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Mammalian Genome
Genes and Phenotypes

Quantitative trait loci for hip dysplasia in a crossbreed canine pedigree

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Abstract

Canine hip dysplasia is a common developmental inherited trait characterized by hip laxity, subluxation or incongruity of the femoral head and acetabulum in affected hips. The inheritance pattern is complex and the mutations contributing to trait expression are unknown. In the study reported here, 240 microsatellite markers distributed in 38 autosomes and the X chromosome were genotyped on 152 dogs from three generations of a crossbred pedigree based on trait-free Greyhound and dysplastic Labrador Retriever founders. Interval mapping was undertaken to map the QTL underlying the quantitative dysplastic traits of maximum passive hip laxity (the distraction index), the dorsolateral subluxation score, and the Norberg angle. Permutation testing was used to derive the chromosome-wide level of significance at $p < 0.05$ for each QTL. Chromosomes 4, 9, 10, 11 ($p < 0.01$), 16, 20, 22, 25, 29 ($p < 0.01$), 30, 35, and 37 harbor putative QTL for one or more traits. Successful detection of QTL was due to the crossbreed pedigree, multiple-trait measurements, control of environmental background, and marked advancement in canine mapping tools.

Introduction

Hip dysplasia in dogs is an inherited, complex, developmental trait characterized by hip laxity and subluxation (Todhunter and Lust 2003). The abnormal mechanical environment results in osteoarthritis, hip pain, and physical disability. Almost all breeds of dog are afflicted with the trait; the prevalence in some breeds is over 50% (Kaneene et al. 1997), making it one of the most common orthopedic traits of dogs. The clinical and radiographic key features of the canine trait are similar to the human condition called developmental dysplasia of the hip or congenital hip dysplasia. The general incidence of hip dysplasia in humans has been estimated at 1-10 per 1000 live births (Weinstein 1996). Familial aggregation and genetic predisposition to developmental dysplasia of the human hip have been reported (James and Wynne-Davies 1969; Beighton et al. 1994; Baronciani et al. 1997; Holderbaum et al. 1999; Sollazzo et al. 2000).

For canine hip dysplasia, estimated heritability ranges between about 0.2 and 0.7, dependent on the breed and pedigree studied and the radiographic method of hip assessment (Todhunter et al. 2003). The inherited characteristic most commonly advanced as a cause of hip dysplasia in both humans and dogs is joint laxity. At the Seeing Eye Inc., Morristown, NJ, heritability for the distraction index (DI), which measures maximum passive laxity of the hip, was approximately 0.5 in German Shepherds and about 0.6 for Labrador Retrievers (www.vet. upenn.edu/researchcenters/pennhip; Leighton et al. 2001). An interstitial duplication of HSA15q24-26 (DUP25) was associated with joint laxity in humans; stratification of these patients according to joint laxity enabled identification of the DUP25 locus which was also associated with social phobias (Gratacos et al. 2001).

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Radiographic measures of hip conformation in dogs include the Norberg angle (Olsson 1961), the dorsolateral subluxation (DLS) score (Farese et al. 1998, 1999) (equivalent to the center edge angle of Wiberg measured in human hips), and the distraction index (DI) (Lust et al. 1993; Smith et al. 1993, 1995; Smith 1997; Smith et al. 2001). Hips with better conformation have high Norberg angles, high DLS scores, and low DIs. The ascertainment reliability of these dysplastic traits for subsequent development of osteoarthritis has been studied extensively (Lust et al. 2001a, 2001b; Todhunter et al. 2003).

To systematically map QTL contributing to hip dysplasia expression, we developed a pedigree founded on dysplastic Labrador Retrievers and trait-free Greyhounds (Todhunter et al. 1999). This pedigree comprised backcrosses of the F_1s to both founder groups and F_2s. Quantitative genetic analysis showed an additive mode of inheritance for the DI and the DLS score and dominance deviation also contributed to variation in the DLS score (Bliss et al. 2002). In a single-marker, linkage-based simulation, it was shown that this experimental pedigree had a power of 0.8, at an α level of 0.05, to detect underlying QTL with a genome-wide screen in the backcross generation (Todhunter et al. 2003b). Microsatellite markers tested had heterozygosities in the F_1 generation ranging from 0.68 to 0.82 (Todhunter et al. 2003a, 2003b).

Based on published linkage and radiation hybrid maps of the dog (Breen et al. 2001; Richman et al. 2001), 240 microsatellite markers that span the genome were selected for genotyping. More recently, a 3300-marker canine map at 1-Mb resolution was made available (Guyon et al. 2003). Using these tools, Chase et al. (2004) discovered QTL on *Canis familiaris* CFA01 that contributed to Norberg angle expression in a pedigree of Portuguese Water Dogs. One QTL linked to marker FH2598 on the telomeric end of CFA1 explained 16% of the variance of the left-hip Norberg angle and marker FH2524, 95 Mb centromeric, was linked to a QTL contributing 15% of the variance to the right hip Norberg angle. The only reported mapping of human hip dysplasia has been for Beukes familial hip dysplasia to human 4q35 (Roby et al. 1999). In this article we describe the identification of QTL for the Norberg angle in addition to several other dysplastic traits in a cross-breed, dysplastic pedigree.

