrated males, 48 sexually

intact males, 31 spayed females, and 48 sexually intact females) were evaluated at the ISU-CVM from January 1, 1996, through December 31, 2002. Twenty-two percent (15 castrated males, 5 sexually intact males, 12 spayed females, and 4 sexually intact females) had RCCL. Mean \pm SD age at the time of definitive diagnosis was 3.53 ± 2.11 years (range, 1.09 to 10.62 years; median, 2.98 years).

Recruitment study—The recruitment study included 411 Newfoundlands, of which 92 (22%; 53 females and 39 males) were RCCL affected and 319 (78%; 182 females and 137 males) were unaffected. Mean \pm SD age of all dogs in the study was 6.51 ± 3.22 years (range, 1.46 to 14.17 years; median, 5.71 years). Mean \pm SD age at the time of diagnosis of RCCL was 5.17 ± 2.86 years (range, 0.84 to 13.88 years; median, 4.07 years).

The pedigree of all study dogs consisted of 11 generations with a range of 1 to 5 offspring represented in each family. The mean inbreeding coefficient, defined as the probability that a mating pair's genes were identical because they were inherited from a common ancestor, was 1.19×10^{-4} in the pedigree overall ($n = 411$). The mean inbreeding coefficient for those dogs that were inbred (ie, inbreeding coefficient > 0 [$n = 17$]) was 0.05 (range, 0.004 to 0.17) within the pedigree population. Heritability, the degree of resemblance for RCCL classification between parents and offspring in this Newfoundland population, was calculated as 0.27. Segregation analysis predicted a major gene effect with a recessive pattern of inheritance. The frequency of the recessive allele was 0.60 with partial penetrance of 51%.

Discussion

Dogs frequently develop RCCL secondary to a minor traumatic event. Owners often describe dogs developing lameness after events such as a running in the backyard, landing from a short jump, or standing up from a recumbent position. Because 1 finding of our study was that RCCL in Newfoundlands is a heritable condition, it is possible that Newfoundlands that develop RCCL from minor trauma may have a mutation in a gene or genes that predisposes the CCL to rupture. This may also be true in other breeds that are predisposed to RCCL and have a similar history. A mutation of 1 or more genes associated with structural components of the CCL or of anything that influences the mechanical or chemical environment of the CCL could predispose the dog to RCCL. It is important to remember that genetic mutations can have varying degrees of phenotypic expression, some with minor clinical importance (eg, hereditary clotting factor XII deficiency) and some with major clinical implications (eg, systemic collagen mutations in Ehlers-Danlos syndrome). Given the mechanical demand on the CCL, it is reasonable to consider that a mutation in a gene associated with, for example, collagen that does not clinically affect skin strength may affect the CCL. Obviously, 1 motivation to investigate the heritability of such a common and economically important condition¹¹ is to identify the etiology and reduce the prevalence of RCCL.

Prevalence of RCCL in the ISU-CVM hospital population was consistent with that reported in the literature.^{1,12} Of course, this represents dogs that were taken to veterinary hospitals and typically had a disease problem, although not necessarily RCCL. Dogs included in the recruitment population, which were obtained by communicating with owners of Newfoundlands, were free of this effect; nevertheless, prevalence of RCCL in the recruitment population was nearly the same as that in the hospital population. We believe that this similarity provides evidence that the prevalence rate was reasonably accurate, the method to classify affected and unaffected dogs was reasonable, and the recruitment technique was not skewed toward finding RCCL-affected dogs.

One limitation of the study was that dogs were evaluated at a variety of ages during the defined study period, and some of the dogs may have developed RCCL later. We attempted to calculate age at onset for both populations. However, the method to do so for the recruitment study was not as accurate; therefore, the mean and median ages were older and likely biased upward, compared with the hospital population. However, another problem was that cases that occurred after the study period would have been missed; if this did not occur, prevalence of RCCL would have been higher. In addition, this could be a cause for error in the genetic analyses. For example, a dog that was 5 years old and unaffected at the time of the study may develop RCCL at 7 years of age. This would help explain the low value for penetrance because dogs that were classified as unaffected during the study period could still develop RCCL. Penetrance is typically calculated as the number of animals that express the phenotype divided by the total number expected to express the phenotype. Because the study was limited to a specific time period, a portion of the dogs may not have reached the critical period or event at which they were most likely to express the phenotype, making the number that was observed with the phenotype lower than expected.

A second weakness of the study was that not all dogs included in the recruitment population had surgical confirmation of RCCL. Given the invasiveness of surgery, this was unavoidable. Magnetic resonance imaging could have been used to noninvasively confirm CCL status, but MRI requires general anesthesia, and given the cost and limited availability of MRI, we thought this was unreasonable. Perhaps the best way to consistently make the diagnosis would have been with a single veterinarian performing a physical examination on each dog and evaluating the stifle joint radiographs of each dog that did not have surgery. Although this would be possible, it would likely limit other aspects of the study design. For example, pedigree data were collected from owners from all parts the country. If data could be collected by only a single veterinarian, pedigree data would have been skewed to populations only within a reasonable travel distance, thus biasing the study population. In addition, this would have lessened the number of pedigrees that could have been studied. We believe that this point makes the inclusion criteria defensible and allows for a reasonable estimate of heritability.

