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Single nucleotide polymorphisms refine QTL intervals for hip joint laxity in dogs

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Summary

Hip laxity is one characteristic of canine hip dysplasia (CHD), an inheritable disease that leads to hip osteoarthritis. Using a genome-wide screen with 250 microsatellites in a crossbreed pedigree of 159 dysplastic Labrador retrievers and unaffected greyhounds, we previously identified putative ($P < 0.01$) QTL on canine chromosomes 11 and 29 (CFA11 and CFA29). To refine these QTL locations, we have genotyped 257 dogs including 105 Labrador retrievers, seven greyhounds, four generations of their crossbreed offspring and three German shepherds for 111 and 171 SNPs on CFA11 and CFA29 respectively. The distraction index (DI, a measure of maximum hip laxity) was used as an intermediate phenotype that predicts whether a hip joint will or will not develop osteoarthritis. Using a multipoint linkage analysis, significant evidence (95% posterior probability) was found for QTL contributing to hip laxity in the 16.2–21 cM region on CFA11 that explained 15–18% of the total variance in DI. Evidence for an independent QTL on CFA29 was weaker than that on CFA11. Identification of the causative mutation(s) will lead to better understanding of biochemical pathways in both dogs and humans with hip laxity and dysplasia.

Keywords hip dysplasia, quantitative trait loci, single nucleotide polymorphism.

Introduction

Hip dysplasia is one of the most common inherited orthopaedic traits in dogs that leads to osteoarthritis accompanied by pain in affected hips (Todhunter & Lust 2003). The phenotypic characteristics of canine hip dysplasia (CHD) are hip joint laxity and subluxation (Todhunter *et al.* 1997; Harcke & Grissom 1999). It has been proposed that abnormalities of supporting structures of the hip, especially the round ligament of the femoral head and the hip joint capsule that hold the femoral head and acetabulum together during development, could result in hip laxity (Riser 1987; Todhunter & Lust 2003).

Studies with pure-breed and crossbreed dogs have demonstrated a strong genetic basis for CHD (Leighton *et al.* 1977; Willis 1989; Burton-Wurster *et al.* 1993; Lust 1997; Bliss *et al.* 2002; Chase *et al.* 2004, 2005). Breur *et al.* (2002) reported that heritabilities for CHD were from 0.1 to 0.68. Four studies using variance estimates (Leighton 1997; Todhunter *et al.* 2003b; Janutta *et al.* 2006) and Bayesian modelling (Maki *et al.* 2004) showed evidence for a major locus contributing to CHD. The underling genetic factors that contribute to CHD are likely to be complicated. Asymmetric effects of QTL on each end of canine chromosome 1 (CFA1) contributed to CHD in the right vs. the left hip joints following a genome-wide screen of 286 Portuguese water dogs using about 500 microsatellite loci (Chase *et al.* 2004). These QTL explained 14–16% of the variation in the left and right Norberg angle, a measure of hip incongruency in hip dysplaisa (Chase *et al.* 2004).

Although dogs with hip laxity do not always have CHD, dogs with CHD have loose hips. Maximum lateral hip laxity in dogs is measured using the distraction index (DI) (Lust *et al.* 1993; Smith 1997; Smith *et al.* 2001), which can be considered an intermediate phenotype for hip dysplasia in dogs.

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To map loci that contributed to hip laxity, we previously conducted a genome-wide screen with 250 microsatellites using 159 dogs developed by crossing a line of dogs resistant to CHD (greyhound) with one susceptible to the trait (Labrador retriever). Twelve chromosomes harboured putative QTL (LOD > 2.0) contributing to CHD as documented radiographically (Todhunter *et al.* 2005). Among these, CFA11 and CFA29 showed significant evidence of linkage (chromosome-wide significance at $P < 0.01$) after accounting for the breed effect (Todhunter *et al.* 2005). In this study, we refined the location of the QTL on CFA11 and CFA29 using SNPs.

