GENETIC ANALYSIS OF CANINE HIP DYSPLASIA

A Dissertation

by

KATE LEANNE TSAI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Veterinary Microbiology

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ABSRACT

Genetic Analysis of Canine Hip Dysplasia. (December 2005)

Kate Leanne Tsai, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Keith E. Murphy

The morphologic variability seen in the domestic dog, *Canis lupus familiaris*, is unique among mammals. Selective pressures imposed by humans have divided dogs into almost 400 separate breeds. Selection has also led to the development of approximately 450 hereditary diseases, many of which are limited to specific breeds. Over half of these diseases present with similar clinical characteristics to those of many human hereditary diseases, making the dog an ideal model for study of the genetic bases of such diseases. Many diseases do not have candidate genes or have too many candidates to characterize. This is exacerbated in complex diseases that are caused by several genes. Whole-genome scans can provide insight into diseases by identifying marker(s) that co-segregate with a disease phenotype. The Minimal Screening Set – 2 (MSS-2) is the most recent set of microsatellites suitable for whole-genome screens. The first objective of this work was to streamline genomic screens in order to efficiently analyze large numbers of animals. To this end, chromosome-specific microsatellite panels were developed for the MSS-2.

Canine hip dysplasia (CHD) is the most common orthopedic disease of the dog. CHD primarily affects medium and large breed dogs, but is found in almost every breed. The major objective of this work was to use linkage analysis to identify chromosomal regions that contain genes that are involved in CHD. Two populations were screened using the MSS-2. The first was a small family of Boykin Spaniels, though no markers

were statistically significant in a whole-genome screen. An outcrossed pedigree of Greyhound/Labrador Retrievers was created for quantitative trait loci (QTL) mapping of CHD. The informativeness of markers in the F2 and backcrossed generations were calculated to show the utility of using such a population. Other factors that affect the power of this pedigree to identify QTL were also highlighted. Chromosomes that were identified in a previous screen as harboring putative QTLs were examined using the chromosome-specific panels to further define and confirm the regions of interest. Although no markers reached statistical significance, several areas of interest were identified.

For Mom

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I would first like to thank my advisor, Dr. Keith Murphy. I was given the opportunity to discover the joys and frustrations of research early in my academic career as a student worker in his lab. The experiences I gained led me to continue research in canine genetics. His guidance throughout my undergraduate and graduate careers has been invaluable. I would also like to thank my committee members, Dr. Kelly Credille, Dr. Sharon Kerwin, and Dr. James Womack, for their encouragement and support.

The members of the Canine Genetics Laboratory, both past and present, have kept me motivated throughout the years. I especially want to thank Dr. Leigh Anne Clark for assisting me in the lab and in life. Her friendship is and always will be treasured. Dr. Melissa Cox helped me adapt to the laboratory setting by answering my many questions and remains a good friend.

There are also several collaborators whose contributions have allowed me to accomplish the work presented herein. Firstly, I must thank Dr. Rory Todhunter of Cornell University for generously inviting me to be a visiting member of his laboratory. This allowed me to contribute to his group's extensive hip dysplasia studies and to gain valuable experience while working with him and his colleagues. Secondly, I am grateful to Dr. Thomas Famula of the University of California-Davis, without whom the statistical analyses necessary for my research could not have been completed. Thirdly, I also thank the scientists at ViaGen, Inc. for their assistance and generous access to their equipment.

My entire family has always supported my educational pursuits. Specifically, I want to thank my husband, Jason Tsai, for believing in me and always pushing me forward when I was ready to give up. I also want to thank my dad, Dr. Richard Willingham, who has encouraged me every step of the way.

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CHAPTER I

INTRODUCTION

Genetics of the dog

The morphologic variability seen in the domestic dog, *Canis lupus familiaris*, is unique among mammals and is the result of defined breeding practices carried out over the centuries since the dog was domesticated. Mitochondrial studies show that the domestic dog diverged exclusively from the wolf (Vila et al. 1997) and recent studies suggest that a single domestication event occurred in eastern Asia approximately 15,000 years ago (Savolainen et al. 2002). There are now approximately 400 recognized breeds (FCI 2005) that have arisen due to the aforementioned selective breeding practices. Most breeds have come into being within the last 250 years (Ostrander and Giniger 1997) because breeders wanted to produce specific breeds that possess certain behavioral and physical traits. In the effort to propagate these specific traits, breeds have been subjected to a tremendous level of inbreeding. Breed clubs create a genetic barrier between breeds resulting in relatively isolated genetic populations that do not interbreed. However, even though breeds are highly differentiated, haplotype sharing shows a low level of diversity among all dogs (Sutter et al. 2004). In fact, breeds exhibit a high level of genetic homogeneity with only 27% of total genetic variation in dogs accounted for by variability among breeds (Parker et al. 2004).

This thesis follows the style of Mammalian Genome.

The consequence of the breed barrier and other selective pressures, such as limited founders, population bottlenecks, and popular-sire effects, is the emergence of more than 450 hereditary diseases in the dog (OMIA 2005). Many of these diseases are found more commonly in one particular breed or group of breeds (Patterson 2000). The dog has emerged as a model organism due to the fact that almost half of canine hereditary diseases are present in the human and many result from mutations in the same genes (Ostrander and Giniger 1997). Additional reasons for use of the dog as a model are that dogs 1) share our environment, and are therefore subject to the same external pressures that affect physiology, 2) receive medical attention at a level second only to humans, so we know more about the pathology of canine infectious and hereditary diseases, and 3) are physiologically more similar to the human than is the mouse. While many rodent models of hereditary disease are available, virtually all are induced using transgenics technology; the dog offers naturally occurring models of many human hereditary diseases. Finally, the dog has a large litter size, short generation time, and detailed pedigrees, making it an invaluable tool.

Genomics of the dog

The dog has 40 chromosomes, the metacentric X and Y chromosomes, and 38 small, acrocentric autosomes that could not be distinguished from each other until the development of chromosome-specific fluorescence in situ hybridization (FISH) paint probes (Breen et al. 1999a, Breen et al. 1999b). This laid the groundwork for the development of a high-resolution map of the canine genome.

The first linkage map of the dog was developed prior to standardization of the karyotype and consisted of 139 microsatellite markers comprising 30 linkage groups (Mellersh et al. 1997). The construction of a radiation-hybrid (RH) cell panel using canine-hamster cell lines (Langston et al. 1997) allowed for the development of a whole genome RH map of the dog, consisting of 400 markers, including 218 genes and 182 microsatellites (Priat et al. 1998, Vignaux et al. 1999). The integration of the linkage and RH maps (Breen et al. 2001) led to the identification of microsatellite markers that were evenly distributed across the genome. The first set of microsatellite markers that provided coverage of the canine genome and was suitable for whole genome scans is termed the Minimal Screening Set-1 (MSS-1) (Richman et al. 2001). The MSS-1 consists of 172 markers with an average polymorphic information content (PIC) value of 0.74 and average spacing of 10 cM with no gaps greater than 20 cM (Richman et al. 2001). This set is multiplexed into 48 multiplex sets to streamline whole genome screens (Cargill et al. 2002).

In 2003, a RH map composed of 3,270 markers that provide 1 Mb resolution of the canine genome was released (Guyon et al. 2003). A subset of highly informative microsatellite markers was characterized to form a more comprehensive screening set with greater coverage than the MSS-1. The Minimal Screening Set-2 (MSS-2) is comprised of 327 markers, including 171 tetra-, 151 di-, and 3 tri-nucleotide repeats with an average heterozygosity value of 0.73 (Guyon et al. 2003). The MSS-2 has an average spacing of 9 Mb and no gaps larger than 17.1 Mb (Guyon et al. 2003). The most recent

RH map consists of 4249 markers and more clearly defines 79 regions of conserved segments between the canine and human genomes (Breen et al. 2004).

The ultimate physical map is now available for the dog. The dog became the fourth mammalian genome to be sequenced, and this highlighted the dramatic growth of canine genetics within the last 6 years. The first large scale sequencing effort in the dog was completed by Celera Genomics. A 1.5X sequence, representing approximately 80% of the genome from a male standard poodle was completed in 2003 (Kirkness et al. 2003). The National Human Genome Research Institute (NHGRI) recognized the need for a publicly available sequence with higher resolution (NHGRI, http://www.ncbi.nlm.nih.gov/genome/guide/dog/). More than 60 breeds were tested to determine the level of genetic homogeneity that exists within the breed. Among the dogs tested, a female boxer named Tasha showed the greatest homogeneity and was chosen for sequencing. Today, a 7.8X sequence of the dog is publicly available (Sutter and Ostrander 2004). The planned sequencing of nine additional breeds (with 100,000 reads) will allow comparison of sequence differences between breeds and will facilitate the construction of a single nucleotide polymorphism (SNP) map (Sutter and Ostrander 2004).

Linkage and QTL mapping in the dog

More than half of the 450 hereditary diseases that affect the dog are transmitted in an autosomal recessive fashion (Ostrander and Kruglyak 2000), making identification of carriers difficult. Also, many hereditary diseases have a late onset, making it difficult

for breeders to prevent the spread of disease alleles. There are different approaches being utilized to identify deleterious genes of the dog. The first approach is candidate gene analysis, which focuses on specific genes that are thought to be involved in the disease process. These candidates are often identified as causative genes in other species with a similar disease phenotype.

Two other approaches, classical linkage and linkage disequilibrium (LD), are recombination mapping strategies that are useful when no, or many, candidate genes exist for a disease. Both utilize meiotic recombination to define a region that is co-inherited, or linked, with a disease gene. Linkage analysis uses multigenerational pedigrees to trace inheritance of chromosomal segments harboring the causative gene. In the dog, it is often possible to collect phenotype information and DNA samples from pedigrees. When samples cannot be acquired from family members, unrelated dogs can be used in a LD mapping approach, which measures co-segregation at the population level. LD mapping assumes that the disease is the result of an ancestral mutation and is the same in all individuals with the trait. LD is 20-100 times more extensive in the dog as compared to the human, with values ranging from less than 1 Mb to greater than 3 Mb in different breeds (Sutter et al. 2004). Therefore, fewer markers are needed to identify a locus in LD.

The above analytical tools are suitable for dissection of simple, Mendelian diseases, but many diseases and traits are complex. Complex traits are governed by the interaction of several genes and the environment, presenting an additional challenge to investigators. Specifically, the mode of inheritance and number of genes involved in a

trait are often unknown. One approach to dissecting such traits is quantitative trait loci (QTL) analysis. Ideally, QTL analysis is carried out using two inbred lines that express opposite extremes of a phenotype with each line assumed to be homozygous for the genes of interest. Upon breeding the two lines and creating a backcrossed generation, polymorphic markers can be analyzed for segregation with the trait of interest (Ostrander and Giniger 1997, Lynch and Walsh 1998).

