Quantitative genetics of traits associated with hip dysplasia in a canine pedigree constructed by mating dysplastic Labrador Retrievers with unaffected Greyhounds

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Canine hip dysplasia (CHD) is a common developmental trait that affects primarily large breed dogs and is characterized by poor hip joint congruity, joint laxity and subluxation, and development of secondary coxofemoral joint osteoarthritis (OA). Hip dysplasia is a quantitative trait, the expression of which is influenced by genetic, nutritional, and possibly hormonal factors. Heritability estimates for CHD range from 0.11 to 0.68. The complex pattern of inheritance of CHD suggests that expression of the trait is controlled by genes located at several quantitative trait loci (QTL). Alleles that contribute to the development of a complex trait such as CHD may act in an additive or dominant fashion, and the magnitude of the effect of an individual locus is independent of its mode of inheritance. Development of CHD undoubtedly results from complex interactions among multiple genetic loci and environmental factors. Nevertheless, a few major QTL are likely to be involved in trait expression, and a single major locus may have a substantial influence on this trait on the basis of biometric methods outlined by Leighton.

Hip dysplasia is most commonly diagnosed by examination of ventrodorsal hip-extended radiographic views of the pelvis. Radiographic criteria for subjective grading of dogs on the basis of identification of dysplastic conformational features and OA have been proposed. Selective breeding programs determined on the basis of this form of radiographic assessment have been applied in a number of dog populations; however, because of the modest sensitivity of this test in immature dogs, success in reducing the prevalence of CHD has been limited. Alternative methods for measurement of the dysplastic phenotype include the distraction index (DI), dorsolateral subluxation (DLS) score, and radiographic determination of the age of detection of femoral capital epiphyseal ossification (OSS). These tests measure different features of hip conformation and differ with respect to their sensitivity and specificity as predictors of development of hip joint OA in an experimental pedigree.

Because neither the genetic potential to develop CHD nor the carrier status of an individual dog can be unequivocally inferred from its phenotype, selective breeding programs determined on the basis of phenotypic evaluation of adult dogs are likely to remain inefficient. Genetic testing would aid breeders and
prospective owners in selection of immature dogs that do not carry susceptibility alleles for CHD. Unfortunately, the molecular genetic basis of CHD is unknown, and elucidation of the QTL that underlie expression of a complex trait such as CHD is a formidable task. Linkage analysis is a statistical method for mapping and identification of QTL that has been used extensively in experimental settings as well as for genetic analysis of several human diseases.20-23 In veterinary medicine, linkage analysis has led to the identification of loci or mutations underlying a number of monogenic disorders. However, linkage analysis for identification of QTL associated with a complex canine trait of clinical veterinary importance has not been reported.

Detection of linkage between a set of polymorphic genetic markers and a complex trait requires that a pedigree be informative with respect to mapped genetic markers and the QTL associated with the trait of interest.27 Informativeness refers to the overall confidence with which the parental source of a marker allele or a QTL can be determined for members of a pedigree. The presence of significant measurable phenotypic differences between members of a pedigree is a necessary condition for observation of linkage and thus is an indication of QTL informativeness. We have developed an outcrossed pedigree for linkage analysis of CHD.28

The purpose of the study reported here was to describe a biometrical model for evaluation of the differences among the founder breed dogs and their crosses in this pedigree with respect to the traits OSS, DI, and DLS score. Our results demonstrate that the pedigree is informative with respect to the QTL underlying expression of these traits. This information provides a necessary foundation for the development of statistical models for linkage analysis that incorporate molecular data from genome-wide analysis of genetic markers.

Materials and Methods

Pedigree—A canine pedigree was constructed for linkage analysis of CHD by outcrossing dysplastic Labrador Retrievers (LR) with unaffected Greyhounds (GH).29 Briefly, trait-free GH (2 males and 5 females) were purchased from racing stock. Dysplastic LR (5 males and 3 females) were selected from an inbred pedigree that has been maintained at the James A. Baker Institute for Animal Health, Cornell University, for the study of CHD since 1968. Seven dogs from the first filial generation (F1) were bred back to GH and LR parents of other F1 litters or intercrossed to encourage maximum recombination across the entire genome (Fig 1). Two GH founder dogs were bred to each other and produced a litter of 9 puppies; measurements from these dogs were used to estimate the phenotype of GH founder dogs at 8 months of age. Siblings of 6 LR founder dogs (n = 22) were similarly used to improve the accuracy of 8-month trait value estimates for this breed.

