Evaluation of quantitative trait loci for hip dysplasia in Labrador Retrievers

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Objective—To identify the quantitative trait loci (QTL) that contribute to hip dysplasia in dogs.

Animals—192 Labrador Retrievers.

Procedures—Hip dysplasia was measured by use of the Norberg angle (NA), dorsolateral subluxation (DLS) score, and distraction index (DI). Genome-wide screening was conducted by use of 276 unique microsatellites. Linkage analysis was performed with a variance-based linear model. Logarithm of the odds (LOD) scores were reported when values were > 2.0.

Results—*Canis familiaris* autosomes (CFAs) 01, 02, 10, 20, 22, and 32 harbored significant QTL at LOD scores > 2.0. Among the 6 QTL, the QTL on CFA02 had not been reported to harbor QTL for hip dysplasia. The highest LOD score of 3.32 on CFA20 contributed to the second principal component of the DLS score and NA of the right hip joint. The QTL that was mapped on CFA01 (LOD score of 3.13 at 55 centimorgans) was located on the same chromosome reported to harbor a QTL for hip dysplasia in Portuguese Water Dogs and German Shepherd Dogs. In this study, CFAs 10, 20, 22, and 32 harbored QTL for hip dysplasia that have been identified in a Labrador Retriever–Greyhound pedigree and in German Shepherd Dogs.

Conclusions and Clinical Relevance—Multiple QTL were clearly involved with hip dysplasia. Identification of these QTL will enable fine-resolution mapping and subsequent assessment of candidate genes within the refined intervals to enable researchers to develop genetic screening tests and preventative and novel therapeutic regimens. (*Am J Vet Res* 2009;70:1094–1101)

Advances in genetic technology have enabled studies on the extent and pattern of genetic variation to be used to identify loci and genes that underlie complex traits. Quantitative trait loci, which contribute to complex traits, have been studied[1–5] in various domestic animal species, especially livestock, in an effort to improve production through genetic marker–assisted selection. In companion animals, HD is arguably the most common inherited orthopedic trait in dogs. It is characterized by laxity and subluxation of the hip joint that leads to osteoarthritis, followed by pain and disability in affected hip joints.[6] Affected dogs become incapable of athletic activity, and this limits their quality of life. Hip dysplasia affects small-, medium-, and large-breed dogs,[7] with an incidence of up to 75% reported for some breeds.[8] Clinical signs are more common in medium- to large-breed dogs.

Selective breeding programs based on clinical evaluation can reduce the incidence of HD, but can-

Abbreviations

CFA	*Canis familiaris* autosome
cM	Centimorgan
DI	Distraction index
DLS	Dorsolateral subluxation
HD	Hip dysplasia
LOD	Logarithm of the odds
NA	Norberg angle
PIC	Polymorphism information content
QTL	Quantitative trait loci

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not eradicate the condition, within a population.[9–13] Quantitative population genetics has been used to improve breeding stock by computing breeding values for an animal based on the genetic correlation among its relatives (a measure of trait heritability) as well as the phenotypes of its relatives. Application of these techniques to reduce HD in dogs through selection pressure has been successful only in closed colonies.[12] Phenotype-based screening methods, which are affected by age at detection and genetic control programs, require many years to be successful. Radiographic methods, such as the NA, DI, and DLS score, were developed to improve the sensitivity and specificity for detection of HD to assist veterinarians, breeders, and the public in diagnosis and detection; however, none of the methods are 100% accurate.[14]

When population genetic information within a breed is not available for use in breeding decisions, molecular genetic testing may help breeders and owners remove dogs from the breeding pool that carry susceptibility alleles for HD. Conversely, a breeder or owner may want to increase protective alleles in the breeding pool by introgression. Identification of susceptibility loci for HD will enable such breeding strategies, potentially improve diagnostic accuracy, and open new avenues for treatment before dogs develop secondary osteoarthritis.