Hip dysplasia in the dog

Pathogenesis

Canine hip dysplasia (CHD) is the most common orthopedic disease of the dog. Clinical signs associated with CHD include lameness, a bunny-hop gait, difficulty rising, and reluctance to jump. It is often a biphasic disease with symptoms showing at the beginning of the disease in a puppy and again when the pain of arthritis begins later in life.

CHD is a degenerative disease characterized by malformation of the hip joint. The inevitable result of CHD is osteoarthritis (OA), also termed degenerative joint disease (DJD). Changes in hip joint laxity and conformation begin in the first few weeks after birth. Lesions on the round ligament of the femoral head may be observed as early as 30 days of age (Morgan and Stevens 1985). It is unknown if changes in tissue surrounding the hip joint lead to increased laxity or if increased laxity causes tissue changes. Laxity in the joint allows the head of the femur to contact the edge of the acetabulum (Lust 1997). The joint capsule becomes inflamed causing it to thicken and

the amount of synovial fluid in the joint increases, a condition called synovitis (Lust and Summers 1981). Mild arthritis develops often leading to the first clinical signs of lameness. The joint then undergoes cartilage fibrillation and erosion, exposing the head of the femur and the acetabular cup, causing the bones to eburnate and resulting in inflammation (Fries 1995). Osteophytes form around the rim of the acetabular cup in an attempt to restore the articular surface (Morgan and Stevens 1985).

Diagnosis and Prediction

Several methods have been developed in an attempt to predict the probability of a dog developing CHD. In young dogs, hip joint laxity can be determined using the Bardens, Barlow and Ortolani tests (Barlow 1962, Bardens 1973, Ortolani 1976). These semi-quantitative tests will generally not give positive results in older dogs with advanced hip dysplasia due to structural changes in the joint. The only definitive manner to diagnose CHD is by observation of arthritis in the hip joint; although, it is important to note that arthritic changes may be the result of something other than CHD. In addition to diagnostic methods there are several radiographic techniques that predict the likelihood of developing CHD, including the ventrodorsal hip extended (VHE) view (Rendano and Ryan 1985), the University of Pennsylvania Hip Improvement Program (PennHip) method (Smith et al. 1990), and the dorsolateral subluxation (DLS) test (Farese et al. 1998).

In an effort to reduce CHD in all breeds, the Orthopedic Foundation for Animals (OFA) in Missouri maintains a database of hip scores that breeders can access. The

scores are based on a 7-level scale that classifies hips as normal (excellent, good or fair), borderline, or dysplastic (mild, moderate or severe) using VHE radiographs. Radiographic evidence of DJD as well as the fit of the femoral head in the acetabulum determines the score. Similar scales exist in other countries, each with its own grading system (Willis 1994). Preliminary evaluations can be done when the puppy is four months of age but cannot completely rule out the disease until two years of age (Smith 1993). The ventrodorsal view (with hips either extended or flexed) is used to calculate the Norberg angle, which is an indicator of subluxation in the hip (Norberg 1962).

Joint laxity can cause instability of the hip leading to incomplete or complete dislocation of the femoral head. The PennHip method quantifies passive laxity in the hip joint using the distraction index (DI). This method measures the level of distraction of the femoral head to determine the probability of developing CHD (Smith et al. 1990). Scores generally range from zero to one. A higher value indicates increased joint laxity and a higher probability of developing CHD. General diagnostic measures have been described with a value less than 0.3 as normal and values above 0.7 as affected (Smith 1993). However, there are breed-specific ranges, so it seems that some breeds may have a tolerance for increased laxity.

Subluxation, or partial dislocation, of the joint may be indicative of minor dysplasia depending on the degree of separation. The DLS test measures the percentage of the femoral head that is covered by the acetabulum while in sternal recumbency. Increased coverage increases the DLS score and indicates a decreased probability of developing CHD (Farese et al. 1998).

Genetics

CHD is a complex trait. The pattern of inheritance suggests that several major and many minor QTL may influence the phenotypic presentation of CHD (Leighton 1997). It is thought that there are genes that increase the risk of developing CHD as well as some that protect against CHD. Environmental influences also have a major effect. For example, caloric intake has been shown to aggravate pre-disposed dogs (Olsson 1980).

While it is known that joint laxity plays a major role in the development of CHD (Henricson et al. 1966, Kealy et al. 1997), there is some question as to whether the laxity leads to development of CHD or unstable hips lead to increased laxity. Smith et al. (1990) identified joint laxity as a heritable trait in the German Shepherd Dog. Studies of the genetics underlying joint laxity and overt CHD include breeding programs in military working dogs and designed pedigrees (Leighton et al. 1977, Todhunter et al. 1999), as well as studies of natural populations with a high incidence of CHD (Wang et al. 1999, Chase et al. 2004).

One major study is being carried out at the James A. Baker Institute for Animal Health at Cornell University. Researchers there maintain an outcrossed canine pedigree of Greyhounds and Labrador Retrievers ideal for whole genome scans (Todhunter et al. 1999, Todhunter et al. 2003). The Greyhound is known for its sound hips, with a DI range of 0.07 to 0.35 (PennHip 2002). In contrast, the Labrador Retriever is prone to CHD, with a DI range of 0.08 to 1.36 (PennHip 2002). A screen carried out at the Marshfield Clinic Center for Medical Genetics on select members of the

Greyhound/Labrador Retriever pedigree allowed for the identification of several putative QTL spanning 12 chromosomes (Todhunter et al. 2005). In addition, the Baker Institute also maintains a multigenerational pedigree of Labrador Retrievers that segregate CHD. Our laboratory has been working with another breed, the Boykin Spaniel (BS), for quite some time (Wang 1990). The BS has a high level of genetic homogeneity due to its small, highly inbred population. Importantly, despite its small size (30-40 lbs) the BS has one of the highest incidences of CHD, ranking 9th of 137 breeds included in the OFA database (OFA).

CHD does not seem to be predisposed to a certain gender or hip. However, Chase et al. detected a higher level of joint laxity in the left hips of Portuguese Water Dogs (PWDs) using the Norberg angle (Chase et al. 2004). They also report the identification of two QTL on CFA01 that asymmetrically affect joint laxity in the right or left hip of the PWD (Chase et al. 2004). The same group identified a marker on CFA03 that is associated with osteoarthritis (Chase et al. 2005).

Human Hip Dysplasia

Human hip dysplasia, or developmental dislocation of the hip affects between 1 and 10 per 1000 live births (Weinstein 1987) and is influenced by both genetic and environmental factors (Carter 1964). Almost 50% of babies born with lax hips are naturally corrected within one week (Barlow 1962). It is more common in females, breech births, and firstborn children. There also seems to be prevalence for the left hip. Initial diagnosis generally made at birth by use of the Ortolani sign. Further investigation

is then done with radiographs and/or ultrasound (Grissom and Harcke 1999). The condition can be corrected by fixing legs in a harness (Pavlik harness) that flexes and abducts the legs.

Specific objectives

The objective of this work is to use linkage analysis to identify QTL that affect the joint laxity, the major factor influencing the development of CHD. To accomplish this goal, a tool that streamlines whole genome screens must be developed and populations suitable for analysis must be identified. To this end, chromosome-specific multiplex sets for the MSS-2 were developed to increase the ease and speed of linkage analysis. Two populations segregating CHD were identified for study, the BS and a designed outcrossed pedigree of Greyhound/Labrador mixes. Finally, linkage analysis was carried out on both populations to identify markers that co-segregate with joint laxity or CHD.

CHAPTER II

CHROMOSOME-SPECIFIC MICROSATELLITE MULTIPLEX SETS FOR LINKAGE STUDIES IN THE DOMESTIC DOG*

Overview

To expedite linkage studies and positional cloning efforts in the dog, Minimal Screening Set 2 (MSS-2) of 327 canine microsatellite markers has been multiplexed into chromosome-specific panels. MSS-2 provides 9 Mb coverage of the canine genome with no gaps larger than 17.1 Mb and is the most recent and comprehensive set of microsatellites available for whole-genome scans. Markers were labeled with fluorescent dyes based on locations and expected product sizes to facilitate the multiplexing of a maximum number of markers for each chromosome. All markers are amplified using a single thermal cycling program and PCR mix and are optimized for resolution on an ABI 3100 genetic analyzer. Sixty-nine chromosome-specific panels were created by coamplification of a maximum number of markers and subsequent coloading of the remaining markers.

* Reprinted from Genomics, 84, Clark LA, Tsai KL, Steiner JM, Williams DA, Guerra T, Ostrander EA, Galibert F, and Murphy KE, Chromosome-specific microsatellite multiplex sets for linkage studies in the domestic dog, 550-554 (2004), with permission from Elsevier.

Introduction

Studies of the dog offer insight into the genetic bases for many hereditary diseases affecting both canine and human populations (Ostrander and Kruglyak 2000; Ostrander et al. 2000; Patterson 2000). Linkage analyses and linkage disequilibrium studies are useful for eventual identification of mutated alleles in the dog, many breeds of which are characterized by small founding populations, high degrees of inbreeding, and popular sire effects. The creation and maintenance of multigenerational pedigrees offers additional advantages compared to studies using human populations. To facilitate linkage mapping and positional cloning studies in the dog, it is necessary to have a defined set of polymorphic markers that provides complete coverage of the genome.

Previously, we multiplexed Minimal Screening Set 1 (MSS-1), a set of 172 microsatellite markers (Richman et al. 2001) selected from a map comprising 600 markers (Mellersh et al. 2000), into 69 reactions, thereby reducing the time, expense, and DNA required for whole-genome screens (Cargill et al. 2002). The most recent version of the canine map has 3270 markers, including 1596 microsatellite markers (Guyon et al. 2003). From this, a superior screening set of microsatellite markers providing increased density and a greater level of informativeness was developed. Minimal Screening Set 2 (MSS-2) comprises 327 microsatellite markers that have an average spacing of 9 Mb with no gaps larger than 17.1 Mb (Guyon et al. 2003). The set includes 171 tetra-, 151 di-, and 3 tri-nucleotide repeats with an average heterozygosity value of 0.73 when analyzed on a panel of unrelated purebred dogs.

In the human, chromosome-specific multiplexed microsatellite sets have been developed to accelerate collection and analysis of data for linkage studies (Reed et al. 1994; Lindqvist et al. 1996). However, no such sets exist for the dog. The development of chromosome-specific multiplex panels will allow rapid screening of those chromosomes presumed to harbor genes of interest as determined through comparative mapping and genomic sequencing.

Materials and methods

Primer pairs were synthesized by Applied Biosystems (PE Biosystems, Foster City, CA, USA) and forward primers were labeled with one of four fluorescent dyes: 6FAM, NED, PET, or VIC. Previously multiplexed microsatellites (Cargill et al. 2002) that were labeled with 6FAM were not relabeled, and those labeled with TET and HEX were relabeled with VIC and NED, respectively, to retain their original dye colors. Dye types for new markers were chosen for even distribution across each chromosome and size range. Primers were diluted to 10 μM. The primer sequences and intermarker distances are available at www.fhcrc.org/science/dog_genome/guyon2003/guyon_data/mss2.html and http://www-recomgen.univ-rennes1.fr/doggy.html.

