

Clinical and genetic assessments of hip joint laxity in the Boykin spaniel

Kate L. Tsai, Keith E. Murphy

Abstract

Canine hip dysplasia (CHD) is characterized by a malformation of the hip joint that leads to joint laxity and consequential degenerative joint disease. The most widely used method for diagnosis of CHD is the ventrodorsal hip-extended radiologic view, commonly referred to as the Orthopedic Foundation for Animals (OFA) method. The method of the University of Pennsylvania Hip Improvement Program (PennHIP), an alternative technique that is based on hip joint laxity, provides a quantitative assessment, the distraction index (DI), of the likelihood of the development of CHD because of increased laxity in the hip joint. Linear regression analysis showed that, across many breeds of dog, the incidence of CHD, as defined by the OFA, is positively correlated with the mean DI, the determination coefficient (r^2) being 26%. We used families of Boykin spaniels (BSs) to determine the level of joint laxity in the breed and to conduct an initial whole-genome screening to identify markers that co-segregate with increased joint laxity. Although there was a positive correlation between the incidence of hip dysplasia and increased joint laxity, we did not find significant linkage in the 28 BSs that underwent genotyping, likely owing to the small size of the pedigree.

Résumé

La dysplasie de la hanche canine (CHD) est caractérisée par une malformation de l'articulation de la hanche qui conduit à une laxité articulaire et une maladie articulaire dégénérative subséquente. La méthode de diagnostic la plus couramment utilisée pour le diagnostic de CHD est un examen d'une vue radiographique ventro-dorsale de la hanche en extension, procédure référée communément comme étant la méthode «Orthopedic Foundation for Animals» (OFA). La méthode du «University of Pennsylvania Hip Improvement Program» (PennHIP), une technique alternative basée sur la laxité de l'articulation de la hanche, fournit une évaluation quantitative, l'index de distraction (DI), de la probabilité du développement de CHD causée par l'augmentation de la laxité de l'articulation de la hanche. Une analyse par régression linéaire a montré que parmi plusieurs races de chiens l'incidence de CHD, telle que définie par la OFA, est corrélée positivement avec le DI moyen, le coefficient de détermination (r^2) étant 26 %. Des familles d'épagneuls de Boykin (BSs) ont été utilisées afin de déterminer le degré de laxité articulaire de cette race et de procéder à un tamisage initial du génome entier pour identifier des marqueurs qui sont co-sélectionnés avec l'augmentation de laxité articulaire. Bien qu'il y avait une corrélation positive entre l'incidence de dysplasie de la hanche et une augmentation de la laxité articulaire, aucun lien significatif ne fut trouvé parmi les 28 BSs soumis au génotypage, probablement à cause de la petite taille du pedigree.

(Traduit par Docteur Serge Messier)

The Boykin spaniel (BS) originated in South Carolina in the early 1900s and is now found throughout the United States, although most of the population is still in and around the Carolinas. The BS is relatively small, weighing 11 to 18 kg, yet has a surprisingly high incidence of hip dysplasia, as reported by the Orthopedic Foundation for Animals (OFA) (1). The breed has a high level of genetic homogeneity, according to analysis of randomly amplified polymorphic DNA (2).

Canine hip dysplasia (CHD) is the most common orthopedic disease of the dog. It is a degenerative disease characterized by malformation of the hip joint. The inevitable result is osteoarthritis, also termed degenerative joint disease (DJD). The presence of osteoarthritic changes is the primary diagnostic criterion for CHD. The methods most commonly used for diagnosis of CHD are radiography with the ventrodorsal hip-extended view, commonly referred to as the OFA method, and the University of Pennsylvania Hip Improvement Program (PennHIP) method (3).

The OFA maintains a registry of dogs that have undergone the OFA method for diagnosis of CHD. A panel of radiologists scores radiographs on a 7-point scale. Before 1980, radiographs had been

submitted to the OFA for only 38 BSs: almost 40% were classified as showing dysplasia, and none received an excellent score. Between 1974 and 2003, the OFA evaluated more than 1400 BSs: approximately 40% were classified as showing dysplasia, and less than 1% received an excellent score. According to the OFA, the BS has the 9th-highest incidence of hip dysplasia (1).

The PennHIP method uses joint laxity to predict development of CHD. Joint laxity can cause hip instability that leads to dislocation of the femoral head. The PennHIP method quantifies passive laxity by measuring the distraction index (DI), the distance of the femoral head from the center of the acetabulum divided by the radius (3). A higher DI indicates a higher probability of osteoarthritis developing as a result of CHD. According to the PennHIP registry, the BS has the 12th-highest mean DI, 0.64 (4).