Investigators in 1 study[15] of an extended pedigree of purebred Portuguese Water Dogs found 2 QTL for HD linked to microsatellites FH2598 and FH2524 on the telomeric end and centromeric end of CFA01, respectively, that were significant at the genome-wide level. Subsequently, in that same pedigree, those investigators found a QTL associated with acetabular osteophyte formation on CFA03.[16] Another group of investigators conducted genome-wide screening in 457 German Shepherd Dogs at 254 microsatellite loci and identified QTL for HD on CFAs 01, 03, 04, 08, 09, 16, 19, 26, and 33, all of which were significant at the genome-wide level.[17] Crossbreeding between trait-free Greyhounds and dysplastic Labrador Retrievers revealed many putative QTL for HD, including 1 each on CFA11 and CFA29 that were significant at a chromosome-wide level.[18] Subsequently, the QTL interval on CFA11 and CFA29 has been narrowed by use of single-nucleotide polymorphism genotyping in Greyhound–Labrador Retriever crossbred dogs and purebred Labrador Retrievers.[19] To confirm the existence of the QTL for HD and identify those loci most likely to be segregating in Labrador Retrievers, a breed with an HD incidence of approximately 20% in the general population,[8] a microsatellite-based genome-wide screen was conducted. The intent of the study reported here was to identify the loci that contribute to HD and then to define the genes at these loci.

Materials and Methods

Animals—A colony of Labrador Retrievers has been maintained at the Baker Institute for Animal Health of Cornell University for more than 40 years. One strain of Labrador Retrievers was bred to maximize the incidence of HD, whereas an unrelated strain was bred to provide a low trait incidence with no secondary osteoarthritis. Six of the Labrador Retrievers in the susceptible strain were also used to develop a crossbreed pedigree with Greyhounds.[20] The study was approved by the Cornell Institutional Animal Care and Use Committee.

Animal pedigree, DNA isolation, and marker selection—One hundred fifteen dogs from the population of Labrador Retrievers were genotyped at 249 microsatellite markers. An additional 77 Labrador Retrievers were genotyped at 323 microsatellite loci.[21] These evaluations used genotyping sets that have 100 microsatellite markers in common.

To improve the accuracy of our statistical analyses and marker order at the time the study was conducted, we used an integrated canine genome map[22] that encompassed 4,249 markers (900 genes, 1,589 microsatellites, and 1,760 bacterial artificial chromosomes) with 3,090 unique positions. Some markers were missing among these marker sets, which resulted in an integrated genome-wide screen with 276 microsatellites from a total of 192 Labrador Retrievers in 7 generations of 35 full-sibling families with 1 to 8 offspring/family (**Figure 1**). None of these dogs had a full set of 3 measures of hip joint traits (ie, NA, DI, and DLS score), and some of them matured before the DLS score and DI were implemented as measures of hip joint conformation. The DI was measured on 162 dogs, the NA on 164 dogs, and the DLS score on 80 dogs. Blood samples were collected from these dogs, and genomic DNA was extracted by use of phenol-chloroform extraction.

Phenotypic assessment—When dogs were 8 months old, they were anesthetized and the traits were measured by use of 3 radiographic methods (NA, DI, and DLS score).[23] The NA was used to evaluate hip joint subluxation[24]: the smaller the angle, the greater the amount of subluxation. The DI, which was assessed in each of these dogs at the University of Pennsylvania by the use of the PennHip[a] method, measured maximum lateral distraction laxity of the hip joint.[25] A DI

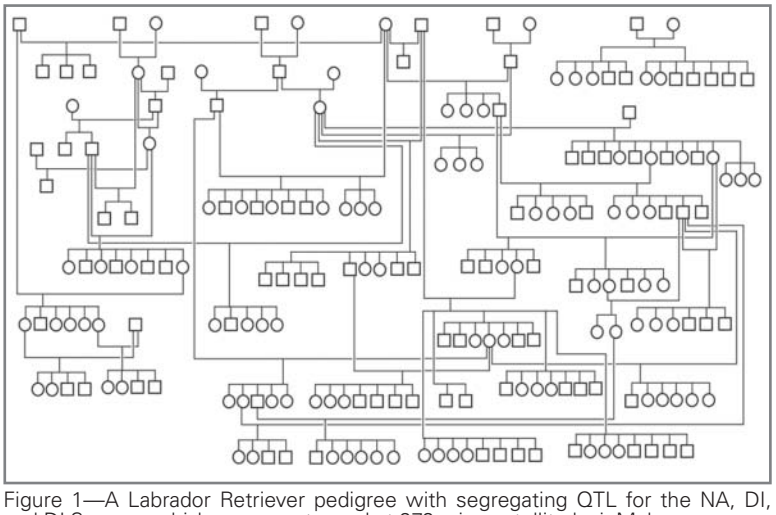

Figure 1—A Labrador Retriever pedigree with segregating QTL for the NA, DI, and DLS score, which were gentoyped at 276 microsatellite loci. Males are represented by squares, and females are represented by circles.