Materials and methods

Animal pedigree. Seven Greyhounds (two males and five females) from racing stock with good or excellent hip conformation, seven Labrador Retrievers (four males and three females) with hip dysplasia, and one female Labrador Retriever from a dysplastic lineage but with fair hip conformation herself were intercrossed. The F_1 families were backcrossed to both founder groups and intercrossed to form F_2 families. All dogs were housed, bred, and fed *ad libitum* for maximal hip dysplasia expression in the same controlled conditions in the same facility (Todhunter et al. 1999). The mean and distribution of the trait measures of the different breed groups have been described (Todhunter et al. 1999; Bliss et al. 2002). Since those publications, additional backcrosses to the Labrador Retriever founders were bred. For QTL detection, we sampled 152 dogs from three generations and 20 families in which there were 1–9 offspring (Fig. 1).

Phenotypes. Dogs' hips were radiographed and the traits were measured under general anesthesia when the dogs were skeletally mature at 8 months of age as described (Bliss et al. 2002). The ventro (antero) dorsal (posterior) hip-extended radiograph (EHR) was used to score the hips for the presence or absence of hip subluxation based on a traditional 7-point scale described by the Orthopedic Foundation for Animals (OFA) (Rendano and Ryan 1985; Henry 1992) (Fig. 2A). From this projection, hip subluxation was also measured as the Norberg angle (NA) (Olsson 1961; Lawson 1963); lower NAs indicate subluxation; high angles indicate hip congruity. The DI, an indicator of maximum passive hip laxity, was measured from the ventrodorsal distraction radiograph by a commercial organization (PennHip, University of Pennsylvania, Philadelphia, PA). High DIs indicate a high probability of development of secondary hip osteoarthritis, while low DIs indicate protection against secondary hip osteoarthritis (Lust et al. 1993; Smith 1997; Smith et al. 2001) (Fig. 2B). The DLS score was measured on the dorsoventral (posteroanterior) radiograph (Farese et al. 1998) (Fig. 2C) with the hips orientated and loaded in a weight-bearing (neutral) position rather than in the extended-hip position. A high DLS score indicates a low probability of developing hip osteoarthritis as a result of hip dysplasia and a low score indicates a high probability of developing hip osteoarthritis. The traditional gold standard of whether a hip is dysplastic is whether it develops secondary osteoarthritis (Lust et al. 2001a, 2001b).

Canine hip dysplasia is usually expressed bilaterally but can be unilateral (Todhunter et al. 1997). The trait measures on each hip are usually different. Because they are not independent measures, the NA, DI, and DLS on the right and left hip were analyzed separately. To account for this asymmetry while

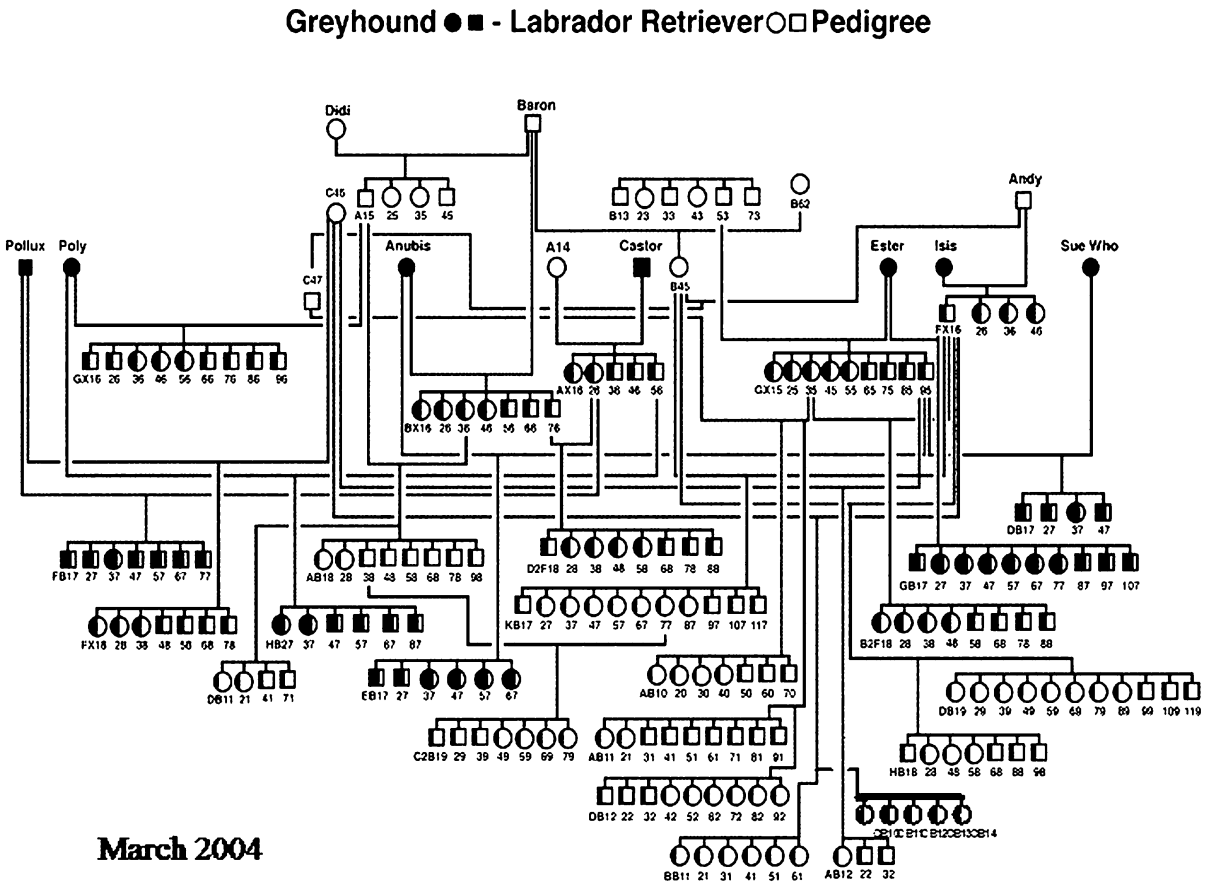

Fig. 1. Diagram of a crossbred pedigree founded on trait-free Greyhounds and dysplastic Labrador Retrievers. Squares and circles represent males and females, respectively. Filled and open portions of each symbol represent the proportion of Greyhound and Labrador Retriever alleles, respectively, possessed by that dog.