A complex trait, CHD has both quantitative trait loci (QTL) and environmental factors contributing to the phenotype (5–7). The pattern of inheritance suggests that several major and minor QTL influence the phenotype (8). Genetic studies have included breeding programs in military working dogs and designed pedigrees (7,9). Additionally, using the German shepherd, Smith et al (3) identified

Department of Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77845-4467, USA.

Address all correspondence and reprint requests to Dr. Keith E. Murphy; telephone: (979) 845-2720; e-mail: kmurphy@cvm.tamu.edu

Received February 3, 2005. Accepted August 3, 2005.

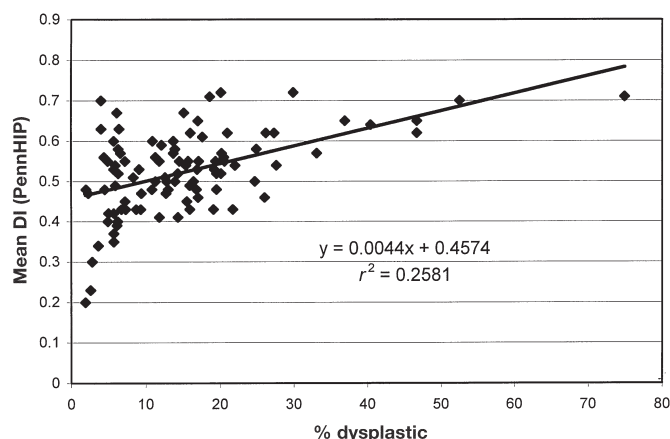


Figure 1. Linear regression analysis of the percentage of dogs with hip dysplasia, as defined by the Orthopedic Foundation for Animals, against the mean distraction index (DI) as determined by the University of Pennsylvania Hip Improvement Program (PennHIP) method, for each breed.

joint laxity as a heritable trait. Dysplasia occurs more often in the left hip in humans (10), but this trend has not been observed in dogs. However, Chase et al (11) detected greater laxity in the left than in the right hip of Portuguese water dogs (PWDs), using the Norberg angle, and identified 2 QTL on canine chromosome 1 (CFA1) that asymmetrically affect joint laxity in the right or left hip of the PWD.

A linkage analysis approach is often taken to identify regions of the genome that co-segregate with 1 or more disease genes. This allows for the identification of candidate genes when there are none or the narrowing of candidate genes when there are many. Microsatellite markers have become the tool of choice for linkage analysis owing to their polymorphic nature and Mendelian inheritance. Microsatellites are tandem repeats of 1 to 6 base pairs that are dispersed throughout the genome. In the dog, the minimal screening set 2 (MSS-2) is the most comprehensive screening set of microsatellite markers available for use in whole-genome scans. The MSS-2 comprises 327 microsatellite markers that have an average heterozygosity value of 0.73 and an average spacing of 9 Mb, with no gaps larger than 17.1 Mb (12). The MSS-2 has been multiplexed into 69 chromosome-specific panels to expedite whole-genome screenings (13). To identify markers that co-segregate with increased joint laxity, a whole-genome screening with the multiplexed MSS-2 was carried out on a small pedigree of BSs.

Because many BSs have been examined with use of the OFA method, and the work reported here used the PennHIP method, it was of interest to define the relationship between CHD and joint laxity. Correlation between the OFA and PennHip methods has previously been defined. However, the criteria differ between studies. For example, Adams et al (14) found a correlation between the DI and DJD but not between the DI and CHD as these conditions were classified. We wanted to define the correlation between the DI and CHD on the basis of the OFA definition of clinical dysplasia. Therefore, we performed linear regression analysis across 96 breeds to compare the incidence of hip dysplasia as defined by the OFA with the degree of joint laxity as defined by PennHIP. Only breeds having 100 or more evaluations from 1974 to 2003 in the OFA registry and represented

by 20 or more dogs in the 2002 PennHIP registry were included. This analysis showed a positive correlation between the mean DI and the percentage of dysplastic dogs, as determined by the OFA assessment (Figure 1), the determination coefficient (r^2) being 26%.

Although the BS is a relatively small dog, the breed is among the top 10 in incidence of hip dysplasia, according to the OFA. The PennHIP method offers a possible explanation: the high degree of joint laxity in the BS predisposes the breed to CHD; hence, the incidence of CHD as determined by the OFA method is increased in this breed.