> 0.7 indicated a loose hip joint, whereas a DI < 0.3 indicated a tight hip joint that was highly unlikely to be dysplastic.[26] The DLS score was used to evaluate passive subluxation of the femoral head when a hip joint was radiographed in a natural weight-bearing position.[27,28] A combination of the NA and DLS score can be used to predict secondary osteoarthritis of the hip joint (which is the traditional expression of a dysplastic hip joint in dogs) better than any single method.[14]

Because these hip joint traits are correlated,[29] a principal component analysis was performed to transform the NA, DI, and DLS score for the right and left hip joints of each dog and the worst and best hip joint (ie, most- and least-affected hip joint, respectively) of each dog into a set of uncorrelated variables (ie, principal components) for each set of traits. Principal components are the linear combination of traits with eigenvectors (or weightings) as the linear coefficients. The main use of principal component analysis is to reduce the dimensionality of a dataset while retaining as much information as possible. The first principal component explains the greatest amount of variation among the measurements, and the second principal component explains the second greatest amount of variation among the measurements; each additional principal component explains a subsequently smaller amount of variation. Our laboratory group has used this approach to map HD traits in another study.[18]

Microsatellite genotyping—Gel-based electrophoresis of microsatellite markers was used to determine the size of microsatellite alleles.[30] The electrophoresis was performed at a commercial laboratory.[b] Additional capillary sequencing of microsatellites was conducted by use of a microsatellite screening set[21] and an automated sequencer[c] at the Core Laboratory Center.

To maximize the accuracy of genotypes, an algorithm[d] was used to detect genotype inconsistencies between parents and offspring. The program was developed by use of a statistical program.[e] The program was developed with the assumption that marker allele size in the grandparents was correct, and the program was used to follow each allele through the pedigree, evaluate for inconsistencies in allele size within a narrow interval (depending on whether the microsatellite was a dinucleotide, trinucleotide, or tetranucleotide), and correct errors. This correction program can reduce the microsatellite allele error rate to 1.07%.[31]

Heterozygosity for each marker was determined by use of the following equation[32]:

$$\text{heterozygosity} = 1 - \sum_{i=1}^{n} p_i^2$$

where p_i is the frequency of allele i and n is the number of alleles.

The PIC for each marker was calculated by use of the following equation:

$$\text{PIC} = (1 - \sum_{i=1}^{n} p_i^2) - 2 \left[\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} p_i^2 p_j^2 \right]$$

where j is the alternate allele.

Statistical analysis—For the linkage map, distances between markers were based on physical distance but expressed as the number of cM.[33] Meiotic recombinations detected in the pedigree were used to map the QTL that contribute to HD traits in a linkage analysis. The QTL mapping was performed separately for each trait of the left and right hip joints, the best and worst hip joints, and their principal components for each dog. A novel module of a program[f] designed to analyze genotype and phenotype data from purebred populations by use of a variance-component approach was used for the statistical analysis.[34] This method used data from marker genotypes and animal pedigree information to calculate the variance and covariance matrices associated with a QTL at a particular position along the genome by use of a 2-step approach described elsewhere.[34] The first step used a multipoint linkage analysis program[g], as described in another study,[35] to estimate identical-by-descent probabilities at each marker position, simultaneously estimating missing marker data and unknown haplotype information. Linear mixed models with residual maximum likelihood were developed to use the identical-by-descent probabilities to model the phenotypic covariance for a putative QTL. By fitting QTL and polygenic effects simultaneously, variance component analysis can generate the proportion of variance explained by the polygenic component and by the QTL. The mixed models used were as follows:

$$Y = (X \bullet \beta) + (Z \bullet \mu) + e \text{ and } Y = (X \bullet \beta) + (Z \bullet \mu) + (Z \bullet \nu) + e$$

where Y is a vector of phenotypic observations, X is an incidence matrix relating the fixed effects, β is a vector of the fixed effects of sex and litter, Z is an incidence matrix relating the genetic effects, μ is a vector of additive polygenic effects, e is a vector of random residuals, and ν is a vector of QTL effects.