maintaining independence between hips within individuals, each trait for the most or least affected hip (low and high) was also analyzed. Because some of these hip traits are correlated (Farese et al. 1999; Lust et al. 2001a; Todhunter et al. 2003c), a principal component analysis was performed to transform the NA, DI, and DLS scores on the right and left sides and on the most and least affected hip into a set of two uncorrelated (orthogonal) variables, called principal components for each particular set of traits (SAS Institute 1988). Principal component analysis (PCA) is a multivariate procedure that rotates the data such that maximum variabilities are projected onto the axes. Essentially, a set of correlated variables is transformed into a set of uncorrelated variables that are ordered by reducing variability. The uncorrelated variables are linear combinations of the original variables and the last of these variables can be removed from a statistical analysis with minimum loss of information. The main use of PCA is to reduce the dimensionality of a data set while retaining as much information as is possible. The first principal component is the combination of variables that explains the greatest amount of variation. The second principal component defines the next largest amount of variation and is independent of the first principal component. There can be as many possible principal components as there are variables. For our data set, the first and second components explained about 70% and about 22% of the variance in the data, respectively. Therefore, we used only the first and second principal components for mapping. In summary, a total of 20 traits were evaluated to identify QTL affecting hip quality.

Genotype determination. Breen et al. (2001) constructed an integrated linkage/radiation hybrid (RH) map of the canine genome. That RH map comprised 1078 microsatellites, 320 dog gene markers, and 102 chromosome-specific markers, and the linkage map contained 354 canine-specific microsatellite markers. These two maps were integrated through sets of common markers distributed on both maps, 251 RH/genetic, 102 RH/cytogenetic, and 52 linkage/