Optimization of multiplex sets was achieved using DNA from a Rough collie and further testing was conducted using a family of Boykin Spaniels. Genomic DNA was extracted from whole blood and buccal cells using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA).

All multiplex sets were amplified with a single stepdown thermal cycling program: 5 min at 95°C followed by 5 cycles of 30 s at 95°C, 15 s at 58°C, and 10 s at 72°C and an additional 30 cycles of 20 s at 95°C, 15 s at 56°C, and 10 s at 72°C, with a final extension of 5 min at 72°C. A single mastermix, excluding primers, was used for all multiplex and individual reactions. Concentrations are 0.113 units/μl Taq DNA polymerase (Fisher Scientific, Pittsburgh, PA, USA) with 1.5× Buffer B (Fisher Scientific), 4.5 mM $MgCl_2$, 1.5× MasterAmp PCR Enhancer (Epicentre Technologies, Madison, WI, USA), 0.75 mM total dNTPs, and 2.8 μl water to bring the final master mix volume to 6.65 μl. One microliter of 50 ng/μl genomic DNA was used in each reaction. Primer volumes vary by multiplex (Table 1), resulting in different final reaction volumes and concentrations.

Multiplex sets were first established by amplifying markers for each chromosome in various combinations. Once the maximum number of coamplified markers was achieved, the remaining markers were amplified individually and coloaded into a multiplex set representing individual chromosomes. Duplex sets were coloaded into other multiplexes on the chromosome, if possible. For ease of genotyping, no markers having the same dye type and product sizes less than 50 bp apart were multiplexed in a chromosome panel.

Table 1: Chromosome-specific panels for the MSS-2, listed by multiplex name[a] followed by marker name,[b] primer amount,[c] and fluorescent label[d]

1.1	FH3413	0.8	P	REN112I02	0.8	V	C01.424	0.8	F	C00901	0.8	P	FH2793	0.6	P	FH2326	1.0	N	
1.2	FH3325	0.8	P	FH3300	0.8	N	C01.251	0.8	N	FH2309	0.8	V	REN143K19	0.6	V				
1.3	FH2663	1.1	F	FH3603	1.5	F	FH3922	1.1	F	FH2294	0.3	N							
2.1	FH3210	0.8	P	REN303H07	0.8	V	REN70M14	0.8	V	FH3965	0.8	F							
2.2	FH2890	0.4	N	C02.609	0.6	P	FH2613	0.6	V	FH2132	0.6	F							
2.3	FH2274	0.8	N	FH2608	0.8	P	C02.342	0.8	F										
3.1	REN161A12	0.6	F	FH3252	0.6	P	FH3464	0.6	V	FH2316*	0.8	N	FH3377	0.6	N				
3.2	FH3115	0.8	N	C03.629	1.2	V	FH2145	0.8	P	REN260I04	0.6	F							
3.3	FH3396	1	F	FH2302	0.6	V													
4.1	REN298N18	0.4	P	REN303C04	0.4	V	FH2732	0.8	F	FH3310	0.8	F	REN74B13	0.6	V	AHT103	0.8	F	
4.2	FH2776	1.0	P	REN195B08	0.8	N	FH2097	0.6	N	G07704	0.6	V							
5.1	FH3004	0.8	F	DTR05.8	0.8	N	FH3978	1.0	P	REN175P10	0.6	V	CPH14*	0.8	N				
5.2	FH3928	0.8	P	FH3320	0.6	N	FH3702	0.8	F	FH3089	0.8	V							
5.3	FH2140	0.8	P	REN285I23*	0.8	V	FH3278	0.8	P	C05.771	0.6	V							
6.1	FH2525	0.8	V	FH2561	1.4	N	FH2734	0.8	V	FH2164*	0.8	V	FH3303	0.5	P				
6.2	FH2576	0.6	F	FH3933	0.8	P	FH2370	0.8	N										
6.3	REN285H12	0.8	F	FH2119	0.8	N	REN111L07	0.8	P										
7.1	REN97M11	0.8	P	FH3972	0.6	N	REN162C04	0.8	V	REN143L20	0.6	P	FH2860	0.4	V				
7.2	FH2226	0.8	N	VIASD10	0.8	P	FH2973	0.8	P										
8.1	FH3241	0.8	P	REN204K13	0.8	N	FH3316	0.8	V	C08.618	0.8	F							

Table 1: Continued

8.2	FH3425	0.8	N	C08.410	0.8	F	REN178J05	0.6	F	FH2989	1.0	V										
9.1	GALK1	0.6	V	FH2263	0.8	N	C09.173	0.4	N	REN54L20	0.4	F	G06401	0.6	P	REN287G01	0.6	N				
9.2	FH2186	1.4	V	REN145P07	0.6	P	FH3835	0.3	F	REN73K24	0.3	V	FH2885	0.4	N							
10.1	FH2537	0.8	N	FH4081	0.8	P	C10.781	0.6	V	ZUBECA1	0.4	N	DTR10.5	0.8	F	FH3381*	0.8	P				
10.2	REN06H21	0.8	P	FH2293	0.8	V	C10.16	0.8	F	FH2422	0.8	N										
11.1	FH3203	0.8	V	REN242K04	0.8	F	FH2004	0.6	F	C11.868	0.8	P	C11.873	0.8	V	DGN13	0.8	V				
11.2	AHT137	0.3	V	FH4031	0.8	P	FH2319	1.0	N	FH2019	0.2	N										
12.1	REN153O12	0.6	F	FH2401	0.6	V	FH3591	0.6	N	G01811	0.6	P	REN94K11	0.6	N							
12.2	REN258L11	0.8	P	REN213F01	0.8	F	FH3711*	0.8	N	FH1040	0.6	V	FH3748	0.8	P							
13.1	C13.391	0.8	N	FH3494**	0.8	V	REN120P21	0.6	F	FH3619	0.6	P	DTR13.6	0.6	F	FH2348	1.2	V	REN227M12**	0.8	P	
	FH3800	0.6	N																			
14.1	FH3951	1	F	FH3725	0.6	P	FH2658	1	P	FH2763	0.4	N										
14.2	C14.866	0.8	F	FH3285	0.8	P	PEZ10	0.8	V													
15.1	FH4012	0.6	P	FH3813	0.8	V	FH2171	0.6	N	CPH4	0.8	N	REN230G12	0.6	F							
15.2	FH3802	0.6	V	REN06C11	0.6	F	FH2360	0.8	P													
16.1	REN214L11	0.4	V	FH2670	0.6	F	REN73O19	0.6	P	REN85N14	0.8	P	FH3592	0.8	V							
16.2	FH2155	1.0	N	REN275L19	0.4	N	FH2175	1.2	F													
17.1	REN240A05	0.6	F	FH3369	0.6	P	REN294E18	0.6	V	FH3995	0.8	F										
17.2	FH3047	0.8	P	FH4023	0.8	P	PEZ8	1.0	N	FH2869	0.6	V										

Table 1: Continued

18.1	FH4060	1.0	N	FH3944*	0.8	P	FH3824	0.4	V	FH3815	0.4	F	REN54P11*	0.8	P	FH2834	0.4	N	REN47J11*	0.8	V
	AHT130	0.6	F																		
19.1	REN213G21	0.6	V	FH3491	0.4	F	FH3313	1.0	P	FH2206*	0.8	P	FH2380	0.6	N						
19.2	FH3299	0.6	V	FH3834	0.6	F	FH3969	0.6	N												
20.1	PEZ19	0.8	N	FH2951	0.6	F	FH2158	1.0	P	REN114M19	0.4	F									
20.2	REN55P21*	0.8	N	REN100J13	0.8	P	REN93E07	0.2	V	AHTk209	1.0	N									
21.1	FH3803	1.0	P	FH2233**	0.8	F	REN118B15	0.3	V	FH2441	0.8	N	REN37A15	0.3	V	FH3398	0.8	P	FH2312**	0.8	N
22.1	REN42F10	0.8	V	FH3355	0.8	V	FH3411	0.8	N	FH3853	0.8	P									
22.2	REN49F22	1.0	N	REN128E21	1.0	P	C22.279	0.4	V	REN78I16	0.6	F									
23.1	FH3078	0.8	P	FH2508	1.0	P	FH2626	0.8	F	REN113M13	0.6	V	REN02P03	0.8	N	REN181K04	0.8	P			
24.1	FH3023	0.8	P	FH2261*	0.8	F	AHT125	0.8	P	FH3287	0.8	F	REN228J19	0.8	V						
24.2	FH3750	0.8	P	FH2159	0.8	N	REN106I06	0.6	V	REN272I16	0.4	F									
25.1	REN54E19	0.8	F	FH3245*	0.8	P	FH2324	0.3	N	FH2141	1.0	N	FH3627	0.3	V	FH4027	0.7	F			
26.1	REN62M06	0.4	N	DTR26.9	0.4	V	FH3426	0.4	V	DGN10*	0.8	P	FH2130	1.0	N	C26.733	0.6	F			
27.1	FH3221	0.8	P	PEZ6	0.6	F	REN181L14	0.4	N	REN72K15	0.4	V									
27.2	FH2289	0.8	P	PEZ16	0.3	N	LEI002	0.3	F	FH3924	0.6	V									
28.1	C28.176	0.8	V	FH3963	0.8	P	FH2585	0.8	F	REN146G17	0.8	V	FH2208*	0.8	N						
29.1	FH2952	0.8	P	FH2364*	0.8	F	REN52D08	0.4	P	REN45F03	0.8	F	FH2385	1.0	V	FH1007	0.3	V			
30.1	FH3489	0.4	F	REN51C16	0.4	P	REN248F14**	0.8	V	FH2290	1.0	N	FH3632**	0.8	P	FH3053	0.4	F			
31.1	FH2189	0.8	N	RVC11	0.6	V	REN43H24	0.6	N	REN109B10	1.2	P	REN110K04*	0.8	P	FH2712	0.4	F			

Table 1: Continued

32.1	REN244E04	0.3	F	CPH2	0.4	V	FH2875	0.6	N	FH3635	1.0	F	FH3236	0.8	N	AHT127	0.3	V	FH3294*	0.8	P
33.1	FH2790	0.4	F	FH3608	0.8	F	FH2361	0.3	V	REN186B12	0.6	V	FH2165*	0.8	N						
34.1	FH3721	0.8	P	REN174M24	0.6	F	REN243O23	0.8	F	REN314H10	0.4	V									
34.2	REN109L16	0.8	N	FH2377	0.8	V	FH3836	0.8	N												
35.1	FH3570	0.8	F	REN282I22	0.4	V	REN94K23	0.6	N	REN112C08	0.6	P									
36.1	REN106I07	0.8	V	FH2611	0.8	P	REN179H15	0.8	P	FH3865	0.8	V	DTR36.3*	0.8	N						
37.1	FH3272	0.8	F	H10101	0.8	V	REN67C18	0.8	P	FH3449	0.8	F	FH2532	0.8	N						
38.1	FH2766	0.8	P	REN02C20	0.8	F	REN164E17	0.8	N												
X.1	FH2916	0.8	F	REN101G16	1.0	N	D04614	0.8	F	REN144O22	0.6	V									
X.2	FH3027	0.8	N	FH1020	0.8	F	FH2985	0.6	N	REN230I20	0.6	V									
X.3	REN130F03	0.8	F	FH2584	0.8	N	REN75A05	0.8	P												
Y.1	REN197E16	0.8	V	REN44K10**	0.6	F	DTRY.13**	0.4	F	REN75H09	0.2	V	REN173O16	0.2	P						

[a] Denoted by chromosome and then multiplex number within chromosome
[b] Marker names; * indicates that the marker is amplified individually and co-loaded into the panel; ** indicates that the markers are co-amplified and then co-loaded into the panel.
[c] Total amount (µl) of primer (forward and reverse) in the multiplex.
[d] F=6FAM, P=PET, V=VIC, N=NED

PCR products were diluted 1:20 with water and resolved with an internal size standard (GeneScan 500 LIZ, PE Biosystems) using an ABI 3100 capillary-based genetic analyzer (PE Biosystems). For coloading, dilutions of 1:10 were made for the multiplex and combined with dilutions of 1:20 for the coloaded marker. Analysis of multiplex sets was done using ABI GENESCAN version 3.7 (PE Biosystems).