The log likelihood ratio was calculated to determine the likelihood of a QTL versus no QTL at a particular chromosome marker position. Two times the difference between the logarithms of the likelihood ratio of the model with and without QTL for the aforementioned equations was used as a likelihood ratio test. Because of software computational limitations, confidence intervals could not be estimated by use of the bootstrap method, which highlighted a problem with this mapping approach. Any LOD scores > 2.0 were reported.

Results

Hip joint traits—Descriptive statistics for hip joint traits of the genotyped dogs were summarized (**Table 1**). The DI and DLS score for some dogs were not available because these dogs were phenotyped before these measures of hip joint quality were implemented. The first 3 principal components calculated from the 6 measurements (DI, DLS score, and NA for the left and the right sides) explained approximately 70%, 15%, and 10% of the variance in these hip joint measurements, respectively. Principal components were also calculated for the trait measurements of the best and worst hip joint of each dog. The dogs in the pedigree (Figure 1) can be grouped in accordance with their susceptibility

Table 1—Descriptive statistics for hip joints of 192 Labrador Retrievers (7 generations that included 35 full-sibling families and 17 loops) genotyped at 276 microsatellite markers distributed over 38 autosomes and the X chromosome.

Variable	No. of dogs	Mean	Median	Range
DI				
Left hip	162	0.53 ± 0.17	0.52	0.18 to 1.00
Right hip	162	0.53 ± 0.17	0.52	0.18 to 1.00
DLS score (%)				
Left hip	80	49.92 ± 13.01	48.05	22.20 to 76.00
Right hip	80	52.09 ± 11.82	50.95	30.90 to 78.20
NA(°)				
Left hip	164	105.29 ± 8.11	107.00	70.00 to 119.50
Right hip	164	106.64 ± 7.81	108.00	68.00 to 123.00
OFA score				
Left hip	79	2.68 ± 1.50	2.00	1.00 to 7.00
Right hip	79	2.66 ± 1.48	2.00	1.00 to 6.00
PC				
1, left hip	76	0.00 ± 0.12	0.16	–2.56 to 2.04
2, left hip	76	0.00 ± 0.12	–0.08	–2.37 to 4.27
3, left hip	76	0.00 ± 0.12	–0.17	–2.18 to 2.21
1, right hip	76	0.00 ± 0.12	–0.04	–4.73 to 2.01
2, right hip	76	0.00 ± 0.12	–0.15	–1.79 to 2.40
3, right hip	76	0.00 ± 0.12	0.07	–2.67 to 2.31

OFA = Orthopedic Foundation for Animals. PC = Principal component.

or resistance to HD by use of DI, DLS score, and NA (**Table 2**). The risk of developing HD on the basis of the DI, DLS score, and NA for each parental group and litter or family was determined (**Tables 3–5**).

Marker information—A total of 276 markers were polymorphic in the microsatellite markers screened for 192 purebred Labrador Retrievers, and the mean number of alleles per marker was 6.4 (range, 2 to 19). The number of alleles (and the corresponding number of markers) was 2 (1), 3 (32), 4 (50), 5 (42), 6 (41), 7 (33), 8 (23), 9 (15), 10 (11), 11 (11), 12 (10), 13 (5), 14 (1), and 19 (1). Mean heterozygosity was 0.61 (range, 0.04 to 0.93). Results for heterozygosity were as follows: < 0.25, 18 markers; 0.25 to 0.50, 62 markers; 0.50 to 0.75, 115 markers; and > 0.75, 81 markers. The PIC, which measures the probability of differentiating the allele transmitted by a given parent to its offspring given the marker genotype of sire, dam, and offspring,[32] had a mean value of 0.60 (range, 0.06 to 0.90). Results for PIC were as follows: < 0.25, 10 markers; 0.25 to 0.50, 69 markers; 0.50 to 0.75, 131 markers; and > 0.75, 66 markers. This set of microsatellite markers spanned the entire genome, with a mean intermarker interval of

Table 2—Number of Labrador Retrievers grouped on the basis of their susceptibility for developing HD of the left or right hip joint.