We assembled partial, multigenerational pedigrees of BSs that segregate CHD. We had collected DNA and PennHIP data previously for each family member (2) and considered DIs greater than 0.60 as indicating affected phenotypes. The MSS-2 reactions were set up as described by Clark et al for 28 BSs (13). Products were resolved with the use of a capillary-based genetic analyzer (ABI 3100; PE Biosystems, Foster City, California, USA). Genotypes were analyzed by means of a commercial software program (Genotyper version 2.0; PE Biosystems). Two-point logarithm of the odds (LOD) scores were calculated for each marker with a statistical software package (Sequential Oligogenic Linkage Analysis Routines; Southwest Foundation for Biomedical Research, San Antonio, Texas, USA); LOD scores were calculated for the left and right hips separately as well as together.

The average DI for the 28 genotyped dogs was 0.55. Genotypes were collected for 272 markers; 37 markers (14%) were noninformative, having only 1 or 2 alleles. Of the LOD scores calculated for 254 markers, the score for marker FH3413, located on CFA1, was highest, at 1.23. This score was correlated with increased laxity in the right hip joint. A LOD score of 3.0 or greater is necessary to infer linkage. The LOD scores for the left or right hip individually varied for many markers, and the highest score was found on the chromosome identified by Chase et al (11).

Although there was a positive correlation between the incidence of hip dysplasia and increased joint laxity, we did not find significant linkage in the BS, likely owing to the small size of the pedigree. Additional pedigree members or increased marker density, or both, would be necessary to identify a region of interest.

Acknowledgments

This work was funded in part by Genetic Savings and Clone, The Iams Company, and the Baptist Memorial Healthcare Foundation. We thank Dr. Thomas Famula for assistance with the statistical analysis and Drs. Xuning Wang, Paul Shealy, and Robert Pernell for technical assistance. We also thank the dog owners for their cooperation.

References

1. Orthopedic Foundation for Animals (OFA) [page on the Internet] Hip Dysplasia Statistics. Available at <http://www.offa.org/hipstatbreed.html> Last accessed November 21, 2005.
2. Wang X, Miller AB, Lepine AJ, Scott JD, Murphy KE. Analysis of randomly amplified polymorphic DNA (RAPD) for identifying genetic markers associated with canine hip dysplasia. *J Hered* 1999;90:99-103.

3. Smith GK, Biery DN, Gregor TP. 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.
4. University of Pennsylvania Hip Improvement (PennHIP). Semi-annual Update: Distraction Index Laxity Profile. San Diego, California: Synbiotics Corporation, 2002.
5. Hedhammar A, Olsson SE, Andersson SA, et al. Canine hip dysplasia: study of heritability in 401 litters of German Shepherd dogs. *J Am Vet Med Assoc* 1979;174:1012–1016.
6. Henricson B, Norberg I, Olsson SE. On the etiology and pathogenesis of hip dysplasia: a comparative review. *J Small Anim Pract* 1966;7:673–688.
7. Leighton EA, Linn JM, Willham RL, Castleberry MW. A genetic study of canine hip dysplasia. *Am J Vet Res* 1977;38:241–244.
8. Leighton EA. Genetics of canine hip dysplasia. *J Am Vet Med Assoc* 1997;210:1474–1479.
9. Todhunter RJ, Acland GM, Olivier M, et al. An outcrossed canine pedigree for linkage analysis of hip dysplasia. *J Hered* 1999;90:83–92.
10. Smith WS, Coleman CR, Olix ML, Slager RF. Etiology of congenital dislocation of the hip. *J Bone Joint Surg Am* 1963;45-A:491–500.
11. Chase K, Lawler DE, Adler FR, Ostrander EA, Lark KG. Bilaterally asymmetric effects of quantitative trait loci (QTLs): QTLs that affect laxity in the right versus left coxofemoral (hip) joints of the dog (*Canis familiaris*). *Am J Med Genet* 2004;124-A:239–247.
12. Guyon R, Lorentzen TD, Hitte C, et al. A 1-Mb resolution radiation hybrid map of the canine genome. *Proc Natl Acad Sci USA* 2003;100:5296–5301.
13. Clark LA, Tsai KL, Steiner JM, et al. Chromosome-specific micro-satellite multiplex sets for linkage studies in the domestic dog. *Genomics* 2004;84:550–554.
14. Adams WM, Dueland RT, Daniels R, Fialkowski JP, Nordheim EV. Comparison of two palpation, four radiographic and three ultrasound methods for early detection of mild to moderate canine hip dysplasia. *Vet Radiol Ultrasound* 2000;41:484–490.