Materials and methods

Dogs

To maximize phenotypic variation for hip dysplasia, a canine pedigree was constructed by crossing eight Labrador retrievers (four males and four females) with hip dysplasia or secondary hip osteoarthritis (Todhunter *et al.* 1999) and seven greyhounds (two males and five females) from racing stock with excellent or good hip conformation (Beling *et al.* 1975). This produced a four-generation pedigree with 159 dogs. The DI was available on 254 dogs that included the two founder lines ($n = 15$), F_1 dogs ($n = 7$), F_2 dogs ($n = 16$), F_1 dogs backcrossed to founder Labrador retrievers ($n = 79$), F_1 dogs backcrossed to founder greyhounds ($n = 33$) and double backcrosses of Labrador retrievers (i.e. two dogs from the F_1 backcross crossed to founder Labrador retrievers, $n = 7$), as well as three German shepherd dogs and 97 unrelated Labrador retrievers. The pedigree structure is illustrated in Fig. S1.

Phenotypes

The DI measures the maximum amount of lateral hip laxity on both the left and right hips and is the distance between the geometric centres of the femoral head and the acetabulum divided by the diameter of the femoral head. The DI ranges from 0.1 to 1.2. In this study, DIs were measured using the PENNHIP program (University of Pennsylvania, http://www.pennhip.org/). Dogs with DIs greater than 0.7 have a high probability of developing HD and subsequent hip osteoarthritis, whereas dogs with DIs less than 0.4 are likely to be unaffected (Lust *et al.* 1993; Smith 1997).

Genotypes

Two hundred and fifty-seven dogs were genotyped. Extraction of genomic DNA from citrated whole blood was performed using the PureGene kit (Gentra). Based on the estimated QTL LOD score peak and profile from the analysis of the microsatellite-based genome-wide screen (Todhunter *et al.* 2005), 171 and 111 SNPs on CFA29 and CFA11 respectively were selected from the CANFAM 1.0 database at the Broad Institute of Harvard/MIT (http://www.broad.mit.edu/mammals/dog/snp/). On CFA29, we selected five SNPs over 100 kb (i.e. 1 SNP/25 kb), then skipped the next 800 kb and selected another five SNPs over the following 100 kb, then skipped the next 800 kb, etc. This selection process was repeated across the QTL on CFA29 to take advantage of both ancient and modern breed linkage disequilibrium (Lindblad-Toh *et al.* 2005). For fine-maping the QTL on CFA11, we selected one SNP every 200 kb with increased density under the QTL LOD score peak (Todhunter *et al.* 2005). The CANFAM 2.0 database was used to orientate the SNP positions. Accession numbers and locations of all SNPs in this study are listed in Table S1.

The Applied Biosystems SNPlex genotyping system, which uses an allele-specific ligation reaction to detect SNPs in genomic DNA, was used. Multiplexing of up to 48 SNPs was achieved by coupling allele-specific oligonucleotides with tag array sequences (ZipCode) and universal PCR amplification following the ligation reaction. After PCR amplification, which incorporated a biotin moiety into the amplicons, ZipCode-containing amplicons were bound to streptavidin-coated microtitre plates and used as capture reagents. Fluorescently labelled ZipChute molecules were captured, each containing a unique ZipCode sequence and engineered to have unique mobility/fluorescent properties, then eluted from the capture plates and analysed by capillary electrophoresis on an Applied Biosystems (ABI) 3730 automated DNA analyser with fluorescent size standards included in every sample. The retention of fluorescent ZipChutes on the streptavidin-coated plates and subsequent detection in the electropherogram indicated the presence of a SNP in the original DNA sample. Conversely, the absence of an individual ZipChute in the electropherogram indicated the absence of a SNP (Schweitzer *et al.* 2006). Data analysis was performed using the GeneMapper™ (ABI) software package.

