Identification of quantitative trait loci for osteoarthritis of hip joints in dogs

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Objective—To identify quantitative trait loci (QTL) associated with osteoarthritis (OA) of hip joints of dogs by use of a whole-genome microsatellite scan.

Animals—116 founder, backcross, F_1, and F_2 dogs from a crossbred pedigree.

Procedures—Necropsy scores and an optimized set of 342 microsatellite markers were used for interval mapping by means of a combined backcross and F_2 design module from an online statistical program. Breed and sex were included in the model as fixed effects. Age of dog at necropsy and body weight at 8 months of age were also included in the model as covariates. The chromosomal location at which the highest F score was obtained was considered the best estimate of a QTL position. Chromosome-wide significance thresholds were determined empirically from 10,000 permutations of marker genotypes.

Results—4 chromosomes contained putative QTL for OA of hip joints in dogs at the 5% chromosome-wide significance threshold: chromosomes 5, 18, 23, and 31.

Conclusions and Clinical Relevance—Osteoarthritis of canine hip joints is a complex disease to which many genes and environmental factors contribute. Identification of contributing QTL is a strategy to elucidate the genetic mechanisms that underlie this disease. Refinement of the putative QTL and subsequent candidate gene studies are needed to identify the genes involved in the disease process. (*Am J Vet Res* 2008;69:1294–1300)

Osteoarthritis is a common disease of diarthrosis (synovial) joints that is characterized by degeneration of articular cartilage, formation of new bone at joint margins, and pain or loss of function. The osteoarthritic disease process involves the entire synovial joint, which encompasses the synovium, fibrous joint capsule, ligaments, articular cartilage, and underlying bone. Development of OA in hip joints is likely to involve multiple factors, including genetic and nongenetic variables. Nongenetic factors that contribute to OA include nutrition, body weight, and physical factors such as joint mechanics. Dogs maintained on a restricted diet during growth and development are less likely to develop hip subluxation and secondary OA in hip and shoulder joints and lumbar vertebrae than are dogs fed an unrestricted diet.[1] Moreover, just as obesity exacerbates OA of hip joints in humans,[2,3] dogs with rapid growth and weight gain are more likely to develop severe OA of hip joints than are dogs on restricted feeding.[4]

Abbreviations

CFA	*Canis familiaris* chromosome
EHR	Extended-hip joint radiograph
OA	Osteoarthritis
PIC	Polymorphism information content
QTL	Quantitative trait loci

Articular cartilage degeneration in dysplastic canine hip joints is defined as secondary OA and is related to hip displacement, which causes impingement of the acetabulum on the femoral head at a site at which the cartilage is prone to lesions.[5] The resultant abnormal focal load and stress leads to erosion of articular cartilage and synovial effusion, followed by subchondral sclerosis and osteophyte formation.[6] Cartilage degeneration is uncommon in nondysplastic hip joints.

The contribution of genetic factors to susceptibility to OA is increasingly recognized. Various studies in humans (eg, epidemiologic, twin-pair, and sibling-risk studies), including genome-wide–scanning[7,8] and candidate-gene[9,10] studies, have provided evidence of an important genetic component in the development of OA in human hip joints. The probability of genetic influence on the development of OA in hip and knee joints in humans was estimated to be as high as 65% in a twin study.[11,12] A study[13] of the inheritance patterns of secondary OA of hip joints

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in related Labrador Retriever, Greyhound, and crossbreed dogs revealed that the inheritance process was additive, which suggested that dissection of the underlying genetic variation into QTL by use of genetic linkage analysis would be possible. In Portuguese Water Dogs, a QT locus for acetabular osteophyte formation in hip joints has been mapped to CFA03,[14] and susceptibility loci for canine hip dysplasia have been mapped to several chromosomes.[15,16]

In dogs with hip dysplasia, the pathologic process that underlies the development of OA in multiple joints[17] is not completely understood, but genetic susceptibility may be involved, and joint biomechanics likely play a major role in the predilection for the development of OA in hip joints. To our knowledge, the role of genetic susceptibility to OA secondary to hip dysplasia has not been elucidated. The incidence and progression of secondary OA in dogs with hip dysplasia vary with breed; large and giant breeds are most at risk.[18] Therefore, genetic variation (breed predisposition) and body weight may be involved in the development and severity of OA of hip joints and in the expression of associated clinical signs.