Dogs were bred by artificial insemination on the basis of an increase in serum progesterone concentrations. Dogs were inseminated between days 3 and 6 after their serum progesterone concentrations reached 1.0 ng/ml. Feeding regimens were designed to achieve maximum growth rate for maximum expression of CHD. Pregnant and postpartum bitches were fed a standard diet of canned and ad libitum dry food. Rice cereal and canned and dry food were introduced into the diet at 4 weeks, and puppies were weaned at 6 weeks of age.

![Figure 1—Diagram of an outcrossed pedigree developed for linkage analysis of hip dysplasia. Squares and circles represent males and females, respectively. Filled and open portions of each symbol represent the proportion of Greyhound and Labrador Retriever alleles, respectively, possessed by that dog. The first letter of a litter indicates the temporal sequence of litter for that year (ie, A is the first litter born, B the second). X = F1 dogs. 2F = F2 dogs. B = Backcross (BC). 2B = BC X BC. The first numeral is the birth order for each dog in that litter and the last numeral is the year born (ie, 5 = 1995, 6 = 1996). For example, FX16 is the first F1 dog in the Fth (6th) litter born in 1996.](image-url)
Dogs were examined regularly for lameness or other clinical signs of OA. The Institutional Animal Care and Use Committee approved all aspects of the study, including provisions for medical treatment of OA.

Detection of OSS—Femoral capital epiphyseal ossification was determined radiographically or ultrasonographically. For radiographic imaging, puppies were positioned in sternal recumbency, and dorsoventral radiographic views of the pelvis were obtained. Imaging was performed every other day starting at 4 days of age until ossification was observed in each proximal femoral chondroepiphysis. For ultrasonographic imaging, puppies were positioned in ventral recumbency. Images of each femoral capital epiphysis were obtained in the transverse view with a 12- to 3-MHz linear array transducer. Imaging was performed every other day from 4 days of age until ossification was observed twice consecutively in each capital femoral epiphysis. Ossification was observed as an echogenic region in the developing capital femoral chondroepiphysis. For all dogs, OSS was expressed as the radiographic age at detection of epiphyseal ossification. For dogs that were examined ultrasonographically, OSS was determined by adding 2 days to the age at which epiphyseal ossification was observed ultrasonographically, as previously described.1

Distraction index—Radiographic views for DI determination were obtained for each dog under general anesthesia at 8 months of age.11 The maximum amount of lateral hip joint laxity was measured, and the DI was determined from these radiographic views.4

Dorsolateral subluxation score—Dorsolateral subluxation radiographic views were similarly obtained under general anesthesia at 8 months of age. Dogs were placed in sternal recumbency with the femora axially loaded in a neutral position, and dorsoventral radiographic views were obtained.16 Percentage of the femoral head covered by the craniodorsal acetabular rim was calculated by 1 author (AJW).

Standard radiographic evaluation—Standard ventrodorsal hip-extended radiographic views of the pelvis were also obtained under general anesthesia at 8 months of age. Hip joint conformation of each dog was assessed by a board-certified radiologist (NLD). Dogs were considered dysplastic if typical radiographic signs of coxofemoral joint subluxation or degenerative joint disease were identified in either hip.