We checked genotyping errors based on parental genotypes (when available) using a minimum correction algorithm (Zhu *et al.*, unpublished data). Polymorphism information content (PIC) (Botstein *et al.* 1980) at each marker locus was calculated for each breed or crossbreed as a measure of the SNP marker informativeness in the pedigree.

QTL analysis

Multipoint linkage analysis using LOKI, version 2.4.5 (Heath 1997) was used to test the probability of linkage to DI. The number of iterations for each Markov chain was 500 000. Estimates of parameters in the model were obtained with a burn-in = 20 000 iterations and continued every ten iterations.

The model for trait y (an $n \times 1$ vector, where n is the number of dogs with a single trait observation) is:

$$y = \mu + X\beta + \sum_{i=1}^{k} Q_i\alpha_i + e$$

where μ is the overall trait mean, β is an $m \times 1$ vector of fixed effects (sex and breed or crossbreed), α_i is a 2×1 vector of allele substitution effects for the ith biallelic QTL, X $(n \times m)$ and Q_i $(n \times 2)$ are incidence matrices for the covariate and the QTL effects and e is the random error. More generally, $\alpha = \begin{pmatrix} a \\ d \end{pmatrix}$, where a and d are the additive and dominance effects of a QTL with possible genotypes A_1A_1, A_1A_2 and A_2A_2 having effects a, d, $-a$ respectively. The genotypes for all loci (markers and QTL) were updated by a 'reverse peeling' algorithm based on the given pedigree (Ott 1989). The number of QTL (k) in the model was treated as a random variable and multiple QTL contributing simultaneously to the total trait variance were allowed. The Bayes factor (BF), a measure of linkage as a function of position along the two chromosomes, was estimated. A BF $\geq$ 20 indicated strong evidence for linkage, 20 > BF $\geq$ 3 indicated moderate evidence for linkage and 3 > BF $\geq$ 1 indicated weak evidence for linkage according to BF calibration tables (Raftery 1996). The Bayesian confidence intervals of 95% and 99% for the QTL location and the percentages of trait variance and genetic variance due to the QTL were also reported.

Results

Our pedigree of 257 dogs included 39 families with 19 loops (123 male and 134 female dogs). Genotypes for 111 and 171 SNPs on CFA11 and CFA29 respectively were included in the LOKI (Heath 1997) analysis. The PICs of CFA11 and CFA29 SNPs in the F_1 and F_2 generations were larger than in the parental lines (greyhounds and Labrador retrievers) as expected (results not shown). The variation of PICs along each chromosome is shown in Fig. 1.

The observed DI of 257 dogs in the analysis was approximately normally distributed (William 1971), with means 0.47 and 0.50 for the left and right hips respectively. The proportion of affection (DI > 0.7) was 11.7% for the left DI (DIL) and 13.2% for the right DI (DIR). The unaffected proportion (DI < 0.4) was 37.7% for DIL and 30.4% for DIR.

Posterior distributions of the number of QTL (k) were estimated for both the left and right hips on CFA11 and CFA29 (Table S2). One (CFA11) or two (CFA29) putative QTL were linked to the trait loci. Across-family results for DI are shown in Fig. 2, with the estimated Bayes factor as a function of the position along CFA11 and CFA29 for DI on both hips respectively. In general, CFA29 had weaker evidence of linkage to DI than CFA11. The presence of one QTL with a posterior probability of at least 70% was found on CFA11 at 19.7 cM with strong evidence (BF = 22) for DIL and at 19.6 cM (BF = 22) for DIR. The 95% and 99% posterior probability intervals for DIL on CFA11 were 4.8 cM (16.2–21 cM) and 1 cM (19.1–20.1 cM) respectively. The posterior probability intervals for the DIR QTL on the same chromosome were similarly located. The left and right symmetric QTL on the same chromosome may be because DIL and DIR were highly correlated (r = 0.753).