Despite advances in genetic investigations of OA, the origins of cartilage degeneration in OA of humans and other animals are poorly understood, and fundamental questions regarding the precise molecular nature of this complex disease remain unanswered. The objective of the study reported here was to increase understanding of the molecular basis of OA in canine hip joints via identification of QTL for secondary OA of hip joints in dogs from the same pedigree by use of a whole-genome microsatellite scan.

Materials and Methods

Animals—An experimental canine pedigree was developed for linkage analysis of canine hip dysplasia and OA from crosses between dysplastic Labrador Retrievers and unaffected Greyhounds.[19] The 9 founder Labrador Retrievers were from a colony maintained at the Baker Institute for Animal Health. All but one of the Labrador Retrievers were dysplastic on the basis of EHR findings[20] at 2 years of age; that 1 dog had fair hip conformation. The 7 founder Greyhounds were acquired from racing stock; all had good to excellent hip conformation on EHRs obtained at 2 to 3 years of age. Greyhounds were chosen for our study because the breed is one of the few in which hip joint conformation is consistently excellent.[21] Dogs from the first filial generation (F_1) were bred back to Greyhound and Labrador Retriever parents or were intercrossed. The pedigree used in this study consisted of 9 Labrador Retriever and 7 Greyhound founders, 93 backcross and F_2 dogs, and 7 F_1 dogs (**Figure 1**). This crossbred pedigree had adequate power to detect linkage for OA secondary to canine hip dysplasia.[22]

Feeding regimens were designed to achieve a maximum growth rate for maximum expression of hip dysplasia and secondary OA. The study was approved by the Cornell Institutional Animal Care and Use Committee.

Phenotypes—All dogs were humanely euthanized with an IV overdose of pentobarbital sodium. Age of dogs at euthanasia ranged from 8 to 132 months. During necropsy, hip joints were examined macroscopically, and a score (ranging from 0 to 4) was assigned to describe the status of each hip joint with respect to OA. A detailed description of the hip OA scoring method is provided elsewhere.[19] In short, the score was determined by taking into consideration the volume of synovial fluid, volume of the round ligament of the femoral head, and degree of perifoveal articular cartilage degeneration, which received a higher weighting than other abnormalities. Left and right hip necropsy scores for each dog were combined to generate 1 value between 0 and 8 (0 = no signs of OA; 8 = advanced OA in both hip joints).

Genotypes—A genome-wide scan was undertaken to identify QTL associated with hip OA secondary to hip dysplasia. One hundred eighteen dogs were genotyped at an optimized set of 342 microsatellite loci distributed on 38 autosomes and the X chromosome. An initial set of 240 markers was selected from the integrated linkage-radiation hybrid map of the canine genome. Genotyping at these 240 loci was undertaken at the National Heart, Lung, and Blood Institute Mammalian Genotyping Service in Marshfield, Wis. To provide better coverage of the genome, the initial set of microsatellite markers was augmented with more markers from the minimal-screen set 2.[23] Of 327 markers available in the second set, 148 markers were common to our initial set of 240 markers. Of 179 markers unique to the second set, 102 markers on 16 chromosomes were genotyped and integrated with the 240 initial markers. The most recent version of the integrated canine genome map[24] was used to determine the marker order and intermarker distances.