Genetic modeling—We developed a statistical model that has allowed us to show the genetic contribution of each founder breed (LR, GH) and crossbreed groups (F1 dogs, F2 backcrosses to Greyhounds) to traits associated with the development of CHD in dogs in this pedigree. This model attributes to each breed or crossbreed that proportion of genes (as a percentage) that has allowed us to show the genetic contribution of each crossbreed group (F1 dogs, F2 backcrosses to Greyhounds, BCL, F1 crosses to Labrador Retrievers, F2 = Second filial generation dogs, BCL X BCL intercross) to traits associated with the development of CHD in dogs in this pedigree. For ease of calculation, we set the F1 genotype to 0, the GG genotype to 1, and the LL genotype to -1 (Table 1). Model 2 thus incorporates an “in-built” contrast between the GG and LL genotypes that allows their relative contribution to additive and dominant components to be tested simultaneously. The dominance effect is assumed to be attributable only to additive effects. Epistatic effects are not accounted for in this model. Point estimates for the mean values for each trait (OSS, DI, and DLS) for each group were calculated as follows:

\[ Y = \beta_0 + \beta_1(gender) + \beta_2(litter) + \beta_3(GL) + \beta_4(LL) + \text{residual} \]

where \( Y \) is the mean value of an individual trait (OSS, DI, or DLS) in a given group of dogs, and \( \beta_0 \) is the overall mean value for a given trait within the entire study population.

The GG and LL indicate the effects of alleles derived from GH or LR founder dogs, respectively, whereas GL represents the effect of alleles derived from the F1 dogs. Litter was modeled as a random variable; all other variables were considered fixed. For the OSS trait, the model was run with and without body weight at detection of ossification as a covariate. Also, to test for a sex effect on OSS, we modeled body weight as the dependent variable. Trait values were averaged over both hips for each dog. Separate analyses for the trait measures on the left and right hips were also performed.

To test specifically for additive and dominant genetic components of each trait, the 3 variables GG, GL, and LL in model 1 were further collapsed into 2 variables, an additive effect (\( \alpha \)) and a dominance effect (\( \delta \) (Table 1)). The model derived from new variables (model 2) was as follows:

\[ Y = \beta_0 + \beta_1(gender) + \beta_2(litter) + \beta_3(\alpha) + \beta_4(\delta) + \text{residual} \]

Litter was again modeled as a random variable; all other variables were considered fixed. The coefficient of the additive effect (\( \beta_3 \)) is the difference between the expected proportion of GG and LL alleles, whereas the coefficient of the dominance effect (\( \beta_4 \)) is the expected probability that a dog is heterozygous for alleles from the GG and LL at any given locus. For ease of calculation, we set the F1 genotype to 0, the GG genotype to 1, and the LL genotype to -1 (Table 1). Model 2 thus incorporates an “in-built” contrast between the GG and LL genotypes that allows their relative contribution to additive and dominant components to be tested simultaneously. The dominance effect is assumed to reflect the collective dominance of alleles carried by a single breed or crossbreed. Epistatic effects are not accounted for in this model. Point estimates for the mean values for each trait (OSS, DI, and DLS) for each group were calculated as follows:

\[ \text{Trait}_{GG} = \beta_0 + (1*\alpha) + (0*d); \quad \text{Trait}_{GL} = \beta_0 + (-1*\alpha) + (0*d); \quad \text{Trait}_{LL} = \beta_0 + (0*\alpha) + (1*d); \]

\[ \text{Trait}_{BCG} = \beta_0 + (0.5*\alpha) + (0.5*d); \quad \text{Trait}_{BCL} = \beta_0 + (-0.5*\alpha) + (-0.5*d); \quad \text{Trait}_{F1} = \beta_0 + (0*\alpha) + (0.5*d); \quad \text{Trait}_{F2} = \beta_0 + (0*\alpha) + (-0.5*d); \]

\[ \text{Trait}_{BCL \times BCL} = \beta_0 + (-0.5*\alpha) + (-3/8*d) \]

where \( \beta_0, \alpha, \) and \( d \) are restricted maximum likelihood estimates (REML) for the overall mean trait value, \( \alpha \) and \( \delta \), respectively. Variances for these point estimates were calculated by use of the following equation:

\[ s'(y) = c' s(b) c \]

where \( c \) and \( s'(b) \) are the column vector of coefficients and the estimated variance-covariance matrix, respectively, of the fixed effects variables in model 2. Statistical analyses were

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Table 1—Coefficients used in a mixed linear genetic model to adjust restricted maximum likelihood estimates of mean trait values for additive and dominant effects in an experimental outcrossed canine pedigree