	DI*			DLS score (%)†			NA (°)‡	
Hip joint	Unaffected	At risk	Affected	Affected	At risk	Unaffected	Unaffected	Affected
Left	9	129	24	31	19	30	105	59
Right	10	124	27	25	24	31	116	48

*At 8 months of age, a dog with a DI < 0.3 has a high probability that it will have normal hip joints, whereas a dog with a DI > 0.7 has a high probability that it will develop hip joint osteoarthritis.[20,27] †At 8 months of age, a dog with a DLS score < 45% has a high probability that it will develop hip joint osteoarthritis, a dog with a DLS score between 45% and 55% is at risk that it will develop hip joint osteoarthritis, and a dog with a DLS score > 55% is resistant to development of hip joint osteoarthritis. ‡A dog with an NA < 105° has a high probability that it will develop hip joint osteoarthritis, whereas a dog with an NA > 105° has a high probability that it will have normal hip joints; outcome for these early predictions is based on detection of osteoarthritis during necropsy or during evaluation of a radiograph.

Table 3—Families (litters) and their parents, number of genotyped offspring, number of phenotyped offspring, and number of dogs in each family of Labrador Retrievers in each risk group for the DI.

	Family No.												
Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
Parent phenotype*													
Sire	UR	AR	RR	RR	RR	RR	RR	UU	RR	NN	UU	NN	NN
Dam	1 = RR	1= RR	1 = RR	1 = AA	1 = RR 2 = RR 3 = NN	1 = RA 2 = NN	1 = RR 2 = RR	1 = RR 2 = UU	1 = AA 2 = RR 3 = RR 4 = RR	1 = RR 2 = NN	1 = NN 2 = NN 3 = RR	1 = RR 2 = NN	1 = RR 2 = NN
No. of litters	2	2	1	1	8	2	2	4	4	3	3	2	1
No. of genotyped offspring	13	13	7	6	41	9	8	22	18	16	16	7	3
No. of phenotyped offspring	4	13	7	3	41	9	7	21	18	16	14	1	1
DI													
Unaffected													
Left	1	1	1	0	0	0	0	1	1	0	1	1	1
Right	2	0	2	0	2	0	0	2	0	0	1	1	1
At risk													
Left	3	9	5	2	36	9	7	15	12	13	13	0	0
Right	2	1	4	3	3	9	6	1	1	1	1	0	0
Affected													
Left	0	3	1	1	5	0	0	5	5	3	0	0	0
Right	0	2	1	0	5	0	1	5	5	6	2	0	0

*For each sire and dam, the first letter represents results for the left hip joint and the second letter represents results for the right hip joint. U = Unaffected hip joint. R = At-risk hip joint. A = Affected hip joint. N = Not available. Left = Left hip joint. Right = Right hip joint.

Table 4—Families (litters) and their parents, number of genotyped offspring, number of phenotyped offspring, and number of dogs in each family of Labrador Retrievers in each risk group for the DLS score.

	Family No.												
Variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Parent phenotype*													
Sire	NN	NN	UU	AA	RR	NN	UU	NN	NN	NN	NN	NN	NN
Dam	1 = NN	1 = NN	1 = AR	1 = AA	1 = NN 2 = NN 3 = NN	1 = NN 2 = NN	1 = UU 2 = UU	1 = NA 2 = NA	1 = RR 2 = AR 3 = AA 4 = NN	1 = AR 2 = NN	1 = NN 2 = NN 3 = NN	1 = NA 2 = NA	1 = NA 2 = NA
No. of litters	2	2	1	1	8	2	2	4	4	3	3	2	1
No. of genotyped offspring	13	13	7	6	41	9	8	22	18	16	16	7	3
No. of phenotyped offspring	0	13	7	6	21	6	7	1	12	5	0	0	0
DLS score													
Unaffected													
Left	0	6	4	1	5	5	3	0	4	1	0	0	0
Right	0	5	4	1	6	5	2	0	7	0	0	0	0
At risk													
Left	0	5	1	1	6	1	1	1	2	0	0	0	0
Right	0	5	2	2	5	1	2	1	3	2	0	0	0
Affected													
Left	0	2	2	4	10	0	3	0	6	4	0	0	0
Right	0	3	1	3	1	0	3	0	2	3	0	0	0

See Table 3 for key.

Table 5—Families (litters) and their parents, number of genotyped offspring, number of phenotyped offspring, and number of dogs in each family of Labrador Retrievers in each risk group for the NA.