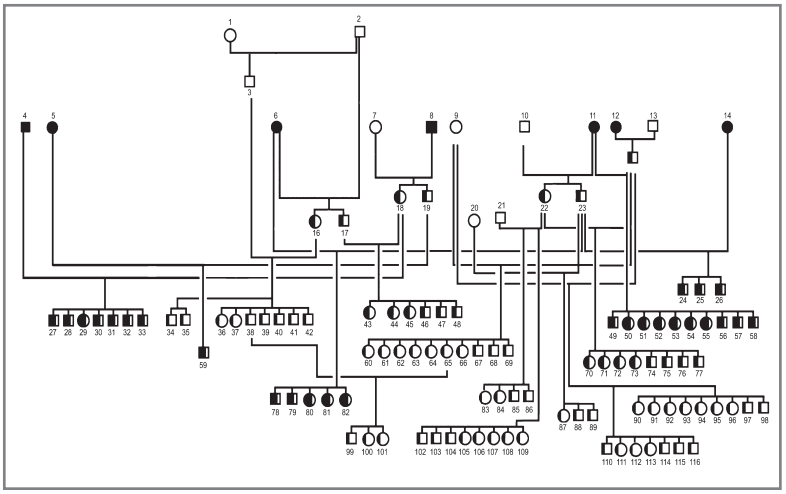

Figure 1—Diagram of a crossbred pedigree that was founded on Greyhounds without hip dysplasia (black symbols) and Labrador Retrievers with hip dysplasia (white symbols) and was used to identify QTL associated with OA of hip joints of dogs. Squares represent males and circles represent females. Filled and open portions of each symbol represent the respective proportion of Greyhound and Labrador Retriever alleles possessed by each dog.

To assess the quality of marker genotype data in our crossbreed pedigree, the total number of alleles and the mean number of alleles per locus were determined for each marker for the 2 founder groups, the F_1 breeders, and the backcross and F_2 groups. The PIC for each marker was determined separately for the 4 groups of dogs by use of the following equation:

$$PIC = 1 - \Sigma p_i^2 - 2 [\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} p_i^2 p_j^2]$$

where p_i is the frequency of the ith allele, p_j is the frequency of the alternate jth allele, and n is the number of alleles.[25]

Statistical methods—Interval mapping using a general linear model was performed by use of the combined backcross and F_2 design module from an online computer program.[26] The outcome of OA of hip joints was analyzed as a multinomial (range of possible values, 0 to 8) and binomial (0 = no OA; 1 = various degrees of OA in 1 or both hip joints) variable. The OA hip scores were binomially distributed (55% of scores = 0; 45% of scores ≥ 1), so the online program for quantitative variables was used because a binomial distribution with a value of p close to 0.5 is well approximated by a normal distribution. Breed (2 founder groups, F_1s, F_2s, and F_1s backcrossed to each founder group) and sex were included in the model as fixed effects. Age at necropsy (≤ 12 months; > 12 months to < 24 months; and ≥ 24 months) and body weight at 8 months of age were also included in the model to adjust for other factors known to affect hip OA. The chromosomal location that yielded the highest F score was considered the best estimate of the QTL position. Estimates were obtained for the additive and dominance effects of the putative QTL at that location. Genome-wide and chromosome-wide significance thresholds for each trait were determined empirically by permuting the genotypes; the threshold was obtained from 10,000 permutations. A value of $P < 0.05$ was considered significant for genome- and chromosome-wide analyses. Data on dog characteristics are reported as mean ± SD.

Results

Characterization of dogs and hip joints—Hip joints of 52 male and 48 female dogs were grossly assessed at necropsy, and the OA scores for all offspring were included in the QTL mapping analysis. Mean ± SD body weight of dogs at 8 months of age was 29.4 ± 4.2 kg for males and 25.0 ± 3.0 kg for females. Fifty-three dogs were ≤ 12 months old at necropsy, 31 dogs were > 12 months but < 24 months old, and 16 dogs were ≥ 24 months old. Seven dogs were older than 36 months of age at necropsy (range, 72 to 132 months). Six of these 7 dogs were Labrador Retriever or Greyhound founders. Mean age at necropsy of 93 dogs (without the 7 F_1 dogs) was 14.1 ± 7.6 months. The 7 F_1 dogs and the founder (parental) phenotypes were not used in the linkage analysis. Osteoarthritis was not detected in 55 dogs at necropsy; 45 other dogs had various degrees of OA in hip joints. The proportions of dogs with OA by age at necropsy were 32.0% for dogs ≤ 12 months old, 58.0% for dogs > 12 months but < 24 months old, and 62.5% for dogs > 24 months old (**Figure 2**). Of 45 dogs with OA, the hip joints of only 16 (35.6%) dogs were affected with OA (7 dogs on the right hip and 9 dogs on the left hip), and 29 dogs had both hips affected. Twenty-four of 45 (53.3%) dogs with OA had necropsy scores ≤ 2; 2 (4.4%) were classified as having advanced OA in both hip joints (**Figure 3**).