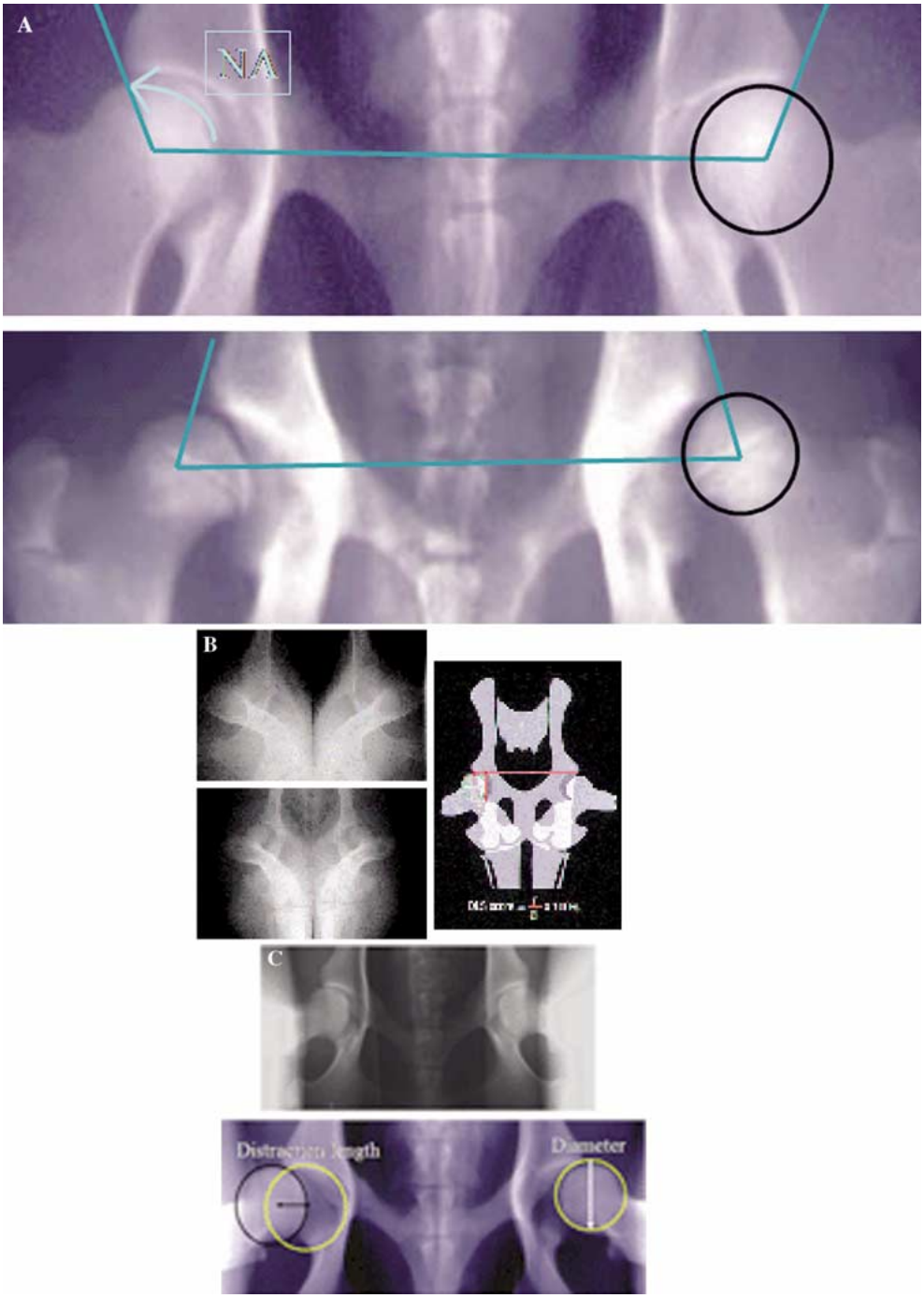

Fig. 2. Images of radiographs (unaffected, top of each panel; dysplastic, bottom of each panel) of the extended-hip method (top) from which a score of hip conformation is derived which ranges from 1 (excellent) to the 7 (poorest) hip conformation. **A**. The Norberg angle is measured from this radiograph by extending a line connecting the geometric centers of each femoral head to the cranio (proximal) dorsal (posterior) acetabulum and measuring this included angle (Olsson 1961). **B**. The dorsolateral subluxation score is measured on this radiographic image (Farese et al. 1998) and is equivalent to the percent of femoral head covered by the acetabulum. The distance of the femoral head is within the acetabulum is expressed as a percentage of the femoral head diameter. **C**. The distraction index is measured on the distraction view as the maximum lateral passive hip laxity (Smith 1997). The distance between the geometric centers of the femoral head and acetabulum is divided by the diameter of the femoral head.

cytogenetic. From this integrated map, we selected 240 microsatellite markers, 142 of which were from the linkage map and 98 of which were from the RH map (Lou et al. 2003). These 240 markers are distributed in 38 autosomes and the X chromosome at an average marker interval of about 10 cM. Based on map

data at the time, we had selected most markers no further than 20–30 cM apart (one intermarker distance was 40 cM and one was 32 cM). Five additional microsatellite markers were identified for CFA37 in collaboration with E. Kirkness, The Institute for Genomic Research, Rockville, MD, and the most likely order and spacing of markers on this chromosome were calculated based on meiotic recombination in our pedigree by multipoint analysis with the program MULTIMAP (Matise et al. 1993). DNA was isolated from peripheral blood by phenol:chloroform extraction, and genotyping on all 152 dogs was undertaken at the NHLBI Mammalian Genotyping Service, Marshfield Medical Research Foundation, Marshfield, WI (Weber and Broman 2001).

The genotypic data was first analyzed to detect Mendelian errors using the software Genoped (http://people.cornell.edu/pages/zz19/research/genoped). Tetranucleotide repeat markers, with high error rates due to small differences in called allele sizes (1–2-bp difference), were corrected assuming that the marker allele size in the grandparents was correct.

Marker map. To improve the accuracy of our statistical analyses and marker order, we used the most recent version of the integrated canine genome map (Breen et al. 2004) which encompasses 4249 markers (900 genes, 1589 microsatellites, and 1760 BACs) with 3090 unique positions.

Statistical methods. QTL mapping was performed using a regression approach originally described by Haley and Knott (1992). A web-based version of this regression interval mapping is available (Seaton et al. 2002). The software, QTL Express (http://latte.cap.ed.ac.uk/), analyzes data from different mating designs including the combined backcross/F_2 design appropriate for our data from crossbreed pedigrees. Briefly, for incremental positions along the marker map, the Identity-By-Descent (IBD) probabilities of QTL alleles are estimated from flanking marker data. Subsequently, a statistical model is fitted to trait observations and the IBD probabilities. The basic statistical model describing the observations was

$$Y = \mu + \beta_1 X_1 + \beta_2 X_2 + e$$

where Y is the observed phenotype, X_1 and X_2 are the probabilities for QTL genotypes conditional on the flanking marker genotypes, i.e.,

$$X_1 = P(QQ|M_iM_j) - P(qq|M_iM_j)$$
$$X_2 = P(Qq|M_iM_j)$$

The regression coefficients β_1 and β_2 measure the difference between the homozygote QTL genotypes (additive effect) and the QTL dominance effect, respectively.

The general linear model allows for fixed effects and covariates to be included. Breed (two founder groups, F_1s, F_2s, and two backcross groups) was always included in the model as a fixed effect.

Every centimorgan, the offspring phenotypes were regressed onto the coefficients of the additive and dominance effects. *F*-test statistics were calculated as the ratio of the residual sums of squares in a model with the QTL (''full'') and a model without it (''reduced'') at each location along each chromosome. Estimates were obtained for the additive and dominance effects of the putative QTL at that location in the backcross/F_2 population. The location giving the highest *F*-ratio statistic was considered to be the best estimate for the position of the QTL. To investigate whether the effect of the putative QTL was different in male vs. female offspring, sex was included in each model but was subsequently dropped from the models because it was not significant.

Chromosome-wide significance thresholds for each trait were determined empirically by permuting the marker data (Churchill 2000); the threshold at $p < 0.05$ and at $p < 0.01$ was obtained from 1000 permutations.