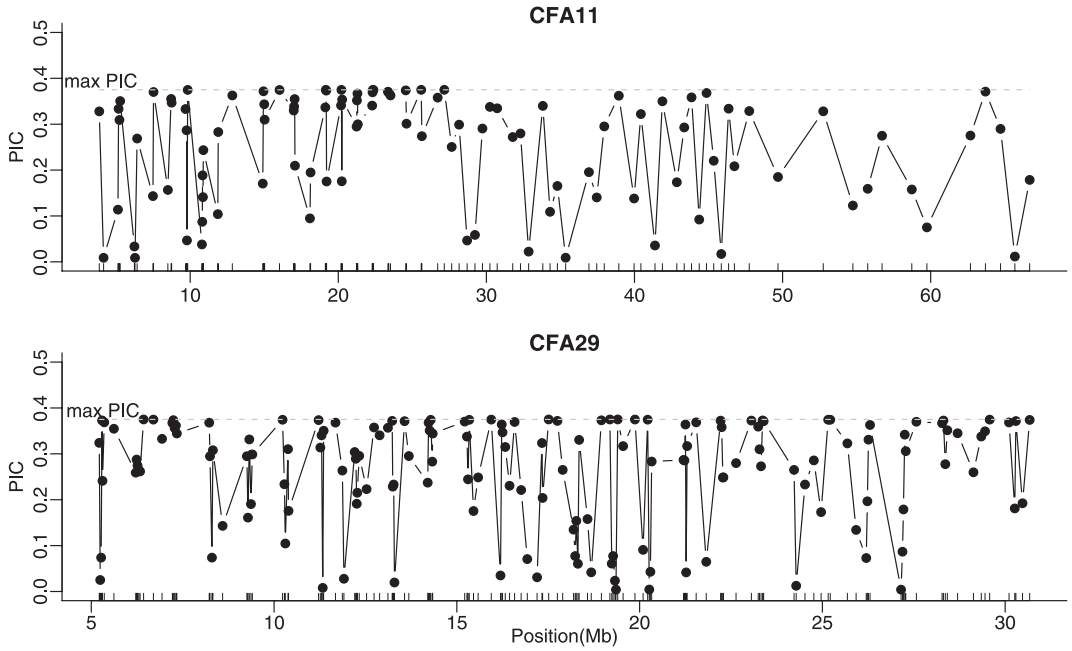

Figure 1 Polymorphic information content of SNPs along CFA11 and CFA29 respectively. Grey upright tick marks on the x-axis represent the SNP marker positions.