	Family No.												
Variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Parent phenotype*													
Sire	AA	UU	AA	UU	NN	UU	AU	UU	AA	NN	AU	NN	NN
Dam	1 = UU	1 = UU	1 = AU	1 = UU	1 = AA 2 = UU 3 = NN	1 = AU 2 = NN	1 = UU 2 = UA	1 = AA 2 = AU	1 = AA 2 = AU 3 = UU 4 = AA	1 = AU 2 = NN	1 = NA 2 = NA 3 = UU	1 = UU 2 = NN	1 = AA 2 =NN
No. of litters	2	2	1	1	8	2	2	4	4	3	3	2	1
No. of genotyped offspring	13	13	7	6	41	9	8	22	18	16	16	7	3
No. of phenotyped offspring	4	13	7	6	41	9	7	21	18	16	14	1	1
NA													
Unaffected													
Left	0	10	4	3	34	7	6	9	14	9	4	1	0
Right	2	8	7	4	3	5	6	1	1	1	9	1	1
Affected													
Left	4	3	3	3	7	2	1	12	4	7	10	0	1
Right	2	5	0	2	8	4	1	8	7	5	5	0	0

See Table 3 for key.

Table 6—Characteristics of QTL of hip joint traits mapped in a Labrador Retriever pedigree by use of a microsatellite-based genome-wide screen.

CFA	Trait	Flanking markers	Interval between flanking markers (cM)	Position (cM)	LOD	Polygenic heritability	QTL heritability	SE
01	PC2, L	FH3300–REN112I02	9.031	55	3.13	0.08	0.31	0.08
01	PC2, R	C01.424–C01.251	7.656	70	2.32	0.08	0.28	0.13
02	PC1, R	FH3965–C02.342	8.244	70	2.01	3.73×10^{-7}	0.51	0.11
10	NA, R	FH2422–DTR10.5	5.460	55	2.66	4.31×10^{-7}	0.26	0.10
10	High NA*	FH2422–DTR10.5	5.460	55	2.85	0.12	0.27	0.11
10	PC2, R	FH2422–DTR10.5	5.460	55	2.33	0.05	0.26	0.12
20	Low DLS score†	FH2158–REN114M19	8.832	60	2.01	3.11×10^{-7}	0.38	0.14
20	Low DLS score†	FH2158–REN114M19	8.832	60	2.27	6.76×10^{-7}	0.39	0.14
20	PC2, R	FH2951–REN100J13	10.432	30	3.32	3.73×10^{-7}	0.47	0.05
20	PC1, R	FH2158–REN114M19	8.832	60	2.44	3.44×10^{-7}	0.54	0.06
22	Low DLS score†	REN49F22–REN42F10	7.740	0	2.10	7.62×10^{-7}	0.34	0.14
32	PC2, R	CPH2–REN41D20	3.066	5	2.35	1.17×10^{-6}	0.36	0.11

The analysis was conducted by use of a variance-component method.
*A high NA was defined as $> 105°$. †A low DLS score was defined as $< 45\%$.
PC2 = Second principal component. L = Left hip joint. R = Right hip joint. PC1 = First principal component.

9.1 cM. Thirteen chromosomes (CFAs 07, 10, 12, 13, 14, 15, 16, 17, 18, 23, 26, 30, and 38) had an intermarker interval > 10 cM.

QTL analysis—Quantitative trait loci were detected for each trait or for the principal components of each trait. Six chromosomes had a putative QTL for 1 or more traits at an LOD score > 2.0 (CFAs 02, 10, 22, and 32) or > 3.0 (CFA01 and CFA20; **Table 6**). There were 3 chromosomes (CFAs 02, 22, and 32) for which a putative QTL for only 1 trait was mapped. However, QTL for several traits were mapped to the same locations on CFA10 and CFA20, which strengthened the assumption that the QTL were not detected by chance. The QTL with the highest LOD (LOD = 3.32) was found for the second principal component of the DLS score and NA for the right hip joint on CFA20. Putative QTL on CFAs 10, 20, and 22 were mapped to the same chromosomal region in the purebred Labrador Retriever pedigree as in the Greyhound–Labrador Retriever pedigree reported in another study.[18] The QTL found on CFA32 at 5 cM in the study reported here was detected at 16 and 20 cM in German Shepherd Dogs in another study.[17] Quantitative trait loci were detected for other traits and for the contralateral hip joint, but only those with an LOD score > 2.0 were reported to reduce the false-positive error rate but still identify those QTL that warrant further investigation.