Results

Three hundred sixteen microsatellite markers from MSS-2 are resolved in 69 chromosome-specific panels (Table 1), providing an average of 1.73 multiplex sets per chromosome. Two hundred ninety-six markers can be coamplified within the chromosome panels. The remaining 20 markers are amplified individually and coloaded into designated panels for resolution in a single capillary. Eight markers are coamplified in pairs and are then coloaded with the appropriate panel. Three markers, FH3245, REN51i12, and, FH2239, could not be coamplified or coloaded in chromosome-specific fashion and must be resolved individually.

All multiplexed markers are amplified using a single PCR mix and thermal cycling program. For multiplexing, 78 primers were labeled with 6-FAM, 82 with VIC, 82 with NED, and 85 with PET. In multiplexes 7.1, 9.1, 12.2, 23.1, and 37.1, two markers with the same dye type have similar product sizes. Eight markers, REN262G24, REN286O18, FH3970, FH2200, REN297D17, FH3939, REN89K14, and FH3399, did not amplify or were not able to be genotyped using these parameters and, hence, were not incorporated into the chromosome-specific panels described here.

Discussion

The most comprehensive screening set currently defined for linkage studies in the dog is MSS-2, which offers 9 Mb coverage and highly polymorphic markers, including 64 markers from the MSS-1. To enhance the utility of MSS-2, we have developed chromosome-specific multiplex sets, which expedite whole genome scans in the dog and have the potential to exclude candidate genes on a given chromosome. Collection of data by chromosome also allows for statistical analysis for individual chromosomes to be conducted before the whole genome scan is complete and will facilitate confirmation of linkage studies, as well as positional cloning efforts.

The multiplex sets minimize the expense, time, and genetic material necessary to collect genotype information for MSS-2. The number of reactions and runs on a genetic analyzer are reduced by 68% and 76%, respectively. Collection of data is further streamlined through use of a single thermal cycling program and PCR mix. To increase the ease with which alleles can be determined, markers having the same dye type and product sizes separated by less than 50 bp were not combined in the same panel. However, similar product sizes from markers having the same dye type were observed in multiplexes 7.1, 9.1, 12.2, 23.1, and 37.1 and alternate dye-types could be selected to eliminate possible genotype error. Observed allelic sizes, figures of panels, overlapping markers, and suggested primer labels are available at www.cvm.tamu.edu/cgr/multiplex.html.

CHAPTER III

CLINICAL AND GENETIC ASSESSMENTS OF HIP JOINT LAXITY IN THE BOYKIN SPANIEL

Overview

Canine hip dysplasia (CHD) is characterized by a malformation of the hip joint that leads to joint laxity and consequential degenerative joint disease. The most widely used method for diagnosis of CHD is the ventrodorsal hip extended view, commonly referred to as the OFA method. An alternative technique, PennHIP, is based on hip joint laxity and provides a quantitative assessment, the distraction index (DI), regarding the likelihood of developing CHD due to increased laxity in the hip joint. Linear regression showed that the incidence of CHD is positively correlated with the mean DI. In an effort to better understand joint laxity and hip dysplasia, we utilized families of Boykin Spaniels (BSs) to determine the level of joint laxity in the breed and to conduct an initial whole genome screen to identify markers that co-segregate with marked joint laxity.

Introduction

The BS originated in South Carolina in the early 1900s and is now found throughout the United States, although the majority of the population is still found in and around the Carolinas. The BS is relatively small in size, weighing between 25 to 40 lbs, yet has a surprisingly high incidence of hip dysplasia as reported by the Orthopedic Foundation for Animals (OFA; http://www.offa.org). From a genetic standpoint, the BS

has a high level of genetic homogeneity based on analysis of randomly amplified polymorphic DNA (Wang et al. 1999).

CHD is the most common orthopedic disease of the dog. It is a degenerative disease characterized by malformation of the hip joint. The inevitable result of CHD is osteoarthritis, also termed degenerative joint disease (DJD). The presence of osteoarthritic changes is the primary diagnostic criterion for CHD. The most common radiographic tests used for diagnosis of CHD are the OFA method and the PennHIP (University of Pennsylvania Hip Improvement Program) method (Smith et al. 1990).

The OFA maintains a registry of dogs that have been radiographed using the ventrodorsal hip-extended view to diagnose the presence of CHD. A panel of radiologists scores radiographs on a 7-point scale. Prior to 1980, radiographs had been submitted to the OFA for only 38 BSs, with almost 40% of those dogs classified as dysplastic and none earning an excellent rating. Over 1,400 BSs were evaluated by OFA between 1974 and 2003. Less than 1% were scored as excellent, while approximately 40% were scored as dysplastic. In summary, the BS has the 9th highest incidence of hip dysplasia according to the OFA. The PennHIP method uses joint laxity to predict future development of CHD. Joint laxity can cause instability of the hip leading to incomplete or complete dislocation of the femoral head. The PennHIP method quantifies passive laxity in the hip joint by measuring the DI, which is the distance of the femoral head from the center of the acetabulum divided by the radius (Smith et al. 1990). A higher DI is indicative of a higher probability of developing osteoarthritis, as a result of CHD.

According to the PennHIP registry, the BS has the 12th highest mean distraction index of 0.64 (PennHip 2002).

CHD is a complex trait with both quantitative trait loci (QTL) and environmental factors contributing to the phenotype (Henricson et al. 1966; Leighton et al.1977; Hedhammar et al. 1979). The pattern of inheritance of CHD suggests that several major and minor QTL may influence the phenotypic presentation of CHD (Leighton 1997). Studies of the genetics underlying joint laxity and overt CHD include breeding programs in military working dogs and designed pedigrees (Leighton et al. 1977; Todhunter et al. 1999). Additionally, using the German Shepherd Dog, Smith et al. identified joint laxity as a heritable trait (Smith et al. 1990). Hip dysplasia in the human occurs more often in the left hip (Smith et al. 1963), but this trend has not been observed in the dog. However, Chase et al. detected a higher level of joint laxity in the left hips of Portuguese Water Dogs (PWDs) using the Norberg angle (Chase et al. 2004). They also report the identification of two QTL on CFA01 that asymmetrically affect joint laxity in the right or left hip of the PWD (Chase et al. 2004).

A linkage analysis approach is often taken to identify regions of the genome that co-segregate with a disease gene(s). This allows for the identification of candidate genes when there are none, or the narrowing of candidate genes if many exist. Microsatellite markers have become the tool of choice for linkage analyses due to their polymorphic nature and Mendelian inheritance. Microsatellites are tandem repeats of one to six base pairs that are dispersed throughout the genome. In the dog, the minimal screening set – 2 (MSS-2) is the most comprehensive screening set of microsatellite markers available

for use in whole genome scans. The MSS-2 is comprised of 327 microsatellite markers that have an average heterozygosity value of 0.73 and an average spacing of 9 Mb with no gaps larger than 17.1 Mb (Guyon et al. 2003). The MSS-2 has been multiplexed into 69 chromosome-specific panels to expedite whole genome screens (Clark et al. 2004). To identify markers that co-segregate with increased joint laxity, a whole genome screen utilizing the multiplexed MSS-2 was carried out on a small pedigree of BSs.

Report

Because many BSs have been examined using the OFA method and work reported here used the PennHIP method, it was of interest to define the relationship between CHD and joint laxity. Correlation between the OFA and PennHip methods has previously been defined. However, the criteria used are different between studies. For example, Adams et al. found a correlation between the DI and DJD, but not for DI and CHD as these conditions were classified (Adams et al. 2000). We wanted to define the correlation between DI and CHD based on the OFA definition of clinical dysplasia. Therefore, linear regression analysis was performed across 96 breeds to compare the incidence of hip dysplasia as defined by the OFA, to the degree of joint laxity as defined by PennHIP. Only breeds having 100 or more evaluations from 1974 to 2003 in the OFA registry and were represented by 20 or more dogs in the 2002 PennHIP registry were included. This analysis showed a positive correlation between the mean DI and the percentage of dysplastic dogs, as determined by the OFA assessment (Figure 1). The determination coefficient of OFA CHD incidence and the mean PennHIP DI is 0.26.

Although the BS is a relatively small breed, the breed is among the top ten most dysplastic breeds according to OFA. The PennHIP method offers a possible explanation for this. That is, the high degree of joint laxity in the BS predisposes the breed to CHD. Hence, the incidence of CHD as determined by the OFA method is increased in this breed.

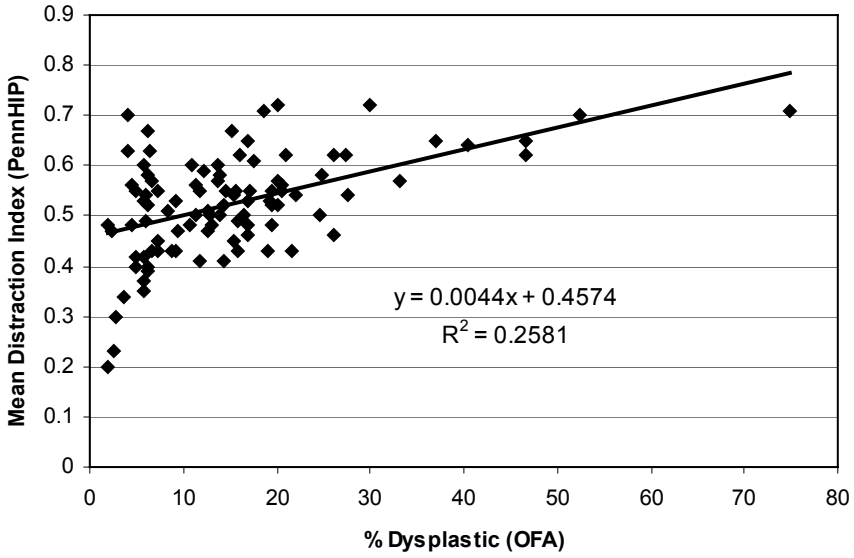

Figure 1. Linear regression analysis of the percent of dysplastic dogs in the OFA registry as compared to the mean DI for each breed.

