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Genetic and Genomic Tools for Breeding Dogs with Better Hips

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Canine hip dysplasia (CHD) is a developmental trait primarily affecting medium- and large-breed dogs. CHD is characterized by faulty conformation and laxity of the hip joint that usually affects both hips. Clinically, the osteoarthritis that results from hip dysplasia is characterized by hind-limb lameness, reduced exercise tolerance, reluctance to jump, poor hind-limb muscle mass, and laxity or pain in the hip joint. CHD can be detected radiographically as subluxation of the affected hip. CHD results in synovitis accompanied by effusion and osteoarthritis of the affected joint. Osteoarthritis is detected on a radiograph as osteophytes around the femoral neck (so-called Morgan's line), femoral head and acetabulum and a flattening of the femoral head with a shallow, open acetabulum. Radiographs are insensitive to the presence of incipient osteoarthritis of upper limb joints like the hip. In addition, the radiographic alterations associated with hip dysplasia can be subtle, and even an "unaffected" dog, as assessed by a radiograph, can still carry some of the mutations that contribute to the disorder.

GENETICS

CHD in dogs is an inherited, polygenic trait in which mutations in several genes [the regions where these genes reside in the genome are called quantitative trait loci (QTLs)] are involved in its clinical expression. The molecular genetic basis of CHD is currently unknown. Many dogs with normal hips on radiographs carry at least a modicum of the trait-causing mutations, but not all that are necessary to cause physical expression of the trait. CHD is a quantitative or complex trait that is expressed as a continuum from imperceptible to severe forms. This continuum in trait expression observed as the hip phenotype represented in the radiographic image is due to the additive nature of the genes and their alleles that underlie the trait. Some alleles increase trait expression, and some contribute resistance to the trait. This continuum of trait expression is affected by environmental influences (such as plane of nutrition and exercise, and many unknown epigenetic factors) that interact with the genetic constitution to affect the degree to which the trait is manifested. CHD has a heritability between 0.25–0.7 depending on the pedigree in which it is estimated and the method used to measure the trait. We have found one gene, fibrillin 2, which has a deletion that segregates with CHD across several breeds represented in our genetic banking archive. Other candidate genes are under investigation. We recently genotyped about 1,000 dogs on the High Density Illumina canine mapping array in order to find markers and genes associated with CHD. Eventually, these genetic mapping experiments will lead to discovery of the mutations that contribute to CHD.

It will take a concerted effort to rid breeds of the genetic mutations that contribute to CHD expression or, conversely, to introduce protective alleles at the loci that cause good hips. Breeding two dysplastic dogs can yield a 75% incidence of CHD in offspring, while mating two unaffected dogs can yield a 25% incidence. Selective breeding using normal dogs from normal parents and grandparents, as well as progeny testing, should decrease the incidence of CHD. The message here is that until we have a genetic test for CHD so we can detect genetically susceptible dogs, the best indication of a dog's genetic makeup is where it came from (its parents and grandparents), what it produces (its offspring), and the phenotype of its siblings or half-sibs. To test whether a sire or dam carries mutations for CHD (even if the dog has OFA - good hips), it should be bred to sires or dams with good hips and the proportion of affected offspring recorded (progeny testing). As many as 20 offspring would be needed to reasonably estimate a dog's genetic value for CHD.

Hip Conformation Screening

Because it is an inherited trait, the traditional strategy to control CHD has been through establishment of registries. Registries can be voluntary or involuntary, and each has its detractors. In the USA, the Orthopedic Foundation for Animals (OFA) has provided a standard for radiographic evaluation of hips based on breed, age, and conformation. Radiographs from dogs under 2 years of age are given a provisional assessment of hip status, and a definitive hip certification is given to dogs 2 years or older. The OFA has far surpassed their one-millionth radiographic submission. Radiographic changes related to the osteoarthritis associated with CHD may not be detected until two years of age or older. The sensitivity of the OFA radiograph at 12 months of age for detecting later development of osteoarthritis in affected hips ranges from 77–99% depending on the severity of the CHD at the earlier age. Ninety to 95% of dysplastic dogs have changes associated with CHD at 12 months of age. However, another study showed that of all dogs developing hip osteoarthritis over their lifespan, only 53% had radiographic evidence of a CHD at 2 years of age. Radiographs are insensitive to the presence of incipient osteoarthritis in the hip. The joint is considered dysplastic when the femoral head conforms poorly to the acetabulum or there are remodeling boney changes at the capsular attachment to the acetabulum or femoral neck. Hip status is graded on a scale from excellent conformation to severe hip dysplasia; there are 7 grades in all.