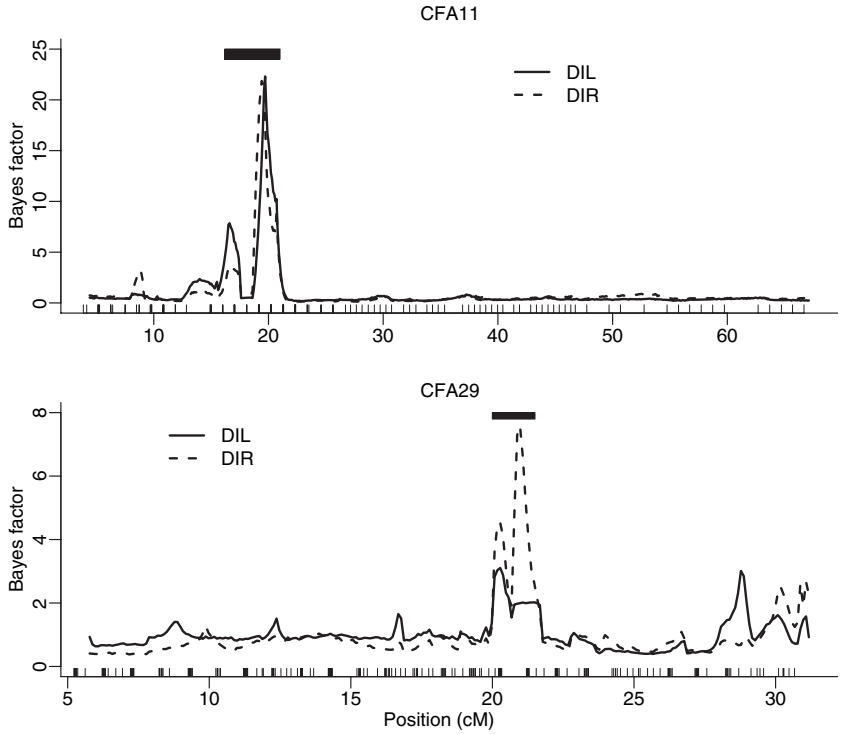

Figure 2 Estimated Bayes Factor for linkage (*y*-axis) as a function of position (cM) along CFA11 and CFA29 (*x*-axis) for the distraction index on left (DIL) and right (DIR) hips. Black horizontal bars above the peaks indicate the 95% posterior probability intervals. Grey upright tick marks on the *x*-axis represent SNP marker positions.

One QTL for DIL on CFA29 at 20.3 cM with moderate evidence (BF = 3.1) and two QTL for DIR on CFA29 at 20.3 cM (BF = 4.5) and at 21 cM (BF = 7.6) were found. The 95% and 99% posterior probability intervals for DIL on CFA29 were 1.4 cM (20–21.4 cM) and 1 cM (20.1–21.1 cM) respectively. Similar posterior probability intervals for DIR were detected on this chromosome. About 15–18% of the total variation (73–75% of the total genetic variance) in DI was explained by the QTL on CFA11. About 11–14% of the total variance (55–72% of the total genetic variance) in DI was due to QTL on CFA29 (Table 1). These percentages were inflated because only two chromosomes were included in the study.

The total amount of variation explained by QTL on both CFA11 and CFA29 simultaneously is not simply the addition of the two percentages (Table 1) due to the possible interactions among loci. To identify these interactions, we jointly analysed CFA11 and CFA29 using LOKI (Heath 1997). Strong evidence of linkage remained on CFA11 while the significance of CFA29 QTL was reduced (results not shown), possibly because of the relatively small effect of the QTL on CFA29 in the presence of CFA11 or the CFA29 effect may be partly mediated through interactions with genes on CFA11.

There was no significant effect of sex on DI. For the left hip DI, we found a significant breed effect ($P < 0.015$), indicating that hip laxities across breeds or generations were not the same. This is not surprising because the variation of hip laxities varies over generations in the crossbreed pedigree.

Table 1 The percentage of total variance in distraction index due to QTL on CFA11 and 29.

Chromosome	DIL[1]	DIR[2]
CFA11	14.7 (75.3)	18.3 (72.9)
CFA29	11.3 (72.3)	13.6 (55.4)
CFA11 + CFA29	15.3 (79.8)	17.6 (72.4)

The percentage of genetic variance due to QTL is in parentheses.
[1]Distraction index on the left hip.
[2]Distraction index on the right hip.

Discussion

The DI, which measures the maximum hip laxity in dogs, is a reliable measure of tight hips (DI < 0.4) and loose hips (DI > 0.7) when measured at 8 months of age. This is the age at which a medium-to-large breed dog reaches maximal skeletal growth. Hip laxity can be considered a major risk factor or intermediate phenotype for CHD and secondary osteoarthritis but alone is not sufficient to make the diagnosis of CHD, particularly for dogs with a DI between 0.4 and 0.7 (Smith 1997). Once osteoarthritis has developed in

a dysplastic hip, secondary remodelling effects of osteoarthritis cause capsular fibrosis, which decreases the maximum hip laxity that can be detected.

Our results are consistent with previous simulation studies which suggested that a major locus for the DI may be involved in CHD expression (Todhunter *et al.* 2003b). In this study, we identified putative QTL on CFA11 and CFA29 associated with DI in 257 crossbreed and pure-breed dogs after controlling for the effects of sex and breed. Compared to our original microsatellite-based study, multipoint linkage analysis reduced the QTL intervals from 20–30 cM to 1–5 cM, and the effective number of QTL is about 1 or 2 for this trait on these two chromosomes, thus providing a reasonable starting point for candidate gene selection.