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Alleles</th>
<th>Genotype effect</th>
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</thead>
<tbody>
<tr>
<td>Greyhound (GG)</td>
<td>GG</td>
<td>0</td>
</tr>
<tr>
<td>Labrador (LL)</td>
<td>LL</td>
<td>0</td>
</tr>
<tr>
<td>F1 (GL)</td>
<td>GL</td>
<td>0</td>
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<tr>
<td>BCL</td>
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<tr>
<td>BCG</td>
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<tr>
<td>BCL X BCL intercross</td>
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GG = Homozygous alleles from Greyhounds. GL = Heterozygous alleles. LL = Homozygous alleles from Labrador Retrievers. F1 = First filial generation dogs. BCG = F1 backcrosses to Greyhounds. BCL = F1 backcrosses to Labrador Retrievers. F2 = Second filial generation dogs. BCL X BCL intercross = 1/16 GG, 6/16 GL (F1), and, 9/16 LL. Additive coefficient calculated as 1/16 - 9/16 = -0.5. Dominant coefficient calculated as 6/16 = 0.375.

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Results

One hundred ninety-three dogs were included in the analysis. Data were available for 162 dogs in the outcrossed pedigree, including LR founder (n = 8), F1 (41), BCG (33), BCL (57), F2 (16), and BCL X BCL intercrossed dogs (7; Fig 1). A litter of 9 GH bred on site from 2 GH founder dogs was used to estimate trait values for GH founder dogs at 8 months of age. In addition, 22 siblings of 6 LR founder dogs were included to improve trait estimates for this breed. Data for OSS, DI, and DLS score were available for 190, 175, and 135 dogs, respectively (Table 2). The DLS scores were unavailable for dogs that reached 8 months of age prior to the development of the DLS technique in 1996.

In the F1 generation, only 1 dog was unequivocally dysplastic on the basis of assessment of standard ventrodorsal pelvic radiographs. No dogs in the BCG generation had evidence of CHD, whereas dogs in the BCL generation had a broad distribution of phenotypes, from excellent hip joint conformation to severely dysplastic with advanced OA.

Summary data from the analysis with model 2 were calculated (Table 2). Results of analyses with model 1 are not shown because the focus of our study was on the additive and dominance effects contained in model 2. The OSS had a significant (P < 0.001) additive genetic effect whereby GH alleles decreased OSS by 3.6 ± 0.9 (REML ± SE) days (Fig 2). However, body weight at OSS had a significant (P < 0.001) effect whereby for every increase in body weight by 1 oz, OSS was delayed by 0.20 ± 0.03 days. Males were significantly heavier at OSS than females (increase of 34.4 ± 1.5 vs 31.8 ± 1.5 oz, respectively; P = 0.004); however, there was no significant additive or dominance effect on body weight itself.

For the DI, the additive genetic effect was significant (P < 0.001) whereby GH alleles decreased the DI by 0.19 ± 0.04. There was no dominance or sex effect on the DI (Fig 3).

For the DLS score, the additive genetic effect was significant (P < 0.001) whereby GH alleles increased the DLS score by 12.5 ± 2.8%. Additionally, there was a significant (P = 0.007) dominance effect whereby GH alleles increased the DLS score by a further 8.2 ± 3.0%. Consequently, the DLS scores for GH, BCG dogs, and the combined F1 and F2 dogs were indistinguishable (Fig 4). Females had higher DLS scores than males, but the difference was not significant (62.1 ± 1.6 vs 59.2 ± 1.6%, respectively; P = 0.06). Separate analyses for the trait measures on the left and right hips produced similar results (data not shown).
as those with DI ability of Retrievers with DI ties to CHD on the basis of the DI. Laboratory parts. In addition, breeds display various susceptibility to later onset of OSS than their nondysplastic counterparts. Because of the dysplastic phenotype into the separate traits OSS, DI, and DLS score that are related to CHD and the eventual development of hip joint OA. The heritability of CHD as a discrete trait has been estimated previously in several natural pedigrees composed of families derived from the same parents or with common sires or dams.\textsuperscript{4,30} In these studies, the diagnosis of CHD in individual dogs has been made on the basis of identification of coxofemoral joint incongruity or osteoarthritic changes on standard radiographic views. The additive genetic variance of CHD in these reports was calculated from a least squares regression of midparental phenotypes on those of the offspring.\textsuperscript{4} Backcrossing, or other forms of inbreeding, is usually discouraged in natural pedigrees because it increases overall homozygosity and may increase the expression of potentially undesirable recessive traits. Therefore, dominance effects may not be directly testable in the natural setting. In our experimental pedigree, F\textsubscript{1} dogs were backcrossed to both founder populations, allowing simultaneous estimation of additive and dominance genetic effects.\textsuperscript{10}