Quantitative genetic variation results from the combined effects of genetic and environmental factors. The QTL heritability ranged from 2.51×10^{-8} to 0.54 (Table 6). The highest QTL heritability was found for the first principal component on CFA02 and CFA20. Mean estimates of QTL location ranged from 3 to 10 cM (as determined on the basis of the flanking marker interval). For QTL with a small effect (QTL heritability = 0.05),[36] there is less power to estimate QTL location than for QTL with a large effect, as indicated by results of our study. No QTL effect with a heritability < 0.25 was mapped or achieved an LOD score > 2.

Discussion

Quantitative trait loci influencing HD expression and osteoarthritis of the hip joint in dogs have been identified on several chromosomes in several studies (CFA03[15]; CFAs 04, 09, 10, 11, 16, 20, 22, 25, 29, 30, 35, and 37[18]; and CFAs 01, 03, 04, 08, 09, 16, 19, 26, and 33[17]). The QTL for osteoarthritis of the hip joints in Labrador Retrievers were mapped on CFAs 05, 18, 23, and 31.[37] Conformation of the hip joints in Portuguese Water Dogs has been measured by use of the NA,[15] and 2 QTL have been identified on CFA01 associated with subluxation of the hip joints. In the purebred Labrador Retrievers reported here, CFA01 also harbored a QTL associated with the second principal component of the DLS score and the NA. A QTL was identified on CFA01 (as determined on the basis of a 5-point scale) from an extended-hip ventrodorsal radiographic view in German Shepherd Dogs.[17] Furthermore, QTL for HD were identified on CFAs 01, 04, 09, and 16 in Labrador Retrievers[18] and German Shepherd Dogs.[17] Additional QTL have been identified on CFAs 01, 20, and 22 in German Shepherd Dogs and Labrador Retrievers. The identification of QTL in several studies in different breeds of dogs provides convincing evidence that at least 1 QTL on each of several chromosomes (CFAs 01, 03, 04, 09, and 16) affects hip joint conformation in > 1 breed of dog. Six Labrador Retrievers in the Greyhound–Labrador Retriever pedigree were included in the pedigree for the purebred Labrador Retrievers. Three putative QTL on CFAs 10, 20, and 22 were the same in the Greyhound–Labrador Retrievers. Analysis of this result suggested that these QTL were likely the same QTL that transferred from the same parents. However, as expected, more QTL were detected in the pedigree for the Greyhound–Labrador Retriever crossbred dogs because a breed resistant to development of HD was crossbred with a breed strain susceptible to the condition in which the trait was segregating, thus broadening the phenotypic extremes and maximizing marker information.

Although discovery of genetic markers and the genes that underlie HD is our major objective, understanding factors that affect linkage mapping power and resolution will influence the confidence of researchers to proceed with fine-resolution mapping and candidate gene screening on the basis of coarse mapping results. The microsatellite markers were sufficiently informative in this population to enable successful mapping. Only 9 markers had < 3 alleles. We did not measure every trait on each dog, which limited the power of the principal components of the traits for mapping. Nevertheless, we detected QTL on CFAs 01, 02, and 32 for principal components of the DLS score and the NA at LOD scores > 2.0. Had we not measured several traits on each dog, we would have detected only 3 of the 6 QTL in this study. The principal components on the traits with the most mapping power (DLS score and NA) in this pedigree can be used in combination to best predict whether a hip joint will develop osteoarthritis.[14]

Results of QTL mapping vary with the statistical analysis used; they also vary on the basis of the structure of the mapping population. There are limits to the ability of researchers to locate and estimate the position of individual and linked QTL. Multiple linked QTL usually bias estimations of QTL location and effects. This bias has a more serious effect on small-effect QTL than on large-effect QTL. Moreover, QTL mapping is also complicated by factors such as QTL environmental interaction or QTL epistasis. The methods used in the study reported here did not specifically test for QTL epistasis, but there are methods for conducting such testing in large populations.[38] The Labrador Retrievers in our mapping population were reared in the same environment and fed for maximal growth.[20] Therefore, this pedigree was not designed to study QTL by environmental interactions. Furthermore, the Labrador Retriever pedigree used for mapping was not structured specifically for genetic mapping studies. Rather, it was constructed for use in evaluating secondary osteoarthritis of the hip joints. As such, the mapping power of this pedigree was not optimized. Even so, analysis revealed that there were several QTL segregating in the Labrador Retriever pedigree, and their effects varied among loci. Moreover, QTL heritability varied among traits, which affected the power to detect QTL. A higher heritability

indicates an increased ability to detect and locate QTL. High heritability for HD has been found in Flat-Coated Retrievers (mean ± SD, 0.74 ± 0.25), Newfoundlands (0.49 ± 0.08), Golden Retrievers (0.34 ± 0.09 and 0.47 ± 0.08 for males and females, respectively), and Labrador Retrievers (0.54 ± 0.21 and 0.60 ± 0.13 for males and females, respectively).[9,39] In contrast, the heritability of HD is low in Gordon Setters (0.20 ± 0.10).[38] The ability to detect QTL in Labrador Retrievers is probably enhanced by the high heritability of HD in this breed.