We have assembled partial, multigenerational pedigrees of BSs that segregate CHD. DNA and PennHIP data were previously collected for each family member (Wang et al. 1999). DIs greater than 0.60 were considered affected phenotypes. The MSS-2 reactions were set up as described in Clark et al. for 28 BSs (Clark et al. 2004). Products were resolved using a capillary-based genetic analyzer (ABI 3100; PE Biosystems). Genotypes were analyzed using a commercial software program (Genotyper version 2.0;

PE Biosystems). Two-point LOD (Logarithm of the Odds) scores were calculated for each marker using a statistical software package (Sequential Oligogenic Linkage Analysis Routines, SOLAR; Southwest Foundation for Biomedical Research). LOD scores were calculated for the left and right hips separately, as well as together.

The average DI for the 28 genotyped dogs was 0.55. Genotypes were collected for 272 markers. Thirty-seven markers (14%) were non-informative, having only one or two alleles. LOD scores were calculated for 254 markers. Marker FH3413, located on CFA01, had the highest LOD score of 1.23. This LOD score was correlated with increased laxity in the right hip joint. A LOD score of 3.0 or greater is necessary to infer linkage. It is interesting that LOD scores for the left or right hip individually varied for many markers and that the highest LOD was found on the same chromosome that was identified by Chase et al. Although there was a positive correlation between the incidence of hip dysplasia and increased joint laxity, we did not find significant linkage in the BS. This is very likely due to the small size of the pedigree. Additional pedigree members and/or increased marker density are necessary to identify a region of interest.

CHAPTER IV

ANALYSIS OF ALLELE FIDELITY, POLYMORPHIC INFORMATION CONTENT, AND DENSITY OF MICROSATELLITES IN A GENOME WIDE SCREENING FOR HIP DYSPLASIA IN A CROSS BREED PEDIGREE*

Overview

Recent advances in genomics resources and tools are facilitating quantitative trait locus mapping. We developed a cross breed pedigree for mapping QTL for hip dysplasia in dogs, by crossing dysplastic Labrador Retrievers and normal Greyhounds. We show that one advantage of using a cross breed pedigree is the increased marker informativeness in the backcross/F_2 population relative to the founder populations. We also discuss 3 factors that affect the detection power in the context of this cross breed pedigree: ability to detect and correct genotyping errors, increasing marker density for chromosomes with a sparse coverage, and addition of individuals to the mapping population as soon as they become available.

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* Reprinted from Mateescu RG, Zhang Z, Tsai K, Phavaphutanon J, Burton-Wurster NI, Lust G, Quaas R, Murphy K, Acland GM, and Todhunter RJ (2005) Analysis of allele fidelity, polymorphic information content, and density of microsatellites in a genome wide screening for hip dysplasia in a cross breed pedigree. Journal of Heredity (in press) by permission of Oxford University Press

Introduction

Our interest is in the clinical characterization and genetic basis for canine hip dysplasia (CHD). To dissect the underlying genetics of this common heritable trait in dogs, an experimental pedigree was established in 1994 by crossing unaffected Greyhounds and dysplastic Labrador Retrievers. This pedigree includes over 150 dogs spanning four generations of backcrosses and intercrosses (Todhunter et al. 1999, Todhunter et al. 2003). Clinical assessment of hips is determined using multiple diagnostic approaches when dogs are 4 and 8 months of age (Bliss et al. 2002, Lust et al. 2001). A genome-wide screen was undertaken on this cross breed pedigree in collaboration with the Mammalian Genotyping Service, Marshfield, WI. At the time of this undertaking, Minimal Screening Set 1 (MSS1) of 172 markers (Richman et al. 2001) and the canine genetic map of 2001 (Breen et al. 2001) were used to develop a 240-marker set for the aforementioned screen with microsatellite markers to be resolved using a gel-based system.

The identification of genes contributing to variation in canine hip dysplasia requires genetic data of high fidelity. The genotypes on 147 cross breeds at 247 loci were assessed for correct Mendelian inheritance patterns and repeatability. Genotyping errors occur when the observed genotype does not correspond to the true underlying genetic information, as a result of a mistake in data entry or a misinterpretation of the pattern on a gel. Even a small number of genotyping errors can have negative consequences, increasing the estimated recombination fraction (Terwilliger et al. 1990)

and reducing the evidence for linkage (Abecasis et al. 2001; Goring and Terwilliger 2000).

In this report, we describe the method used to identify reading errors in marker allelic size and the approach taken to correct such errors. A comparison of the microsatellite informativeness between the pure breeds and the cross breed dogs is also presented. We further show, using one chromosome as an example, the effect on QTL mapping resolution of correcting genotyping errors, of including additional markers and of adding more dogs to the analysis.

Materials and methods

Pedigree

Seven Greyhounds (2 males and 5 females) with excellent hip conformation chosen from racing stock along with 7 Labrador Retrievers (3 males and 4 females) with hip dysplasia and secondary hip osteoarthritis and one female Labrador Retriever with an intermediate phenotype but from a dysplastic lineage were selected as founders for our pedigree (Todhunter et al. 1999). The Greyhound (G) is one of the few breeds in which hip dysplasia is rare as evidenced by hip joint conformation consistently scored as "perfect" (Beling et al. 1975) or "normal" (PennHIP™ hip registry, Malvern PA) (Cardinet GH et al. 1983). The Greyhound founders were assumed to be homozygous for alleles protective against hip dysplasia and the dysplastic Labrador Retrievers were assumed to be homozygous at the loci contributing to hip dysplasia for statistical

mapping purposes. The cross breed pedigree consists of 4-generations (G and L founders, F_1, backcrosses to both founders, and F_2 individuals) comprising 159 dogs.

DNA and Genotyping

DNA was isolated from peripheral blood by phenol-chloroform extraction. Gel-based electrophoretic separation was used to size microsatellite alleles at the NHLBI Mammalian Genotyping Service, Marshfield Medical Research Foundation, Marshfield, WI (Weber and Broman 2001) and capillary electrophoresis was used at Cornell University at the Bioresource Center on an Applied Biosystems Incorporated 3730 sequencer.

Microsatellites

From the integrated canine genetic map (Breen et al. 2001), we selected 240 microsatellite markers, 142 from the linkage map and 98 from the RH map (Lou et al. 2003). Seven additional microsatellite markers were identified for CFA37 (personal communication with E. Kirkness, The Institute for Genomic Research, Rockville, MA). The most likely order and spacing of markers on this chromosome were calculated based on meiotic recombination in our pedigree using multipoint analysis available from MULTIMAP (Matise et al. 1993). This marker set covers approximately 80% of the estimated length of the canine genome (2.4 Gb).

Allele Check

The QTL mapping programs require marker genotype data in a pedigree to follow the Mendelian inheritance principles. Therefore, the first step in data editing is to check for Mendelian inheritance errors. We developed a program (ZZ, http://www.people.cornell.edu/pages/zz19/research/genoped) that checks the genotypes in the pedigree for inconsistencies between parents and offspring.

Allele Correction

The second step in data editing is correcting these errors. A program to accomplish this task (RM) identifies all possible alleles for a given marker in the grand parent's generation, assumes that these alleles are measured without errors, follows each allele through the pedigree and checks for inconsistencies within a narrow interval. Finally, inconsistencies are corrected. The range was defined as ± 2 bp relative to the grand parent allele size for the tetranucleotide and ±1 bp for di- and trinucleotide repeat microsatellites. These errors were corrected by calling the alleles that differ by 1 or 2 bp as one allele. The errors outside these ranges were left uncorrected, resulting in missing data.

Polymorphic Information Content

To assess the quality of marker genotype data in our cross breed pedigree, the total number of alleles and the mean number of alleles per locus were determined for each marker for the two founder groups, the F_1 breeders and the BC/F_2 population. The

differences in observed allele frequencies for pairwise comparisons between the Labrador Retriever and Greyhound founders were tested at each locus. Because the sample size is small relative to the size of markers contingency tables, a chi-square test may not be valid and a more appropriate test of no association would be Fisher's exact test.

The Polymorphism Information Content (PIC) for each marker was determined separately for the four groups of animals using the following equation:

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

where p_i is the frequency of the i^{th} allele and n is the number of alleles (Botstein et al. 1980).

To analyze the change in the PIC between the four different breed groups, the following statistical model was used:

$$Y_{ij} = \mu + \text{Breed}_i + e_{ij}$$

where

Y_{ij} = PIC for marker $_j$ in breed $_i$,

μ = overall mean,

Breed_i = breed (i = Labrador Retriever, Greyhound, F_1, backcross),

e_{ij} = error associated with ij^{th} observation, assumed to be normally distributed N (0, σ^2_e).

QTL Mapping

QTL mapping was performed using a regression approach originally described by Haley and Knott (1992). A web-based version of this tool is available (Seaton et al. 2002). The software, QTL Express (http://latte.cap.ed.ac.uk/), analyzes data from different mating schemes including the combined backcross/F_2 design employed for our cross breed pedigree. Hip traits measured and analyzed included the distraction index (DI, a measure of hip laxity), the dorsolateral subluxation (DLS) score and the Norberg angle. Chromosome-wide significance thresholds for each trait were determined (Churchill and Doerge 1994); the threshold at $p<0.05$ and $p<0.01$ was obtained from 1000 permutations.

Results

Allelic Size Error Detection

The error rate was 4.92% (out of 36,309 genotypes) for all 247 markers (Table 2). The highest average error rate at 16.08% was for chromosome 37. Average error rates for chromosome 4, 7, 25 and 32 were > 10%. Fourteen other chromosomes had one or more markers with error rates > 30% (data not shown). Only chromosome 38 and the Y chromosomes were free of marker error. The marker with the highest error rate was FH2532 on CFA37 with 68% errors and this marker alone accounted for 89% of all genotyping errors associated with this chromosome (data not shown). Tetranucleotide markers had a disproportionate number of errors compared to the di- and trinucleotides.

Table 2. Distribution of genotyping errors on initial and corrected data following a genome-wide screen with 247 markers on 159 dogs from a cross breed pedigree.