Marker informativeness—Mean number of alleles for the 342 microsatellite marker loci in the entire population was 4.3 (range, 1 to 15); only 6 (1.7%) markers were fixed for the same allele. Summary statistics for PIC, including the mean number of alleles per locus, median, 25% and 75% quartiles, and range for the 2 founder breeds and F_1 and backcross generations, were summarized (**Table 1**). On the basis of established criteria for construction of a genetic linkage map,[25] 230 (67%) markers were deemed highly informative (heterozygosity ≥ 0.59), 87 (26%) markers were moderately

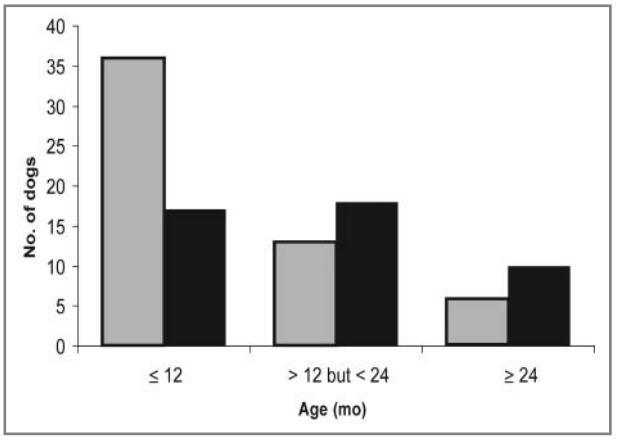

Figure 2—Distribution of dogs (n = 100) from a crossbred pedigree founded on Greyhounds without hip dysplasia and Labrador Retrievers with hip dysplasia and in which OA was (black bars) or was not (gray bars) detected via necropsy, by age at necropsy.

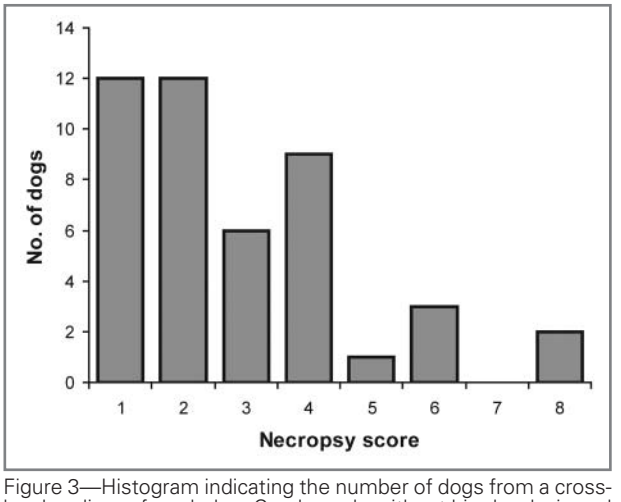

Figure 3—Histogram indicating the number of dogs from a crossbred pedigree founded on Greyhounds without hip dysplasia and Labrador Retrievers with hip dysplasia with OA of hip joints, categorized by necropsy score. Necropsy scores ranged from 0 (no signs of OA) to 8 (severe OA).

Table 1—Descriptive statistics from a whole-genome scan involving 342 microsatellite markers screened in 116 dogs from an experimental canine pedigree representing Labrador Retriever and Greyhound founders and F_1 and backcross generations.

		No. of alleles per marker			PIC		
	No. of dogs	Mean	Maximum	Median	25%	75%	Range
Labrador Retriever	9	3.53	9	0.54	0.37	0.65	0–0.84
Greyhound	7	4.01	9	0.57	0.37	0.68	0–0.86
F_1	7	4.19	10	0.59	0.46	0.71	0–0.88
Backcross	93	5.33	15	0.63	0.46	0.72	0–0.89

Table 2—Parameter estimates for significant ($P < 0.05$) QTL for necropsy score as a multinomial (scale of 0 to 8) or binomial trait identified via chromosome-wide *F* ratio tests performed during a genome-wide screen for QTL for OA in hip joints of dogs.