The PennHIP™ radiographic method measures the maximum amount of lateral (distraction) hip joint laxity (distraction index). There is a positive relationship between the distraction index and subsequent development of osteoarthritis. PennHIP radiographs include an OFA-style ventrodorsal projection, a compression and a distraction projection. The OFA-style film is evaluated for hip congruity and osteoarthritis. Labrador retrievers with a low distraction index (less than 0.3) at 8 months of age have about a 90% chance of being normal, while those with distraction indices greater than 0.8 have about a 90% chance of being dysplastic and succumbing to secondary hip osteoarthritis. Most breeds have similar ranges and relationships between the distraction index and the development of hip osteoarthritis. When choosing between dogs for breeding, preferentially breed dogs with the lowest distraction indices of the available pool. The optimum age for PennHIP™ screening is at early maturity (8–12 months of age for medium- to large-breed dogs).

A radiographic imaging position called the dorsolateral subluxation (DLS) test was developed at Cornell with an eye to improving the accuracy of hip evaluation. The PennHIP™ method finds dogs with laxity (a risk factor for CHD), but not all dogs with hip laxity develop secondary hip osteoarthritis. Their hips presumably function normally when they ambulate. We developed a method in which the hips are imaged in their normal functional position. The dogs are imaged in ventral recumbency under heavy sedation (or general anesthesia). The stifles are flexed and positioned under the hips so that the ischiatic table is superimposed over the stifles. The DLS score equates with the proportion of the femoral head covered by the dorsal acetabular rim. We compared the sensitivity (the percentage of dogs with osteoarthritis that were correctly identified) and specificity (percentage of dogs without osteoarthritis that were correctly identified) between the OFA-like extended-hip radiograph, the distraction index, and the dorsolateral subluxation score (DLS). For a single test, the DLS score is the most accurate in detection of both affected and unaffected dogs. A combination of the DLS score and Norberg angle gave the best estimate of a dog's likelihood of developing subsequent osteoarthritis than any single test, including the DLS test. The Norberg angle is a measure of femoral head coverage on the OFA style extended-hip radiograph. The Norberg angle ranges from near zero for severely subluxated hips to about 120° in the "best"-hipped dogs. An angle over 105° seems to be preferable. So we are back to

needing two methods to adequately describe hip conformation. This conclusion is supported by principal component analysis of the OFA score, the Norberg angle, the DI, and the DLS score to measure hip conformation.

My recommendation for selection of a young to early mature pet dog (not for breeding) with optimal hip quality is to examine the dog, palpate the hips for pain and Ortolani sign (under sedation), and to confirm physical findings with at least one radiograph - either a DLS or an extended hip radiograph. If the extended-hip (OFA style) image demonstrates subluxation (dysplasia), then no need to go further. If the dog has a "normal" extended hip projection but a positive Ortolani test, then that dog is at least susceptible to secondary osteoarthritis, if not dysplastic, and to document the laxity, should have a laxity imaging projection like the DLS method. A dog with optimal hip conformation should have palpated normally and have a DLS score over 55% or a DI below 0.4.

The dilemma is how to select puppies with optimum hip conformation. For those breeds with moderate to high risk for hip dysplasia, select pups from breeders where rigorous selection practices are employed (phenotypes recorded on both the sire and dam lines) so the buyer can review the breeding history. Both sides of the pedigree should be available. Information about results of previous breedings is very helpful (roughly 15–20 offspring of the same parents must be evaluated before one can have reasonable knowledge of the genetic quality of the same parents). Application of estimated breeding values (EBVs) for CHD will result in faster gain than basing breeding decisions on phenotype alone. Finally, marker-assisted selection will improve genetic quality for complex or polygenetic traits like hip dysplasia far faster than them breeding "better than the average" and can replace the use of EBVs for those who can't access them or estimate them with much accuracy (which is the case for most breeders not aligned with service organizations like the Seeing Eye or Guiding Eyes for the Blind or military establishments).

Breeding Values for US Pure-Breed Dogs Derived from the OFA Public Database

The breeding value in its earliest use was also called the selection index. The selection index was based on integration of genetic (pedigree relationships) and phenotypic information (OFA hip scores in our case) from each animal and its relatives and yields better results than phenotypic selection alone for desirable traits. The accuracy of the selection index of a subject increases when the OFA scores from its close relatives (e.g., progeny and ancestors) are included in the estimation. The selection index was developed into the Best Linear Unbiased Prediction (BLUP). The BLUP breeding strategy has been used successfully for genetic improvement, particularly in livestock, and has also been applied in closed colonies of dogs with substantial success. Variance components attributable to additive genetic and residual effects were estimated for the OFA hip and elbow scores and pedigrees. Genetic parameters, including the additive genetic variance and the residual variance were estimated using the REML procedure. Heritability (h2) is the proportion of additive variance over the total variance, which is the sum of additive variance and residual variance. The general concept is to select dogs with the lower EBVs, as these are the individuals with the lowest or best hip and elbow conformation.