		Raw data (n=147)		Corrected data (n=159)	
CFA	# markers	Errors	Rate (%)	Errors	Rate (%)
1	11	54	3.5	8	0.46
2	11	101	6.38	15	0.86
3	9	85	6.6	22	1.54
4	8	118	10.17	45	3.54
5	10	40	2.83	7	0.44
6	6	80	9.43	18	1.89
7	10	180	12.57	15	0.94
8	6	52	6.06	2	0.21
9	7	37	3.65	34	3.05
10	7	46	4.65	26	2.34
11	7	82	8.18	20	1.80
12	9	63	4.89	5	0.35
13	5	1	0.14	3	0.38
14	7	70	7.09	40	3.59
15	7	27	2.69	2	0.18
16	4	12	2.09	1	0.16
17	5	29	4.18	2	0.25
18	7	49	4.97	12	1.08
19	5	9	1.29	9	1.13
20	5	55	7.74	54	6.79
21	5	51	7.31	14	1.76
22	6	58	6.84	13	1.36
23	6	21	2.45	3	0.31
24	4	5	0.88	5	0.79
25	6	95	11.14	5	0.52
26	5	14	1.96	0	0
27	6	13	1.50	7	0.73
28	6	19	2.21	11	1.15
29	4	3	0.53	3	0.47
30	7	24	2.35	3	0.27
31	5	5	0.69	4	0.50
32	4	72	12.83	7	1.10
33	5	53	7.36	10	1.26
34	4	24	4.15	0	0
35	4	3	0.54	0	0
36	2	3	1.05	3	0.94
37	13	114	16.08	0	0
38	4	0	0	0	0
X	5	0	0	0	0
Total	247	1788	4.92	522	1.07

The error rate for each chromosome after running the Allelic Size Correction program is shown in Table 2. The average error rate for the corrected data was 1.07% and the total number of errors was reduced by 70%. All chromosomes, except CFA20, have an average error rate below 3% and 5 chromosomes have zero errors.

Marker Informativeness

Summary statistics for PIC, including the mean number of alleles per locus, the median, 25% and 75% quartiles and the range for the two founder breeds, F_1 and backcross generations, are listed in Table 3. The marker distribution by number of alleles in the cross breed pedigree is shown in Figure 2. A decrease in proportion of markers with fewer than 3 alleles and an increase in those with more than 3 alleles is observed in the F_1 and BC generation as compared to the two founder breeds. The increase in the number of alleles per microsatellite locus translates into an increase in the PIC (Table 3).

The increased informativeness of the markers in a cross breed pedigree is one of the advantages of using a cross breed pedigree for linkage mapping and is associated with an increase in proportion of moderately and highly informative markers in F_1 and backcross generations relative to the founders (Table 4). Approximately 60% of the markers in the backcross generation demonstrated high information content compared to only 39% in the Labrador Retriever and 48% in the Greyhound founders.

Table 3. Descriptive statistics including number of individuals (n), the mean and maximum number of alleles per marker, the median, 25% (Q1) and 75% (Q3) quartiles and the range for the polymorphism information content (PIC) of 247 microsatellite markers screened on 159 dogs in an experimental canine pedigree representing the Labrador Retriever (L) and Greyhound (G) founders, F_1 and backcross (BC) generations.

		No. of alleles		PIC			
	n	Mean/marker	Maximum	Median	Q1	Q3	Range
L	9	3.61	8	0.54	0.37	0.65	0-0.84
G	7	4.1	9	0.58	0.39	0.70	0-0.86
F_1	7	4.3	10	0.62	0.46	0.72	0-0.88
BC	136	5.58	16	0.63	0.46	0.71	0-0.89

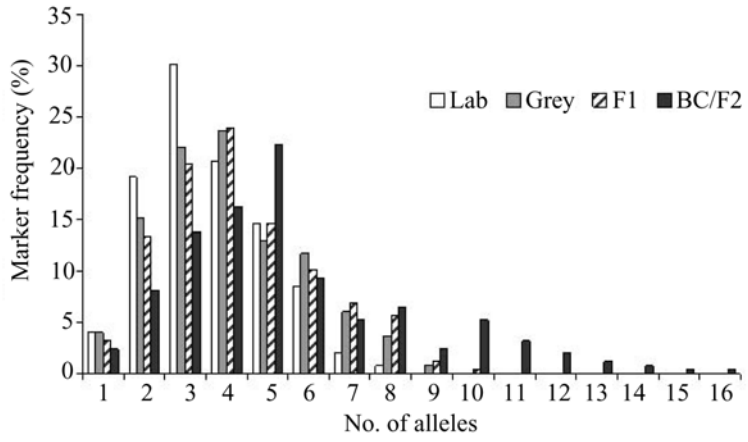

Figure 2. Marker distribution versus number of alleles in the Labrador Retriever (Lab) and Greyhound (Grey) founders, F_1 and backcross/F_2 (BC/F_2) populations.
□ Lab ▒ Grey ▨ F_1 ■ BC/F_2

Table 4. Descriptive statistics for 247 microsatellite markers in the Labrador Retriever (L), Greyhound (G), F_1 and backcross/F_2 (BC/F_2) populations.

	Range of PIC values		
	< 0.30 (uninformative)	0.3 - 0.59 (moderately informative)	> 0.60 (highly informative)
L	37 (15.04%)	114 (46.34%)	95 (38.62%)
G	34 (13.82%)	94 (38.21%)	118 (47.97%)
F_1	27 (10.98%)	90 (36.58%)	129 (52.44%)
BC/F_2	22 (8.94%)	76 (30.89%)	148 (60.16%)

The differences in observed allelic frequencies for pair wise comparisons between the Labrador Retriever and Greyhound founders were tested at each locus. Four out of 247 markers (REN193A22, REN166C13, AHTH134Ren and RENo2C20) were monomorphic in both founder breeds. For 162 of the remaining 243 markers, the allelic frequencies were significantly different between the two founder populations (the Fisher test for 127 markers had $P < 0.01$ and for 35 markers had $0.01 < P < 0.05$). The most prominent markers (with highest Fisher test statistic) were REN41D20, REN150M24, REN130F03, LEI002 and FH2060. We also evaluated the difference in number of alleles between the two founder breeds for each marker. In the Greyhounds, 24.4% of the

markers had up to three fewer alleles, 28% had the same number and 47.6% had up to six more alleles relative to the Labrador Retriever founders.

The least square means and standard deviation for PIC in the two founder breeds, F_1 and backcross generations are shown in Table 5. Pair wise comparison of PIC least squares means shows that the two founder breeds were not different (P = 0.1). However, PIC was significantly higher in F_1 and backcross individuals (P < 0.01) relative to the Labrador Retriever Retriever founders and in backcross (P < 0.01) relative to the Greyhound founders.

Table 5. Least square means (LSM) and standard deviation (SD) for PIC in the Labrador Retriever founders (L), Greyhound founders (G), F_1 and backcross/F_2 (BC/F_2) populations.

	PIC		Het	
	LSM	SD	LSM	SD
L	0.50	0.19	0.55	0.20
G	0.53	0.21	0.58	0.21
F_1	0.57	0.20	0.62	0.20
BC/F_2	0.58	0.20	0.63	0.19

Factors Affecting Mapping Power on CFA06

CFA06 is used as an example to illustrate the importance of correcting genotype errors, of marker density and of the number of observations on the ability to detect QTL. Six markers covering 45.5 cM of CFA06 were initially genotyped on 147 dogs from the cross breed pedigree. Checking the data for Mendelian-inheritance errors, 33 and 54 errors were identified for marker FH2164 and marker FH2561, respectively. To analyze the data using QTL Express, all errors were changed to missing values (QTL Express requires genotypes at each marker to be consistent with Mendelian inheritance). An interval mapping analysis was performed and the values of the F test for three traits (DI on the left side and DLS score on both right and left side) at each location along CFA06 are plotted in Figure 3A. From these results, we would conclude that there is no QTL affecting any of these traits on CFA06.

In the next step, errors for markers FH2164 and FH2561 were corrected using our Allelic Size Correction program. The 33 errors for marker FH2164 and 53 of the 54 errors for marker FH2561 were successfully resolved. The remaining error for marker FH2561 is likely a mutation and, therefore, it was set as a missing value. Interval mapping analysis was performed again using the corrected data set and the plot of the F test statistic is shown in Figure 3B. Note the rise of the F test value for all 3 traits at the right telomeric end of the chromosome, with the F-test for the left DI trait approaching significance. These results suggest that a QTL around the 45 cM position might reside on this chromosome. Note that most of the corrected errors (53) were for FH2561 marker which is located at 45 cM.

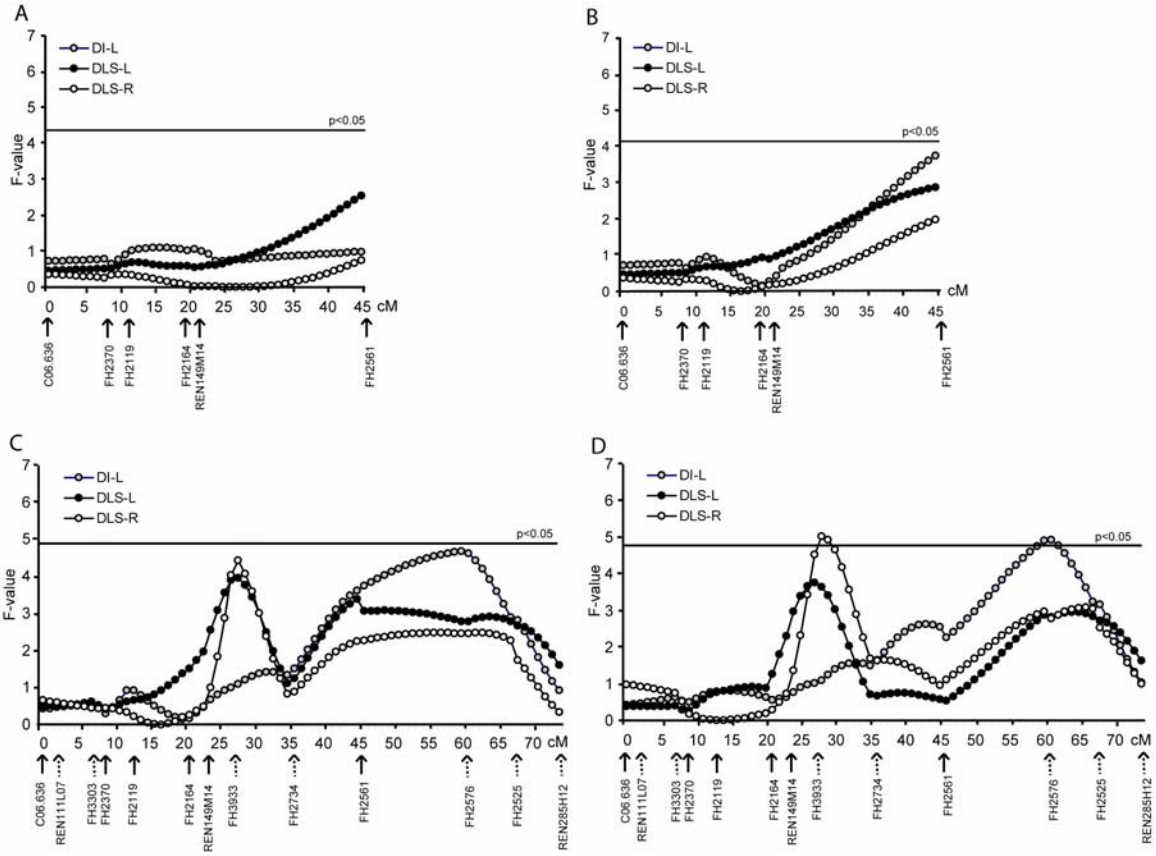

Figure 3. F-value plots for left distraction index (DI), left and right dorsolateral subluxation (DLS) score from interval mapping on CFA06. The position of the initial six markers is indicated by the solid arrows and the position of additional 7 markers by interrupted arrows.

left DI left DLS right DLS

A. Mendelian-inheritance errors on markers FH2164 (33) and FH2561 (54) were transformed into missing values. No F statistic reached the chromosome-wide threshold indicated by the horizontal bar.