Results

Genome-wide screening set and marker heterozygosity. Based on the criteria of Botstein et al. (1980), 166 markers (69%) were highly informative (heterozygosity > 0.59), 58 markers (24%) were moderately informative (0.3 < heterozygosity < 0.59), and 16 markers (7%) were uninformative (heterozygosity < 0.3). Based on the most recent integrated dog map (Breen et al. 2004), the greatest distance between the markers we selected was 35 cM. This 240-marker set was estimated to cover about 2000 cM or 80% of the canine genome (based on a 2400-cM canine genome length). The allele calling error rate over all markers was estimated at about 6%, but most of these errors occurred in 20 markers as a result of errors of 1–2 bp in allele size estimation of tetranucleotides. The errors made in the remainder of the markers averaged 1% error. Repeat genotyping of parents with each litter also enabled some of the genotyping errors to be detected. Newer iterations of the Marshfield canine microsatellite genotyping set do not include these uninformative markers and the size of this set has been increased to at least a 250-marker set.

Table 1. Parameter estimates for the QTL with F tests significant at $p < 0.05$ (chromosome-wide) for several hip dysplasia traits following a genome-wide screen for QTL

						QTL effect estimates (SE)		
Trait[a]	*CFA*[b]	*Bracketing markers*	*Position*	*F*	*LOD*[c]	*a*	*d*	*Mean*
NA, L	4	FH2457-AHT103	0	5.31	2.21	−2.25 (0.92)	1.15 (0.96)	108.23 (1.93)
PC2[d]	9	REN177B24-REN278L10	1	5.47	2.27	0.4 (0.18)	−0.24 (0.19)	0.1 (0.31)
NA, Hi	10	FH2422-C10.16	27	5.72	2.37	1.12 (0.83)	−2.2 (0.86)	113.69 (1.63)
DI, L	11	FH2096-AHT137	63	6.41*	2.64	−0.01 (0.03)	0.11 (0.03)	0.22 (0.06)
DI, Lo	11	FH2096-AHT137	64	5.96	2.47	−0.03 (0.03)	0.09 (0.03)	0.18 (0.06)
PC2[e]	16	REN292N24-C16.147	0	3.72	1.57	0.37 (0.21)	−0.26 (0.22)	0.12 (0.34)
NA, Lo	16	REN292N24-C16.147	0	4.66	1.95	1.78 (1.17)	−2.12 (1.19)	110.71 (2.12)
NA, R	16	REN292N24-C16.147	0	3.35	1.42	1.08 (1.12)	−2.06 (1.15)	112.56 (2.05)
NA, Hi	16	FH2175-REN130B10	10	3.27	1.38	0.75 (0.82)	−1.47 (0.84)	113.02 (1.62)
NA, L	16	FH2175-REN130B10	10	3.81	1.61	1.51 (0.99)	−1.42 (1.01)	111.32 (1.95)
DLS, R	16	FH2175-REN130B10	10	2.61	1.11	0.95 (2.04)	-3.79 (2.09)	69.03 (4.04)
DLS, Hi	20	FH2158-REN193A22	0	5.17	2.15	−2.77 (2.28)	5.39 (2.38)	65.56 (4.41)
DLS, Lo	20	FH2158-REN193A22	0	4.66	1.95	−1.88 (2.32)	5.79 (2.42)	63.8 (4.48)
DLS, R	20	FH2158-REN193A22	0	5.34	2.22	−2.92 (2.3)	5.45 (2.4)	63.54 (4.44)
DLS, Hi	22	FH2538-C22.279	6	4.69	1.96	5.27 (2.29)	−2.68 (2.41)	72.7 (4.05)
DLS, L	22	FH2538-C22.279	7	4.69	1.96	6.03 (2.5)	−2.5 (2.66)	72.42 (4.4)
NA, Hi	25	FH2006-FH2087L	12	3.91	1.64	0.002 (0.9)	2.44 (0.96)	109.55 (1.79)
PC1[f]	25	FH2006-FH2087L	18	3.83	1.61	−0.09 (0.16)	0.4 (0.18)	0.09 (0.32)
NA, R	29	REN45F03-FH2328	7	3.43	1.45	−2.24 (0.91)	−0.04 (0.96)	109.63 (1.9)
NA, Hi	29	REN45F03-FH2328	8	3.56	1.50	−1.82 (0.77)	2.1 (0.81)	110.93 (1.62)
NA, Lo	29	FH2328-FH2609	9	6.84*	2.81	−2.17 (0.91)	1.65 (0.95)	106.77 (1.93)
NA, L	29	FH2328-FH2609	12	6.29*	2.60	−1.95 (0.94)	1.86 (0.99)	107.96 (1.92)
DLS, L	29	FH2328-FH2609	9	3.67	1.55	−3.35 (2.02)	2.89 (2.12)	64.47 (4.3)
PC2[g]	29	REN45F03-FH2328	3	3.94	1.66	−0.49 (0.18)	−0.23 (0.19)	−0.4 (0.33)
PC2[e]	29	REN45F03-FH2328	7	3.94	1.66	−0.38 (0.17)	0.15 (0.18)	−0.42 (0.32)
PC2[f]	29	FH2328-FH2609	19	3.76	1.58	−0.49 (0.19)	−0.04 (0.2)	−0.51 (0.32)
PC1[d]	29	FH2328-FH2609	9	3.64	1.54	−0.22 (0.16)	0.28 (0.17)	0.21 (0.3)
PC2[d]	29	FH2328-FH2609	23	4.73*	1.98	−0.42 (0.19)	0.23 (0.2)	−0.56 (0.32)
DI, R	30	REN105i08-REN248F14	18	4.95	2.06	0.01 (0.04)	−0.13 (0.05)	0.39 (0.06)
PC1[e]	30	REN105i08-REN248F14	18	3.98	1.67	−0.14 (0.21)	0.6 (0.24)	0.05 (0.31)
DLS, Hi	35	REN214H22-REN01G01	18	3.86	1.63	−3.97 (2.58)	4.46 (2.62)	64.09 (4.11)
PC2[f]	37	CE3707-CE3711	42	4.64	1.94	0.29 (0.18)	−0.3 (0.18)	−0.02 (0.32)
PC2[g]	37	CE3707-CE3711	43	4.73	1.98	0.28 (0.19)	−0.34 (0.19)	0.07 (0.33)