			Additive effect		Dominance effect		Effect of sex			
CFA	Trait	Flanking markers	Estimate	SE	Estimate	SE	Estimate	SE	F score	LOD
CFA05	NS(0/1)	FH3004–REN42N13	–0.30	0.10	–0.27	0.10	–0.36	0.09	6.45	2.60
CFA18	NS(0-8)	FH2429–AHT130	1.17	0.36	–0.46	0.38	–1.44	0.43	6.77	2.73
CFA23	NS(0/1)	C23.745–FH2001	–0.09	0.11	0.40	0.12	–0.34	0.09	6.71	2.70
CFA31	NS(0/1)	FH2189–RVC11	–0.29	0.10	0.07	0.10	–0.35	0.09	5.36	2.19

Additive (a) and dominance (d) QTL effects correspond to genotype values of d, +a, and –a for heterozygotes, dogs that inherited 2 Greyhound QTL alleles, and dogs that inherited 2 Labrador Retriever alleles, respectively. Positive additive effects indicate Greyhound alleles that conferred protection against secondary hip OA; negative additive effects indicate Labrador Retriever alleles that were permissive for the disease. A protective or improving effect would decrease the necropsy score.

LOD = Log of the odds ratio score.

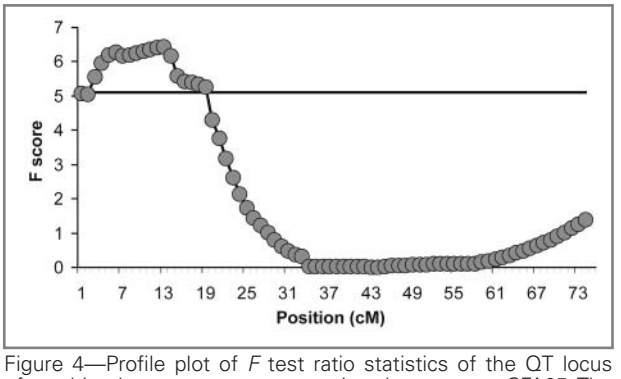

Figure 4—Profile plot of *F* test ratio statistics of the QT locus of combined necropsy score on canine chromosome CFA05. The peak of the curve is the best estimate of the QTL position, which is indicated in cM on the x-axis. Threshold at a chromosome-wide significance level ($P < 0.05$) is indicated by the solid horizontal line.

informative (heterozygosity > 0.3 but < 0.59), and 25 (7%) markers were uninformative (heterozygosity ≤ 0.3).

Mapping of QTL—Although genome-wide significance was not obtained, 4 chromosomes contained putative QTL for secondary OA of canine hip joints at the chromosome-wide significance threshold. The location and magnitude of the QT locus identified on chromosome 5 were graphically displayed (**Figure 4**). Sex was a significant variable for all 4 QTL identified in this study (**Table 2**). Females had a lower necropsy score than males. For example, for the QTL on CFAs 5, 23, and 31, the mean decrease in necropsy score in females compared to males ranged from 0.34 to 0.36. Age at necropsy and body weight at 8 months of age had no significant effect on the model.

The QTL locations with the highest *F* scores and variable estimates obtained at those locations for chromosomes with a significant QTL for secondary hip OA on the binomial or multinomial scale were summarized (Table 2). The QTL identified on chromosomes 5, 23 and 31 had a negative additive effect, whereas the locus identified on chromosome 18 had a positive additive effect, conferring protection against OA of hip joints.

Discussion

Secondary OA is a complex trait that is influenced by several genes and environmental factors. Identification of the QTL that contribute to OA of hip joints is a step toward revealing the genetic mechanism that underlies the associated phenotype. Subsequent refinement of the putative QTL will aid in the identification of polymorphisms or mutations in candidate genes that contribute to disease susceptibility, which should open new opportunities for more effective treatment and prevention strategies. The genes within the QTL that account for substantial amounts of variation could be used for early identification of dogs at risk of developing OA in hip joints, which would allow for early initiation of preventive measures to reduce that risk.