B. Mendelian-inheritance errors on markers FH2164 (33) and FH2561 (54) were corrected. No F statistic reached the chromosome-wide threshold indicated by the horizontal bar, but there was an increase in F-test values at the far right end of the chromosome.

C. Interval mapping after correction of Mendelian-inheritance errors on markers FH2164 and FH2561 and addition of 7 markers. The F statistic for the 3 traits are approaching the chromosome-wide threshold.

D. Interval mapping after correction of Mendelian-inheritance errors on markers FH2164 and FH2561 and addition of 7 markers and 12 backcross individuals. The F statistic for the left DI and right DLS score are exceeding the threshold of significance at $\alpha = 0.05$.

The approximate length of CFA06 is 87 cM and the above six markers provided coverage for only half of the chromosome. Moreover, the distribution of the six markers was not optimal because of a large interval of 22 cM between markers REN149M14 and FH2561.

The most recent version of the canine map contains 3,300 markers at 1 Mb resolution (Guyon et al. 2003). That work included a description of Minimal Screening Set 2 (MSS2), which is comprised of 327 markers. To expedite linkage studies and positional cloning efforts, the MSS2 was multiplexed into chromosome-specific panels (Clark et al. 2004). To better characterize this chromosome and to be able to confirm or reject the possibility of a QTL, seven more markers from MSS2 were genotyped on the same 147 individuals. The coverage of CFA06 was extended to 74.4 cM by the addition of 3 markers at the right telomeric end of the chromosome. The coverage was also improved by adding 2 highly informative markers in the 22 cM interval between markers REN149M14 and FH2561. A complete description of the 13 markers is provided in Table 6. Interval mapping analysis was performed using the new data set and the plot of the F test statistic is shown in Figure 3C. The addition of the 3 markers at the telomeric end of the chromosome resulted in a peak definition for the left DI at 60 cM with an F test value close to the chromosome-wide significance threshold of 0.05. This result supports the presence of a QTL for this trait suggested by the previous analysis using the initial 6 markers.

Table 6. Location (cM), polymorphism information content (PIC) and number of alleles for 13 markers on CFA06 on the first 147 individuals and on the final 159 individuals from the cross breed pedigree.

		n = 147 dogs		n = 159 dogs	
Marker	cM	PIC	# allele	PIC	# allele
C06.636	0	0.13	3	0.13	3
REN111L07	3.6	0	1	0	1
FH3303	7.4	0.86	12	0.86	12
FH2370	8.8	0.80	10	0.85	11
FH2119	12.3	0.75	5	0.76	7
FH2164	20.4	0.71	6	0.72	8
REN149M14	23.4	0.54	3	0.54	3
FH3933	27.5	0.73	8	0.73	8
FH2734	35.2	0.66	4	0.66	4
FH2561	45.5	0.81	9	0.80	11
FH2576	60.6	0.81	13	0.81	13
FH2525	67.1	0.62	7	0.62	7
REN285H12	74.4	0.18	2	0.18	2

Interestingly, this analysis suggests a QTL at 28 cM on CFA06 for both left and right DLS score. A closer examination of the markers in this chromosomal region shows that the only marker available in this area from the initial set of 6 markers is not very informative (REN149M14), having only 3 alleles segregating in the population and a PIC of 0.54. This explains why the analysis with the first 6 markers showed no QTL present in this region. The addition of two highly informative markers at position 27 and 35 cM (FH3933 with a PIC = 0.73 and FH2734 with a PIC = 0.66) revealed the presence of a QTL in this region.

This work was expanded with the addition of 12 new BC individuals that were genotyped for all 13 markers. To determine whether this addition strengthens the data, interval mapping analysis was performed using all 159 individuals and the plot of the F test is shown in Figure 1D. The addition of 12 individuals increased the peak F-value for both left DI and right DLS score above the threshold of significance at $\alpha = 0.05$ and refined the definition of the peak for the left DI.

These results suggest that a QTL is segregating on CFA06 for both the DI and DLS score. This example is a clear illustration of the increase in power to detect QTL obtained by careful correction of genotyping errors, by strategic addition of more markers to provide a better coverage of the chromosome and by addition of more individuals in the analysis.

Discussion

Genotyping errors occur when observed genotypes do not reflect the true alleles. These errors arise from mistakes in data entry, sample mishandling or errors introduced by the genotyping process itself (Ewen et al. 2000; Ewen et al. 2000). Genotyping errors are detrimental to linkage analyses (Goldstein et al. 1997; Cherny et al. 2001); therefore, the identification of errors is necessary for accurate analysis of data. While it is recognized that large genotyping data sets will likely contain errors, and the inclusion of incorrect data can result in erroneous conclusions (Abecasis et al. 2001; Terwilliger et al. 1990), little attention is generally given to correcting the identified errors. The cleaning of genotype data should be an integral and important component of a successful genome scan for QTL detection. Too often data editing is limited to identifying Mendelian inheritance errors and changing the markers with errors into missing values in order to be able to run the analysis. Described herein is the importance of detecting genotyping errors, even those with modest error rates. In our study, an error rate of 4.87% was detected following a whole-genome screen with 247 microsatellite markers on 147 dogs from a cross breed pedigree and was reduced to 1.07% with the use of an Allele Correction program. Correction of genotyping errors increased the detection power of linkage analysis and allowed detection of an underlying QTL on CFA06 that did not show initial evidence for harboring QTL.

The increased informativeness of the markers in a cross breed pedigree is a major advantage of using a cross breed pedigree for linkage analysis of complex traits. Evidence for this in our pedigree is that there is an increase in proportion of markers

with higher number of alleles in the F_1 and BC/F_2 generation compared to the two founder breeds.

Fine mapping of putative QTL is time consuming and costly. Therefore, it is useful to exclude certain chromosomes and chromosomal regions with a high level of confidence. The marker set used in this study was chosen to provide optimal coverage of the canine genome. Even so, intervals between some markers exceeded 10 cM. Addition of more markers in these regions will improve the mapping resolution or result in a definite exclusion of these chromosomal regions for further fine mapping. Also, the addition of highly informative markers in a 22 cM interval on CFA06 allowed us to map a QTL at a chromosome-wide significance level of 0.05.

One of the major issues when envisaging positional cloning of the mapped QTL is the poor mapping resolution that is typically achieved after the initial genome-wide screen. Confidence intervals for the location of the QTL are of the order of 20 to 30 cM and typically contain as many as 500 to 1,000 genes. This is due to the limited number of recombination events in the available pedigree, and the fact that many QTL effects are likely to reflect the combined action of multiple linked genes. The resolution of QTL mapping is limited by the information gained from observing the genotypic states of the markers (Darvasi et al. 1993). The observed recombinants can be limited by both small sample size and missing genotypic data. In one example, the addition of 12 backcross individuals increased the detection power of linkage analysis and helped to narrow the QTL region on CFA06.

In conclusion, by maximizing the information for QTL mapping, even in a less than optimum genome-wide screen, it is possible to detect putative QTL in dog pedigrees. This can be achieved by creating a cross breed pedigree which will result in increased marker informativeness. Detection and correction of genotyping errors is a crucial step in ensuring that maximum information is extracted from available data. The addition of additional markers in regions with poor coverage and addition of new individuals as they become available would also increase the power of the analysis.

CHAPTER V

COMPREHENSIVE SCAN OF CHROMOSOMES HARBORING PUTATIVE QUANTITATIVE TRAIT LOCI FOR CANINE HIP DYSPLASIA

Overview

Canine hip dysplasia (CHD) is a disease of the hip joint characterized by hip laxity and subluxation. CHD is widespread, affecting nearly every breed and more than half of the populations of several breeds. To investigate the genetics underlying CHD, an informative outcrossed pedigree was established using non-dysplastic Greyhounds and dysplastic Labrador Retrievers. Previously, a genome-wide scan using these kindred identified 12 chromosomes harboring putative quantitative trait loci (QTL). A chromosome-specific scan using multiplexed microsatellite markers was undertaken to further define and corroborate the identification of QTL involved in CHD.

Introduction

Canine hip dysplasia (CHD) is characterized by malformation of the hip joint. It predominantly affects medium and large breed dogs, but is present in almost all breeds. CHD is rare in sight hounds such as the Borzoi and the Greyhound, which are known for their tight hips (Todhunter and Lust 2003). CHD is generally a bilateral disease but can present unilaterally (Todhunter et al. 1997). The genetics underlying CHD are complex and several major and many minor quantitative trait loci (QTL) likely play roles in the

overall phenotype (Henricson et al. 1966; Leighton et al.1977; Hedhammar et al. 1979). Heritabilty estimates for CHD range from 0.11 to 0.68 (Bliss et al. 2002).

There are several radiographic techniques that predict the development of CHD in young dogs based on hip conformation. The dorsolateral subluxation (DLS) test measures the percentage of the femoral head that is covered by the acetabulum while the dog is in sternal recumbency. An increased coverage increases the DLS score and represents a decreased probability of developing CHD (Farese et al. 1998). The DLS score is 83% sensitive and 84% specific (Lust et al. 2001).

The PennHip method uses the distraction index (DI) to measure passive laxity in the hip. Specifically, the DI measures the distance of the femoral head from the center of the acetabulum divided by the radius (Smith et al. 1990). Scores generally range from zero to one, with a higher value indicating an increased joint laxity and therefore a higher probability of developing CHD.

The Norberg angle (NA) is another indicator of subluxation that measures the angle of the femoral head in the acetabulum. A NA greater than 105° is considered normal. As the femoral head shifts out of the acetabulum, the angle decreases, indicating joint laxity (Morgan et al. 2000).

Cornell maintains a pedigree of outcrossed Greyhounds and Labrador Retrievers (Todhunter et al. 1999). The pedigree was determined to have a power of 0.8 with an α level of 0.05 to detect QTL in a genome screen in the backcross generation using a single-marker, linkage-based simulation (Todhunter et al. 2003). In an effort to identify QTL involved in joint laxity and development of CHD, a whole-genome screen was

carried out at the Marshfield Clinic for Medical Genetics on 152 members of the designed pedigree (Todhunter 2005). Twelve chromosomes harboring putative QTL, some with a protective effect and others that appear to worsen the phenotype, were identified (Todhunter 2005).