[a]L = left, R = right, Lo = low, and Hi = high, DI = distraction indices, DLS = dorsolateral subluxation, NA = Norberg angle, PC1 = first principal component, and PC2 = second principal component.
[b]CFA = chromosome for *Canis familiaris*.
[c]LOD = logarithm of the odds.
[d]Principal component of left distraction index, left dorsolateral subluxation score, and left Norberg angle.
[e]Principal component of high distraction index, low dorsolateral subluxation score, and low Norberg angle (the worst hip combination).
[f]Principal component of low distraction index, high dorsolateral subluxation score, and high Norberg angle (the best hip combination).
[g]Principal component of right distraction index, right dorsolateral subluxation score, and right Norberg angle.
*Chromosome-wide p value <0.01.

QTL mapping. Twelve chromosomes were identified to harbor putative QTL for one or more traits at the 5% chromosome-wide significance level (Table 1). Canine chromosomes 11 and 29 harbor QTL that exceeded the threshold at the 1% level. QTL for the DLS score were detected on five chromosomes, for the NA on five, for the DI on two, and for different principal components on six. Eight of the 12 chromosomes harbored putative QTL for more than one trait. The same QTL on these eight chromosomes appear to affect multiple traits because they each mapped to the same chromosomal location and contributed similar additive effect on the traits in both magnitude and direction (Table 1). Identification of QTL contributing to multiple traits and measured on different radiographs taken in three different body positions provides some assurance that the QTL are real.

Examination of chromosomes harboring putative QTL revealed that on most of these chromosomes, many of the other traits analyzed indicated the likely presence of an underlying QTL in the same position even though QTL for these traits did not reach statistical significance. On CFA 22, for example, one QTL that contributed to the low DLS score and the DLS score on the left hip was identified at 22 cM. A high *F* statistic at the same position was obtained for the NA (low, high, on the right and on the left side)

and for some principal components without reaching the significance threshold (Fig. 3A-C). This pattern, where many traits suggest the presence of a QTL but only one or two traits reached significance thresholds, applied to most of the chromosomes where putative QTL were identified.

Table 1 presents the QTL location giving the highest *F*-test statistic and the parameter estimates obtained at this location for chromosomes with a significant QTL. Additive (*a*) and dominance (*d*) QTL effects correspond to genotype values of $+a$, d, and $-a$ for individuals having inherited two Greyhound QTL alleles, heterozygotes, and individuals with two Labrador Retriever alleles, respectively. Positive additive effects indicate that the Greyhound alleles confer protection against hip dysplasia while negative additive effects indicate that the Labrador Retriever alleles were permissive for the trait. A protective or improving effect would increase the DLS score and the NA and decrease the DI. QTL for several traits (on CFA10, 11, 16, and 22) had alleles with a protective effect, while QTL identified on CFA4, 20, 29, 30, and 35 had alleles conferring a worsening influence on hip conformation.

An examination of the additive (i.e., half the difference between homozygotes) and dominance effects (i.e., the difference between the heterozygote and the mean of the homozygotes) revealed that QTL on five chromosomes (CFA4, 9, 16, 22, and 29) had a marked additive effect (greater than 2 mean standard errors), while QTL on another five chromosomes (CFA10, 11, 20, 25, and 30) had a marked dominance effect. The QTL on CFA11, 25, and 30 had a completely dominant effect indicated by a large dominance (d/a) ratio.

Discussion

This is the third report of successful QTL mapping in dogs. Both this screen and that reported by Chase et al. (2004) in Portuguese Water Dogs focused on hip dysplasia, a common, inherited orthopedic trait in dogs (Todhunter and Lust 2003). The other paper by Chase et al. (2002) dealt with QTL mapping of skeletal morphologic traits in the same Portuguese Water Dog pedigree in which they mapped the QTL for hip dysplasia.

Twelve chromosomes exhibited evidence for the presence of putative QTL based on the limited number of markers and the statistical methods used. The power of this pedigree for successful detection of QTL was a result of diversity in founder trait characteristics and advancement in canine mapping tools. Advantages of the pedigree include the careful trait ascertainment (Lust et al. 2001a, 2001b), the measurement of multiple traits that were shown to be clearly inherited (Bliss et al. 2002; Todhunter et al. 2003a), its specially constructed, crossbred nature, and the controlled environment in which the dogs were bred and reared to maturity. The crossbred nature of the pedigree optimized the informativeness of the microsatellites (Todhunter et al. 2003a), maximized the spread of the trait measures compared with that expected for a purebreed pedigree e.g., of only Labrador Retrievers, and introduced alleles at the QTL contributing to trait expression that clearly confer susceptibility to, and protection from, hip dysplasia.

Several features of this analysis begin to explain the complicated nature of canine hip dysplasia. The multiple QTL that contribute to trait expression clearly show the polygenic underpinnings of the variation in hip dysplasia phenotype. Measuring multiple traits for hip dysplasia allowed us to detect the maximum number of QTL with this coarse genome-wide screen. Significant QTL on four chromosomes were identified for at least two traits while significant QTL on eight chromosomes were unique to different traits. However, on most of the chromosomes harboring QTL for only one trait, many other traits exhibited a high *F*-test statistic without reaching statistical significance. The contributing effect of the same QTL to expression of several dysplastic traits provides supporting evidence that the mapping is detecting loci that truly contribute to hip dysplasia expression. However, our conclusions must be tempered by the knowledge that mapping in modest-sized pedigrees inflates parameter estimates compared with that which might be observed in large populations. In addition, the limited number of recombinations and markers used reduced mapping resolution and accuracy.