Strong evidence of linkage on CFA11 was found when CFA11 and CFA29 were analysed simultaneously. Furthermore, these QTL explain about 15–18% of the total variation in the trait. Detecting QTL in a 1-cM region with a 99% posterior probability interval demonstrated the advantage of using a crossbreed pedigree and our SNP selection strategies. Moreover, the 99% posterior probability interval on CFA29 was only slightly narrower than that of the 95% interval, while on CFA11, the 95% posterior probability interval was wider than that of the 99% interval. The narrowness of the 95% posterior probability interval indicates that the SNP selection strategy on CFA11 (1 SNP/200 kb) provided at least as much power for narrowing the QTL interval as the strategy used for CFA29, assuming marker loci in this region were linked to the disease loci.

QTL were found around the same location on CFA11 (19.6–19.7 cM, BF = 22) when analysing DIL and DIR independently, indicating symmetric effects that affect hip laxity in both the right and left hip joints of the dog. However, we observed evidence for QTL at different locations on CFA29 for DIL and DIR. This may or may not be the true asymmetric effect of QTL because under these peak regions on CFA29, the densities of SNP markers were not the same. However, asymmetric effects of QTL that affect laxity in hip joints of dogs are not a novel phenomenon. Chase *et al.* (2004) found Portuguese water dogs had greater subluxation (measured by the Norberg Angle) in the left hip than the right hip and two separate QTL were associated with each hip on CFA1. No QTL on CFA1 reached chromosome-wide statistical significance in our microsatellite-based genome-wide scan (Todhunter *et al.* 2005). In contrast to Chase *et al.* (2004), our study used both crossbreed and pure-breed dogs.

Several factors affecting the accuracy of positioning QTL for CHD can be assigned into experimental–statistical analysis and genetics effects. Experimental factors include pedigree informativeness, accuracy of the phenotype measurements, genotyping accuracy, density of informative SNPs and the power of the statistical method. Power is affected by the heritability of the trait, the number of effective genes contributing to the trait, the size of the effect of each gene and epistasis. This pedigree, with crossbreeds and a portion of unrelated Labrador retrievers, is an extension of the one used for microsatellite-based interval mapping (Todhunter *et al.* 2005) and for which we demonstrated sufficient power for detection of QTL (Todhunter *et al.* 2003a). Genotyping errors may reduce statistical power or bias the inferences in the analysis (Abecasis *et al.* 2001). We detected genotyping errors based on the initial marker genotypes before linkage analysis was undertaken. The PIC of each SNP along the chromosomes as well as the narrowed QTL detection also illustrated a good strategy of marker selection in this study. Saturation SNP genotyping in additional pure-breed dogs should provide definitive evidence for quantitative trait nucleotides on CFA11 by further localizing the interval based on association or LD analysis preparatory to candidate gene selection and positional cloning of the contributing genes. Increasing the density of SNPs helps to refine the QTL locations; however, the extent of LD is negatively correlated with increasing distance (Dawson *et al.* 2004; Ke *et al.* 2004).

Candidate genes in the 99% posterior probability interval for the QTL on CFA11 between 19.1 and 20.1 cM include *membrane-associated RING-CH protein III*, *MEGF10* (*multiple EGF domains 10*) and a gene similar to hypothetical protein MGC12103. The 95% posterior probability interval for this QTL on CFA11 contains at least ten genes. The next step will be saturation mapping in this refined region within and across dog breeds to further pinpoint the putative genes or mutations and within the refined QTL on CFA29 linked with hip laxity.

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Supplementary material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2007.01691.x

Figure S1 Pedigree structure of 257 dogs in the study. Highlighted are the affected dogs with squares for males and circles for females.

Table S1 Accession numbers and location of SNPs included in the analysis.

Table S2 Estimates of the posterior distribution of the number of QTL (*k*).

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