The experimental pedigree used in our study was developed for linkage analysis of CHD. To improve the likelihood of disclosing the QTL underlying the expression of CHD, we have decomposed the dysplastic phenotype into the separate traits OSS, DI, and DLS score. The diagnostic reliability of measures of these traits has been evaluated in several studies. Dysplastic German Shepherd Dogs\textsuperscript{17} and LR\textsuperscript{18} had significantly later onset of OSS than their nondysplastic counterparts. In addition, breeds display various susceptibilities to CHD on the basis of the DI.\textsuperscript{31,32} Labrador Retrievers with DI < 0.3 at 8 months of age had a probability of > 80\% of not developing hip joint OA, whereas those with DI > 0.7 had a similar probability of developing hip joint OA.\textsuperscript{33} Finally, results of a recent study\textsuperscript{34} indicate that a clear dose-response relationship exists between the DLS score and development of hip joint OA. In that study, the odds ratios for presence of a cartilage lesion at necropsy at 8 months of age in 106 dogs were 0.2, 2.6, and 8.0 for dogs with DLS scores > 55, 45 to 55, and < 45\%, respectively. All 8-month-old dogs with DLS scores < 42\% had characteristic perilveal cartilage lesions at necropsy.\textsuperscript{11} These results, in conjunction with the segregation of the loci underlying these traits observed in the pedigree of our study, support our strategy of decomposing the dysplastic phenotype into separate measurable traits for linkage analysis.

These results lend support to our power analysis for detecting linkage to OSS, DI, and DLS score.\textsuperscript{2} In that analysis, Monte Carlo breeding simulations of the founder and F\textsubscript{1} dogs were conducted to project backcross phenotypes, and the power to detect linkage between a single genetic marker and the individual traits was calculated. The analysis assumed a normal distribution of phenotypic values for OSS, DI, and DLS scores in the founder and F\textsubscript{1} populations. Independent variables included the recombination fraction (θ) of a hypothetical QTL associated with a given genetic marker, the mean heterozygosity (h) of the F\textsubscript{1} dogs over all marker loci, and the effect sizes for each trait (calculated as the difference in the trait means between 2 breeding groups divided by the SD). The analysis estimated that the BCG has greater power for detection of linkage to the DI and OSS than the reciprocal backcross. Conversely, the BCL was predicted to be more powerful (all other factors being equal it will require fewer dogs) for detection of linkage to the DLS score than the reciprocal cross. Specifically, on the basis of h = 0.75, θ = 0.05, and the observed effect sizes in the founder and F\textsubscript{1} populations, the analysis predicted that 35 BCL would be required to achieve a power of 0.8 for detection of linkage to the DLS score in that population. In contrast, detection of linkage to the DLS score in the BCG population at that power would require 120 dogs. Conversely, 35 and 45 BCG dogs, or 100 and 110 BCL dogs, would be required to obtain equal power for detection of linkage to the DI and OSS, respectively, in those populations. As illustrated (Fig 4), with respect to the DLS score, F\textsubscript{1} dogs were indistinguishable from GH founder dogs. Therefore, on the basis of the genetic model described here, it would be easier to separate the mixture of distributions in DLS scores as a function of marker alleles from a cross between the F\textsubscript{1} dogs and LR than between the F\textsubscript{1} dogs and GH.