An investigation[13] of the mode of inheritance of OA of hip joints in the crossbreed pedigree (Greyhounds crossed with Labrador Retrievers) used in our study revealed that OA is additively inherited, without dominance; each Labrador Retriever allele increased the necropsy score for OA by 0.7 to 0.9 points (on a 4-point scale), compared with a Greyhound allele. Sex was a significant variable in that study, but age at necropsy had no relationship with necropsy score. In the present study, body weight at 8 months of age and age at necropsy, known to be important factors in OA, were not significantly associated with OA. However, because 84% of dogs were < 24 months old at the time necropsies were performed and hip joints were evaluated, age

and body weight may not have been identified as risk factors for OA in our study because these factors may not be important in young dogs.

Results of the study reported here suggested that the crossbred pedigree we used is suitable for investigating the genetic basis of secondary OA of hip joints. The diversity in founder trait characteristics and advancements in canine mapping tools may have enhanced the power of the experimental pedigree used in this study[22] to successfully detect QTL. Advantages of the pedigree include the facts that traits were carefully ascertained,[27–29] the crossbreeding strategy was specifically engineered for this purpose, and the environment in which dogs were bred and reared was controlled. The crossbred nature of the pedigree also optimized the informativeness of microsatellite markers[29]; maximized the range of OA scores, compared with those expected for a purebred pedigree (eg, of only Labrador Retrievers); and introduced alleles at the QTL that contributed to trait expression and clearly conferred susceptibility to, or protection from, secondary OA of hip joints. For example, the QT locus on CFA18 had a strong effect. Dogs that carried both alleles at this QT locus from Greyhound founders had a necropsy score > 1 point lower than the mean scores in this pedigree. On the other hand, dogs that carried both Labrador Retriever alleles at this locus had a necropsy score that increased by > 1 point than the mean score in the pedigree.

The number of markers and statistical methods used in the present study helped identify 4 chromosomes that contained QTL that were likely to underlie the expression of OA in canine hip joints. A genome-wide analysis[16] of the crossbred pedigree identified 12 chromosomes containing putative QTL for hip dysplasia. None of the chromosomes containing putative QTL for OA of hip joints were significantly associated with the expression of hip dysplasia. The differences in chromosomal locations of the QTL identified in dogs with hip dysplasia and QTL in those with OA suggested a different genetic mechanism associated with hip dysplasia and secondary OA of hip joints. However, the power to detect QTL through the genome-wide scan used in our study could have been lower than the power of the analysis in which the QTL for hip dysplasia were identified. The smaller number of dogs with OA necropsy data (93 dogs, compared with 159 dogs in the linkage analysis for hip dysplasia) and the categoric nature of OA data, compared with quantitative hip dysplasia phenotypes, are just 2 factors that may have contributed to differences in putative QTL for the 2 traits. Only QTL with major effects on the phenotype would likely be detected with the methods and number of dogs used in this analysis, whereas a locus with a smaller effect (so-called polygene) that possibly contributed less than 5% to the disease variance would not have been detected. Another explanation of the difference in loci associated with the development of OA in hip joints, compared with those apparently associated with the development of hip dysplasia, may be the difference between a passive evaluation of a hip phenotype based on a radiograph versus a dynamic evaluation that reflects a functional assessment. Presumably, functional subluxation of a dysplastic hip would eventually result in OA of hip joints. As it stands, there is no easy means of measuring dynamic hip function. All available methods for hip evaluation are passive except for clinical assessment of the ambulating dog.

Linkage analyses of the formation of caudal acetabular osteophytes in hip joints of Portuguese Water Dogs identified a QTL on CFA03 associated with the OA phenotype,[14] but such a locus was not identified on that chromosome in our study of OA of hip joints. Because the incidence and progression of OA vary with breed, the set of genes contributing to the disease in Portuguese Water Dogs and Labrador Retrievers may be different or background polygenes may affect the ability to detect major genes. Moreover, mean age of dogs in the 2 studies was different. Although the median age of Portuguese Water Dogs was 6 years (range, 1.7 to 17 years), the median age of dogs in the present study was 0.83 years (range, 0.67 to 3 years); only 1 dog was 11 years old at the time of necropsy. Therefore, our study may have identified QTL involved in the early stages of OA of hip joints, and the QT locus on CFA03 described in another report[14] may be associated with later stages of the disease. In addition, the 2 studies used different criteria to determine whether a joint had changes indicative of secondary OA. We used evidence based on gross evaluation at necropsy. The other research group[14] used subjective radiographic evidence of secondary OA. Both studies may have included dogs that had false-negative test results for OA. For example, dogs euthanized at a young age may not have developed gross cartilage lesions and capsular changes, and radiography is insensitive to early OA-related changes that may be obscured by muscle in synovial joints like hip joints. Additionally, bone must undergo a 30% to 40% change in density (eg, osteophytes must form) before OA-related changes can be detected radiographically. Therefore, OA must be severe to observe these alterations.