Quantitative trait loci were detected in our Labrador Retriever population for individual traits and their principal components. The QTL for the second principal component of the left and right hip joints for the DLS score and NA on CFA01 were estimated to be in approximately the same chromosomal position (55 cM for the left hip DLS score and NA and 70 cM for the right hip DLS score and NA). Such colocalization of the QTL for left and right hip joints and detection of a QTL on CFA01 for HD in 3 separate studies,[15,17] including the study reported here, strengthens support for the existence of a QTL on that chromosome and indicates that it would be easy to justify use of that QTL for positional cloning. The support for the presence of a QTL on CFA10 and CFA20 was also strengthened by the colocalization for individual traits and principal components of the DLS score and NA. The QTL on CFAs 02, 22, and 32 were only detected for a single trait in 1 hip joint of the dogs.

Small-effect QTL can be detected with higher power when the family is large enough,[37] and this may explain the reason that we did not detect some QTL for HD that had been identified in other populations, such as German Shepherd Dogs.[17] Also, mapping QTL in purebred dogs may reveal different major and minor QTL, compared with those within a crossbred population based on the same purebred dogs. Some QTL alleles may be fixed or not segregating in 1 population and therefore will not be detectable. The genetic background of a particular breed will also affect the ability to detect QTL and influence or modify QTL expression.

We did not find significant QTL on CFA11 and CFA29 that reached a statistical threshold at the 1% value, as reported in crossbred dogs.[18] In the crossbred dogs, these QTL reached an LOD score of approximately 3. Some markers were missing in the current analysis, especially on CFA29. The QTL on CFA11 and CFA29 had a high QTL heritability (range, 0.31 to 0.46) associated with the NA, but the QTL did not reach an arbitrary LOD score > 2 for reporting. Finding the balance between declaring a statistically significant mapping result and ignoring a potentially important QTL for further investigation is controversial. Our analysis was conducted on a chromosome-by-chromosome basis, and the LOD score and heritability associated with the putative QTL were provided by the software. An LOD score of 3.0 to 3.3 has traditionally been considered a score that has genome-wide statistical significance because it indicates the probability that a type 1 error was made is < 1/1,000 tests. For a strict Bonferroni correction for multiple testing of 276 individual microsatellite markers, as was conducted in the study reported here, the genome-wide *P* value would be < 0.003. An LOD score of 2 indicates that the probability of a type 1 error is < 1/100. Therefore, we chose to report an LOD score that balanced the type 1 and type 2 error rates. It is still unclear whether a QTL can influence 1 side of an animal (in this case the right or left hip joint) more than the other side. Such a QTL has been reported[15] for the NA in Portuguese Water Dogs; thus, there is precedent for the higher LOD scores for QTL on the left or right hip joint, as we reported here for these Labrador Retrievers. Nevertheless, it is also possible that the effect for a specific side was false or that the power was insufficient to detect the QTL on both sides for the same trait.

The objective of QTL mapping is to apply genetic testing and marker-assisted selection that may improve screening in extremely young animals. It should assist in preventing carriers with mutant alleles from entering the gene pool during breeding, thereby decreasing the incidence of the trait. Moreover, estimates of the QTL location and effect can be used for fine-resolution mapping and positional cloning of candidate genes.

a. PennHIP, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa.
b. NHLBI Mammalian Genotyping Service, Marshfield Medical Research Foundation, Marshfield, Wis.
c. ABI 3770 sequencer, Applied Biosystems, Foster City, Calif.
d. Zhiwu Zhang, Cornell University, Ithaca, NY. Available at: www.people.cornell.edu/pages/zz19/research/genoped. Accessed Nov 1, 2008.
e. SAS, version 9, SAS Institute Inc, Cary, NC.
f. QTL Express, University of Edinburgh, Edinburgh, Scotland.
g. LOKI, University of Washington, Seattle, Wash.

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