In hindsight, the marker coverage on some of these chromosomes was not sufficiently dense for optimum coarse screening. Until fine mapping is completed within this pedigree and across pedigrees, these QTL could contain more than one gene contributing to each trait or there may be more than one QTL in some of these intervals. At present, it appears that each of these QTL inform the different traits to one extent or another. Partial correlation of some of these traits might also explain the overlap. The DI, for example, explains about 35% of the variation in the DLS score (Lust et al. 2001a). Detection of QTL for different traits in different chromosomes may also be explained by sampling variation. Some traits may have greater power than others for QTL detection. With the data in hand, we postulate that it is the particular haplotype over all of these QTL that the dog carries that contributes to the observed hip

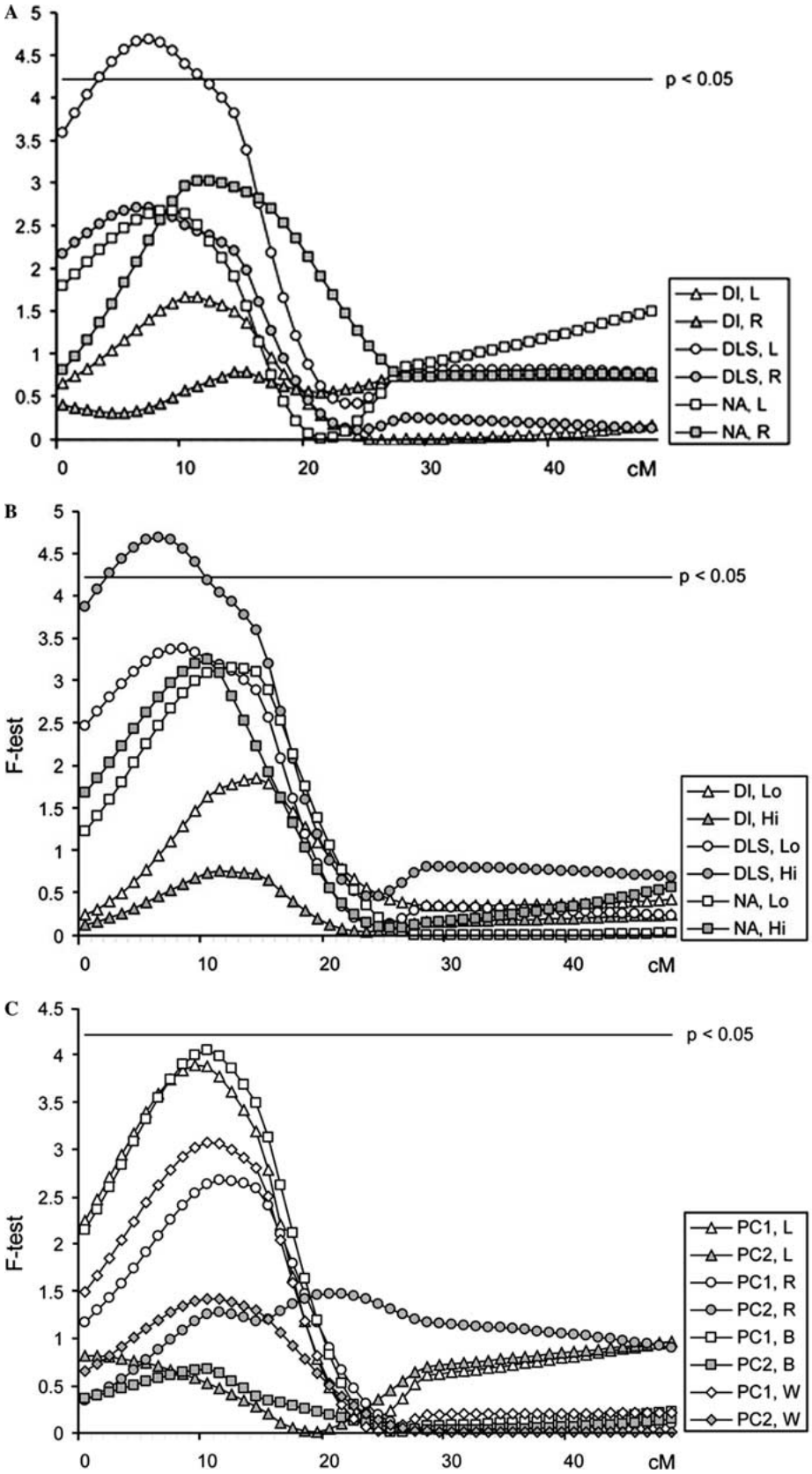

Fig. 3. A. Profile plots of *F*-test statistics (vertical axis) of the QTL underlying the distraction index (DI), dorsolateral subluxation score (DLS), and Norberg angle (NA) on the left and right hip on CFA22. The peaks represent positions of the QTL in cM on the *X* axis across the chromosome. The threshold at a chromosome-wide significance level of 0.05 is drawn as a solid line across the plot. **B**. Profile plots of *F*-test statistics (vertical axis) of the QTL underlying the low and high distraction index (DI), dorsolateral subluxation score (DLS), and Norberg angle (NA) on CFA22. The peaks represent positions of the QTL in cM on the *X* axis across the chromosome. The threshold at a chromosome-wide significance level of 0.05 is drawn as a solid line across the plot. **C**. Profile plots of *F*-test statistics (vertical axis) of the QTL underlying the first and second principal components on the left (L) and right (R) side and most affected (W) and least affected (B) hip. The threshold at a chromosome-wide significance level of 0.05 is drawn as a solid line across the plot.