The significance of the dominant component for the DLS score is an important finding with regards to linkage analysis and indicates that the BCG when used alone will provide little or no information regarding the QTL underlying this trait. Both additive and dominance genetic effects on a particular trait are important components of statistical models developed for linkage analysis. The power of the test of significance of a QTL is a function of many factors including the number of progeny in the population, the magnitude of the QTL effect, the pedigree structure, and the dominance of the QTL.\textsuperscript{23} For additive QTL, an F\textsubscript{2} intercross pedigree design is more powerful than a backcross. However, for dominant QTL, an informative backcross can be twice as powerful as an intercross.\textsuperscript{23} It is important to note...
that our model does not specify the mode of inheritance of any putative QTL associated with these traits. Rather, the significance of the dominant component in our model represents a net dominance, over all contributory loci, of GH alleles over LR alleles with respect to the DLS score. Nevertheless, these results suggest that a backcross design may be the most efficient pedigree structure for detection of QTL underlying the DLS score. This is consistent with the results of our recent power analysis. Fortunately, the DLS score has also been shown to have higher sensitivity and specificity for diagnosis of CHD and coxofemoral joint OA in this pedigree than the DI or standard ventrodorsal radiographic hip joint scoring.\footnote{Lonsdale R, Todhunter R, Williams A, et al. Ultrasound assessment of femoral head epiphyseal ossification and subluxation in Labrador Retrievers (abstr), in Proceedings. Am Coll Vet Radiol Annu Sci Meet 1998;37.}

A limitation of the model described here emerges from the fact that LR and GH founder dogs in the pedigree of our study were selected from separate inbred canine populations. We have assumed that generations of inbreeding have resulted in different alleles becoming fixed at 1 or more controlling loci in each respective founder population. Fixed alleles have no effect on phenotypic variation within a given population.\footnote{Distl O, Grussler W, Schwarz J, et al. Analysis of environmentally-conditioned and genetic influences on the frequency of hip joint dysplasia in German Shepherd dog. Zentabl Veterinarmed [A] 1991;38:460–471.} However, in an outcrossed pedigree, if a fixed allele within 1 founder population is dominant and has a large effect, it will exert a major impact on the mean differences between founder breeds, as well as on the relative phenotypes of the various progeny. The model we used assumes such a locus. Therefore, no conclusions can be drawn regarding additive or dominance genetic effects on the DI, OSS, or DLS score within either founder breed. Moreover, because the traits we examined are understood to be polygenic, our results represent estimates of the typical additive or dominance effects of LR versus GH alleles on individual trait values within the various crosses of the pedigree. Epistatic effects are not accounted for in this model.

Demonstration of strong genetic control over these traits, as shown in this outcrossed pedigree, is an important prerequisite for mapping the positions of underlying QTL by use of molecular genetic marker data. In principle, current statistical methods for QTL mapping could be used for linkage analysis of CHD in the pedigree of our study.\footnote{Howell Book House, 1989.} However, conventional methods generally require large sample sizes to detect multiple QTL or QTL with small to modest effects. In experimental pedigrees of higher mammalian species it is often difficult to generate adequate sample sizes within a single family. One alternative involves a combined analysis of F2 dogs along with dogs from backcross breeding to various founder parents. Our pedigree is well suited for this type of analysis. Theoretical work suggests that inclusion of multiple families in an integrated mapping strategy can improve the power of QTL detection and the precision of QTL effect estimation.\footnote{Smith G, Biery D, Gregor T. New concepts of coxofemoral joint stability and the development of a clinical stress-radiographic method for quantitating hip joint laxity in the dog. J Am Vet Med Assoc 1990;196:59–70.} An additional challenge imposed by the fact that the founder dogs of the pedigree of our study were selected from separate populations is the possible existence of multiple alleles at any given locus as well as varying degrees of heterozygosity over different loci. If marker and QTL loci segregate in different patterns, correct determination of parental linkage phases over all loci is crucial for precise QTL localization. A model based on Markov chain statistical methods was recently developed for simultaneous estimation of linkage and linkage phases for all possible different marker types (fully vs partially informative, dominant vs codominant).\footnote{Goldsmith L, Lust G, Steinetz B. Transmission of relaxin from lactating bitches to their offspring via suckling. Biol Reprod 1994;50:238–265.} The genetic model described here provides a necessary first step toward accurate mapping of QTL segregating in multiple families. This model should prove to be a useful tool for analysis of complex canine traits in this and other experimental pedigrees.

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