The level of inbreeding was investigated because it increases the level of homozygosity in the population and increases the potential for deleterious recessive alleles to be expressed. Steps were taken to make the recruitment population as complete as possible, but some individuals were missing. Several nearly complete families were included, which supported the level of inbreeding that was detected. The low level of inbreeding in the recruitment population suggested that the high prevalence of the disease was not attributable to inbreeding, although inbreeding is only 1 potential genetic explanation for high prevalence of a disease.

Heritability in the narrow sense is calculated as the additive genetic variance divided by the phenotypic variance. A higher value means that the phenotype can be primarily explained by the genotype of the individual, whereas a lower value indicates that other factors, such as environment, have a bigger influence on the trait. In effect, this is also a measure of how much the offspring resemble the parents or the probability that an offspring will develop RCCL if one knows the RCCL status of the parents. In this population of Newfoundlands, the heritability coefficient was calculated as 0.27, which was considered a moderate value for heritability and, thus, amenable to change with selective breeding. This implies that 27% of the phenotypic expression of RCCL is attributable to genetics and, therefore, 73% of the phenotype is attributable to environmental effects. Possible environmental effects include body condition score, level of exercise, diet, housing conditions, and neutering status. Aside from neutering status, the authors did not obtain information concerning other environmental factors of the dogs that participated in the recruitment study.

Fitting a single locus model resulted in an estimated frequency of 60% for the mutant allele *m* and penetrance values of 0.0%, 4.1%, and 50.8% for genotypes ++, +*m*, and *mm*, respectively. This suggested a predominantly recessive mode of inheritance with 51% penetrance for the homozygous recessive genotype. On the basis of this assumption of monogenic inheritance, expression of RCCL would require 2 copies of the mutant allele and the environment would permit only 51% of these dogs to express the condition. Therefore, approximately 96% of the heterozygotes, or carriers, would be clinically healthy yet could transmit the abnormal form of the gene to their offspring. However, the method used assumed that penetrance was random with respect to the rest of the genome, and this may not have been true. It is proposed that the results indicated a likely mode of inheritance that can be useful in designing more efficient gene mapping experiments and in making sensible breeding decisions.

Penetrance is related to the number of affected individuals that express the phenotype but is not related to the level of expression. We classified RCCL as a binary trait because the dogs were either unaffected or expressed the RCCL trait. Thus, it was considered an all-or-none trait. Penetrance of 51% suggests that a breeding program based solely on dogs that do not express the trait will have a limited impact on reducing prevalence of the trait because many of these dogs may

have the undesirable allele. This confirms the importance of identifying a genetic marker for the disease. In addition, because RCCL occurs at various ages, it presents a dilemma for the owner of a dog with a recessive genotype (eg, having 2 copies of the RCCL allele) as to how best provide appropriate management of the dog to delay or even help prevent development of RCCL.

Quick elimination of carriers is not recommended because of the unknown effects the trait-causing gene may have on other traits (ie, pleiotropy). Furthermore, knowing the genetic basis for RCCL could lead to the development of a preventative treatment for dogs with the recessive genotype or curative treatment for dogs that have developed RCCL. In addition, a breed of dog that has RCCL with high frequency could serve as an animal model for studying the genetic cause of RCCL in young female athletes. Rupture of the anterior cruciate ligament occurs in approximately 38,000 women/y in the United States.¹³ Athletic women are 2 to 8 times as likely to injure their anterior cruciate ligament as are athletic men^{14,22} and usually sustain the injury via minimal trauma through noncontact mechanisms (eg, jumping).^{13,23,24} Results of the present study suggest that Newfoundlands fit this model because of the high prevalence (22%) of RCCL in the hospital population and recruitment study population. In addition, RCCL in a purebred dog is similar to the injury in athletic females in that the injury is usually caused by a minor traumatic incident, there are breed and sex predispositions to high prevalence of RCCL, and this human cohort has a similar clinical course when affected.

Medical and surgical management of RCCL in dogs cost the public more than a billion dollars each year,¹¹ and many causes for RCCL in dogs have been suggested. On the basis of results of the present study, breeders should use caution in considering the use of Newfoundlands that have RCCL for breeding.

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- b. Pedigraph, version 1.1, Department of Animal Science, College of Agriculture, Food and Environmental Sciences, University of Minnesota, Saint Paul, Minn.
- c. MTDFREML, USDA, Agricultural Research Service, Lincoln, Neb.

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