phenotype. We previously concluded that the DI and DLS scores were controlled by a single locus (Todhunter et al. 2003a). This analysis was based on the traits and genetic relationships of dogs in the crossbreed pedigree and the Labrador Retrievers related to the founders of the crossbreed that we used for QTL mapping in this report. The assumption of a very large population for estimation of gene numbers contributing to trait expression was not met in that analysis. This estimate was based on a biometric, not a molecular, method. Until finer mapping, composite mapping, and mapping in purebreeds is complete, we still cannot rule in or out the possibility that a major locus is involved (Leighton et al. 2001), and our simulations have suggested that a major locus for the DI may be involved (Todhunter et al. 2003b).

Some QTL alleles conferred protection against hip dysplasia, e.g., the QTL on CFA22 which increased the DLS score by 6% compared with the overall trait mean at the locus. Conversely, the QTL on CFA20 conferred susceptibility for hip dysplasia by decreasing the DLS score by approximately 2% compared with the mean. The QTL conferring protection and susceptibility for hip dysplasia were detected with approximately equal distribution over all traits, i.e., it did not appear that a protective QTL for one trait was purely Greyhound in origin or that a susceptibility QTL for another trait was purely Labrador Retriever in origin (Table 1). A salient question that remains is which alleles will be segregating in a pure Labrador Retriever breed in the general population. Some of these QTL alleles may be fixed at these loci in the founder breeds. Answers to this will come from the fine mapping and association studies in purebreeds. Even though the incidence of hip dysplasia in Greyhounds is very low (http://www.offa.org/index/html), it is unlikely that the dog-breeding community will allow introgression of protective Greyhound alleles (by breeding to Greyhounds) into other purebreeds that are susceptible to hip dysplasia.

In recent years, new genomics resources and tools (including the receptivity of the Mammalian Genotyping Service, Marshfield, WI, to canine genotyping) have enabled the identification of QTL contributing to quantitative traits in many species. Principal component analysis of skeletal morphologic traits was used to uncover nine loci that contribute to skeletal morphology in a pedigree of 330 Portuguese Water Dogs (Chase et al. 2002). However, because there are no previous genome-wide scans reported for the hip traits we measured, except for the NA, it remains to be seen how many of these 12 putative QTL have biological underpinnings. Analyses of the NA in the Portuguese Water Dogs pedigree revealed a QTL on CFA01 (Chase et al. 2004). In our study CFA4, 10, 16, 25, and 29 harbored QTL for the NA but CFA1 did not. In addition, Chase et al. (2004) used a genome-wide level of significance. In our study, we used a less stringent, chromosome-wide significance level, albeit permutation-testing based. We argue that such an approach may have inflated the type I error rate, but it enabled us to not exclude putative QTL suitable for further investigation. We performed interval mapping, chromosome by chromosome, rather than use a composite mapping approach, which also suggests the application of a chromosome-wide, rather than a genome-wide, significance level. Likely explanations for the different results of Chase et al. (2004) and the study we report would be different QTL that segregate in a crossbred pedigree compared with purebreed Portuguese Water Dogs, differences in marker coverage of the genome between the two studies, in the power of the two pedigrees to detect QTL, sampling variation, and the measurement of multiple traits. The results from our study demonstrate that there is additional detection power in the use of more than one quantitative trait in complex trait mapping. In addition, the use of principal components enabled the detection of a QTL on CFA4 and provided corroborating evidence for the presence of a QTL on three other chromosomes.

Fine mapping within this pedigree, other pedigrees, and across breeds in order to further refine the map locations of these QTL will be a necessary next step to validate these results (Glazier et al. 2002). The fine-mapping strategy will be based partly on chromosomal regions in which we have mapped QTL with high *F* statistics. Other criteria would include corroborating evidence of QTL conferring susceptibility for human hip osteoarthritis or laxity based on canine-human synteny and on future information provided by other investigations of canine hip dysplasia.

In summary, fine mapping in this and other canine pedigrees will help direct the search for genetic variants and genes that contribute to hip dysplasia and secondary hip osteoarthritis. Fine mapping will necessitate genotyping additional microsatellites in the crossbreed dysplastic pedigree, linkage or joint linkage and linkage disequilibrium mapping (Lou et al. 2005) in Labrador Retrievers, association mapping in litters, and linkage disequilibrium mapping across different unrelated dogs and breeds using single nucleotide polymorphisms. Candidate gene screening could accompany fine mapping. Although the genetic variants themselves that contribute to, or protect against, hip dysplasia and related forms of

osteoarthritis may be different in dogs compared to humans, many of the biochemical pathways responsible for hip development and osteoarthritis expression are likely to be the same so that understanding the responsible pathway in the dog will help in the search for the equivalent human mechanisms.

Note added in proof: Maki et al. (Heredity 92: 402–408, 2004) reported evidence for major genes for hip dysplasia in four Finnish dog populations based on Bayesian segregation analysis. Chase et al. (Am J Med Genet 135A: 334–335, 2005) identified a QTL contributing 16% of variance to hip osteoarthritis secondary to hip dysplasia on CFA03 in Portuguese water dogs.

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