The MSS-2 is the most comprehensive screening set of microsatellite markers now available for use in whole genome scans. The MSS-2 is comprised of 327 microsatellite markers that have an average heterozygosity value of 0.73 and an average spacing of 9 Mb with no gaps larger than 17.1 Mb (Guyon et al. 2003). The MSS-2 has been multiplexed into 69 chromosome-specific panels to expedite whole genome screens (Clark et al. 2004).

A scan utilizing the chromosome-specific panels of the MSS-2 to further define the 12 chromosomes harboring putative QTL identified by Todhunter et al. (2005) was carried out. Five additional chromosomes were selected that were suggestive of linkage but did not reach a significance level of $p<0.05$ (Todhunter, personal communication) or have been implicated in other work (Chase et al. 2004, Chase et al. 2005).

Materials and methods

MSS-2 reactions were set up as described by Clark et al. (2004) for the following chromosomes: 1, 2, 3, 4, 5, 7, 9, 10, 11, 12, 19, 25, 26, 29, 32, 37, and X. A total of 161 markers were analyzed generating over 25,000 genotypes. Samples were resolved with an internal size standard (GeneScan 500 LIZ, PE Biosystems) using an ABI 3730

capillary-based Genetic Analyzer (PE Biosystems). Genotypes were analyzed using GeneMapper v 3.5 (PE Biosystems).

Two-point LOD (Logarithm of the Odds) scores were calculated using a statistical software package (Sequential Oligogenic Linkage Analysis Routines, SOLAR; Southwest Foundation for Biomedical Research). SOLAR does not assume a mode of inheritance allowing the program to analyze the data for recessive, dominant and additive modes. The likelihood maximized by SOLAR is the normal. However, phenotypic traits are often not normally distributed. For that reason LOD scores were generated using both the $\log_{10}$ and the natural log (ln) of the likelihood ratio. LOD scores for each trait (DLS, DI, and NA) were calculated for the left and right hips separately. The markers included in the Marshfield screen were re-analyzed using the SOLAR program and are included in this work.

Results

LOD scores were calculated for 159 members of the Greyhound/Labrador Retriever pedigree. A significant LOD was not detected for any of the traits. All LOD scores greater than one are reported in Tables 7 and 8. Forty markers for different traits had a LOD between 1.0 and 2.0 when combining the $\log_{10}$ and ln analyses. Six markers with various traits had a LOD score between 2.0 and 3.0, with the highest score of 2.69 located on CFA02 for marker FH2225. Interestingly, four of six markers on CFA29 had LODs greater than one for NA of the left hip. The markers with LODs greater than one did not change between the log and ln methods for this chromosome. One marker on

CFA30, REN50N18, has a LOD greater than one for both the DI and DLS traits in both hips (using the log data). All but 5 of the markers with LODs greater than one calculated with the $\log_{10}$ also had a LOD greater than one using the ln method.

Table 7. LOD scores calculated with Log_{10}

Trait	Chromosome	Marker	LOD
DI - left	CFA02	AHT132	1.57
	CFA02	FH2225	2.69
	CFA02	FH3006	1.26
	CFA20	CPH16	1.60
	CFA30	REN50N18	1.9
DI - right	CFA10	FH2537	1.56
	CFA30	REN50N18	1.68
DLS - left	CFA04	FH2732	1.33
	CFA05	CPH18	1.71
	CFA07	REN97M11	1.23
	CFA08	FH3316	1.01
	CFA12	FH1040	1.14
	CFA30	LEI-1F11	1.07
	CFA30	REN50N18	2.07
DLS - right	CFA30	REN50N18	1.76
NA - left	CFA09	REN145P07	1.63
	CFA29	CPH9	1.10
	CFA29	FH2328	1.09
	CFA29	FH2609	1.15
	CFA29	REN45F03	1.31
	CFA33	REN291M20	1.03
NA - right	CFA23	FH2626	1.07
	CFA29	FH1007	1.35
	CFA31	REN265M13	1.03

Table 8. LOD scores calculated with ln			
Trait	**Chromosome**	**Marker**	**LOD**
DI - left	CFA02	AHT132	1.05
	CFA02	FH2225	2.00
	CFA02	FH3006	1.14
	CFA02	REN70M14	1.03
	CFA02	REN150M24	1.11
	CFA07	VIASD10	1.53
	CFA17	REN310J13	1.18
	CFA20	FH2528	1.49
	CFA23	FH2508	1.30
DI - right	CFA01	REN47D17	1.07
	CFA10	C10.16	1.30
	CFA10	FH2537	1.34
	CFA10	REN06H21	1.09
	CFA15	REN06C11	1.52
	CFA31	REN265M13	1.16
	CFA32	AHT127	1.99
	CFA36	REN85C13	1.38
DLS - left	CFA04	AHT103	2.18
	CFA04	FH2732	2.29
	CFA05	CO5.377	1.15
	CFA05	CPH14	1.89
	CFA05	CPH18	2.38
	CFA05	REN175P10	1.95
	CFA08	FH3316	1.25
	CFA12	FH1040	1.47
	CFA18	AHT130	1.46
	CFA20	FH2528	1.35
	CFA29	REN52D08	1.57
	CFA30	LEI-1F11	1.53
	CFA30	REN50N18	1.97
DLS - right	CFA04	FH2732	1.27
	CFA30	REN50N18	2.16
NA - left	CFA09	REN145P07	1.59
	CFA29	CPH9	1.09
	CFA29	FH2328	1.11
	CFA29	FH2609	1.16
	CFA29	REN45F03	1.29
	CFA33	REN291M20	1.02
NA - right	CFA23	FH2626	1.06
	CFA29	FH1007	1.34
	CFA31	REN265M13	1.05

Discussion

In an effort to further define regions harboring QTL for CHD (Todhunter et al. 2005) a chromosome-specific scan was carried out on a Greyhound/Labrador Retriever outcrossed pedigree. A statistically significant LOD score was not found for any markers. However, several areas of interest were highlighted. For example, Todhunter et al. (2005) identified a QTL on CFA29 with a $p<0.01$. A LOD higher than one was calculated for four of six markers on CFA29. It is possible that the use of multi-point analysis in which neighboring marker information is taken into account will boost the scores to a significant level. By normalizing the data using both the $\log_{10}$ and the ln, we expect to find the same regions highlighted. The $\log_{10}$ analysis overlapped the ln data almost completely (with the exception of 5 scores) indicating that these could be true markers segregating with the trait of interest.

The scores show the value of using multiple traits to scan for a complex disease since several markers showed suggestive results with one trait but not another. SOLAR will be used to analyze the segregation of a low or high DI, DLS and NA in an effort to identify other putative QTL or corroborate interesting data we have generated thus far.

Due to the special design of the outcrossed pedigree, other modeling techniques, such as QTL Express (http://latte.cap.ed.ac.uk/), that include the population structure as a fixed effect are being utilized for analysis of these data. Analyses of the remaining chromosomes not included in the initial screen and of a pure bred population of Labrador Retrievers, created with many of the same founders in the outcrossed pedigree, are also underway.

CHAPTER VI

CONCLUSIONS

The dog plays a role in so many facets of human society. They are hunters, protectors, and most of all our companions. Our laboratory's main goal is to improve the quality of life for dogs. However, an important ancillary aim is to provide new insight into human diseases by dissecting the genetics of those hereditary diseases that affect the dog and human. The specific aims for this work are to develop an efficient genomic tool for use in whole genome screens and to identify genomic regions that are influential in the development of CHD.

CHD is a complex disease that affects most breeds. Joint laxity is an important physical and genetic component of CHD (Henricson et al. 1966, Kealy et al. 1997). Radiographic methods that score hip conformation in young dogs, and therefore, predict the likelihood of later development of CHD have been based on measuring joint laxity in the hip (Smith et al. 1990, Farese et al. 1998, Morgan et al. 2000). There has been little success using selective breeding practices to minimize the incidence of CHD. The identification of genes that are involved in joint laxity and therefore CHD will allow breeders to screen for susceptible dogs prior to breeding.

Chapter II describes the development of a tool for use in whole genome scans of the dog. A set of microsatellites was multiplexed into chromosome-specific panels (Clark et al. 2004). The MSS-2 is composed of 327 microsatellites that provide 9 Mb coverage of the canine genome (Guyon et al. 2003). Primers were labeled with

flurorescent dyes (6FAM, VIC®, PET®, NED®) allowing co-resolution of markers. Labels were selected based on chromosomal location and product size to allow minimal overlap of products. Reactions were optimized for resolution on an ABI 3100 Genetic Analyzer. Sixty-nine chromosome-specific panels were developed for the MSS-2. This work reduces the number of necessary reactions by 68% and runs on a genetic analyzer by 76%.

The multiplexed MSS-2 panels were used in a whole genome screen for joint laxity in the BS as described in Chapter III. Genotypes were generated for 28 BSs for 254 of the MSS-2 markers. Laxity was measured using the DI from the PennHip method. Two-point LOD scores were calculated for each marker using SOLAR. The highest LOD score was for a marker located on CFA01, but did not reach statistical significance. Two methods to diagnose and predict future development of CHD were compared and found to be positively correlated.

Chapter IV describes the power that can be gained by using a designed pedigree. The PIC for markers used in a whole genome scan of an outcrossed Greyhound/Labrador Retriever pedigree increased in the F1 and F2/BC generations. The need for stringent genotyping corrections is also shown. Using an Allele Correction Program, genotyping errors were reduced, allowing the identification of a putative QTL. Increasing genome coverage with additional markers allows for more opportunity to detect a QTL as well. It is also important to note that inclusion of new family members can increase the power of the pedigree to find an association with a QTL.

Chapter V describes a scan utilizing the chromosome-specific panels of the MSS-2 to further define 12 chromosomes harboring putative QTL identified by Todhunter et al. (2005). Five additional chromosomes were selected that were suggestive of linkage but did not reach a significance level of $p<0.05$. LOD scores were calculated for 159 members of the Greyhound/Labrador Retriever pedigree. Although none of the scores reached statistical significance, several indicated positive results in many of the same regions previously identified. Specifically, four of six markers on CFA29 had LOD scores greater than one for the NA of the left hip. The scores also show the utility of using multiple traits to scan for a complex disease since several markers showed suggestive results with one trait but not another.

In summary, the goal of this work was to define the genetics underlying CHD, specifically joint laxity. In an effort to efficiently perform genome screens, a set of chromosome-specific multiplexes was developed. A full genome screen and a partial chromosome-specific scan were unable to identify statistically significant regions linked with the inheritance of CHD. Due to the special design of the outcrossed pedigree, other modeling techniques that take the population structure into account are being utilized. Analysis is now underway of the remaining chromosomes not included in the initial screen and of a pure bred population of Labrador Retrievers created with many of the same founders in the outcrossed pedigree.

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