Several advances in identification of loci for human susceptibility to OA have been made in the past few years. Genome-wide linkage scans were completed, and novel genes were subsequently identified. Completion of sequencing of the canine genome has facilitated comparison of canine and human genomes, allowing additional investigation of the canine QTL identified in the present study. The region on CFA18 (flanked by markers AHT130 and FH2429 and identified as containing a putative QTL for OA of hip joints in our study) is syntenic to region 11q12.2-q12.3 on human chromosome 11 that was fine mapped as a primary locus for susceptibility to OA of hip joints.[7,30] Similarly, the region on CFA19 between markers FH2279 and Ren91I14 (a region that approached significance for containing a putative QT locus in the current study but did not pass the threshold) is syntenic to human 2q14.2-2q21.1 adjacent to region 2q24.3-2q31.1, which has been linked to a locus for OA of hip joints that segregates in various human populations.[31] Several investigators have identified polymorphisms in individual genes that are associated with OA of hip and knee joints in humans. One research group reported[32] an association between the frizzled B–related gene and OA of hip joints in females. Frizzled B codes for secreted frizzled-related protein 3, an antagonist of Wnt signaling. The Wnt signal transduction pathway is critical for healthy development

and is also active in adult tissues. Secreted frizzled-related protein 3 helps to maintain articular cartilage, and the associated alleles at frizzled-related protein B reduce the activity of this important protein. A Japanese group[33] has reported an association of the asporin gene with OA of knee and hip joints and an association of the calmodulin 1 gene with OA of hip joints. Asporin is a cartilage extracellular protein that regulates the activity of transforming growth factor-β. Calmodulin is an intracellular protein that interacts with proteins involved in signal transduction. The alleles of the asporin and calmodulin 1 genes that are associated with the development of OA reduce the ability of chondrocytes to express the genes encoding aggrecan and type II collagen. Because aggrecan and type II collagen are essential structural components of articular cartilage, these alleles of asporin and calmodulin 1 genes are predicted to adversely affect the maintenance of articular cartilage. The identification of polymorphisms in frizzled B–related gene, asporin, and calmodulin 1 genes that are associated with OA of hip and knee joints suggests that polymorphisms in signal transduction pathways are a major component of susceptibility to OA. This is an exciting observation because signal transduction pathways are malleable and therefore potentially amenable to intervention and modification.[34]

Secondary OA of hip joints in dogs is a complex disease, and more than 1 physiologic or metabolic pathway is likely involved in the etiology and progression of the disease. Refinement of putative QTL will likely narrow the chromosomal regions enough that strong candidate genes can be investigated. Refinement of putative QTL could be undertaken through single nucleotide polymorphism association analysis in the crossbred pedigree used in our study and in other Labrador Retrievers.

Dogs from the population in the present study were used in a separate microarray experiment in which a commercial canine genome microarray was used to identify genes differentially expressed in cartilage lesions from dogs with OA of hip joints and in cartilage from hip joints of clinically normal dogs.[35,a] Subsequent quantitative reverse transcription–PCR assay confirmed the results of the microarray study for 2 selected genes: mitogen-inducible gene 6 or gene 33 and dynein cytoplasmic light chain.[36,b] We selected the top 200 genes identified by the microarray experiment as greatly upregulated or downregulated in articular cartilage lesions versus healthy cartilage and placed them on the genome. Some genes that were upregulated or downregulated were located on CFA05 or CFA18, in the vicinity of the QTL locations for OA that were identified in the present study. Five of the 10 genes on CFA18 were located within 10 cM of the QTL peak, and 5 of 12 genes on CFA05 were located within 20 cM of the QTL peak. Integration of different but complementary genomic approaches will help identify strong candidate genes to be explored for their contribution to the biochemical mechanism of OA of canine hip joints.

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