We derived EBVs (a measure of a dog's genetic potential to produce offspring with optimal characteristics for an inherited trait) and inbreeding coefficients for CHD in Labrador retrievers based on OFA hip scores in the OFA database and provided them to the public in 2010 (www.vet.cornell.edu/research/bvhip/). The OFA hip scores and pedigrees of the Labrador retrievers in the public database were used for the genetic evaluation. Dogs were scored by the OFA radiologists into seven categories: excellent, good, fair, borderline, mild, moderate, and severe hip dysplasia. The first three categories (excellent, good, and fair) are generally

considered "normal" dogs, although they will carry some of the mutations that contribute to hip dysplasia. The last three categories (mild, moderate, and severe) are considered "dysplastic" dogs. This analysis was undertaken independently of the OFA. The seven hip score categories were replaced with 7 numerical scores, starting with excellent as 1 and ending with severe as 7. A numerical value of 2 was assigned to the combined category of "normal." Our analysis of the Labrador retriever OFA hip breeding values over that period showed that there has been slow but consistent genetic improvement (Hou *et al. PLoS One.* 2010). The explanation and methods that form the basis of the breeding values available in the search page of this website were published in the *American Journal of Veterinary Research* in 2008 by Zhang *et al.*, and a PDF of that paper is available in the publication section of my research home page in Clinical Sciences at Cornell University.

Since 1974, the Orthopedic Foundation for Animals (OFA) has provided a voluntary registry where the scores of hip and elbow radiographs of individual dogs and their pedigrees have been deposited. Following on from the research we reported on Labrador retriever hip EBVs, we calculated estimated breeding values (EBVs) and inbreeding coefficients for a total of 1,264,422 dogs from 74 breeds that included at least 1,000 individuals. The analysis was performed with a bivariate (used both hip and elbow scores) mixed model across these 74 breeds to improve the accuracy of the EBVs, to compensate for the deficiency in voluntarily reporting bias in the OFA public registry, and to provide an estimate of genetic correlation between the hip and elbow scores. There were 760,455 and 135,409 dogs with their own hip and elbow scores, respectively. The incidences of CHD and elbow dysplasia were 0.83% and 2.08% across the 74 breeds (21 breeds for elbow dysplasia) and ranged from 0.07% to 6%, and 0.5% to 8% within breeds, respectively. These incidences were far lower than the incidence reported in the hip and elbow dysplasia summary statistics by breed in the OFA web page (www.offa.org/stats_hip.html). The heritability of hip and elbow scores was estimated at 0.23 and 0.16, respectively. Over the 40 years since 1974, the genetic improvement for hip scores was 0.1 hip units or 16.4% of the average phenotypic standard deviation across the 74 breeds, which corresponded to a drop in the overall incidence of CHD of 3.37% clinically. For elbow scores, the genetic improvement was 0.0021 elbow units or 1.1% of the phenotypic standard deviation across the 21 breeds. Both genetic improvements were likely underestimated due to the inevitable bias against reporting osteoarthritic records. Genetic change in EBVs for hip and elbow scores was breed specific; some breeds improved their genetic quality and some demonstrated little improvement, while in a few breeds, genetic quality deteriorated. We concluded that distinct breeding selection goals should be directed at improving the genetic quality based on each breed's genetic characteristics and breed requirements, and we provide the first national hip and elbow EBVs by which to do so. The genetic and residual correlations between hip and elbow scores were 0.12 and 0.08, respectively. The weak positive genetic correlation suggested that selection based on hip scores would also slightly improve elbow scores, but it is necessary to allocate effort toward improvement of elbow scores alone (Hou *et al. PLoS One.* 2013; in press).

These estimated hip and elbow breeding values and inbreeding coefficients will be accessible in this Cornell hip dysplasia website. The dogs with low breeding value (low OFA score means a better hip) and with higher accuracy (more related dogs measured, the higher the accuracy) are the most desirable for breeding purposes. Low accuracy means that not many dogs were available to estimate the breeding value.

Inbreeding

Inbreeding occurs when a mating is made with a relative or the parents shared common

ancestors. The closer an individual dog is to its ancestors with other dogs and the more common ancestors, the stronger the inbreeding. The most severe inbreeding occurs in a sibling to sibling mating or offspring to their parents. These matings commonly occur in an effort to preserve features of a breed or line within a breed and is referred to as "line breeding." The degree of inbreeding can be mathematically expressed as an inbreeding coefficient. The inbreeding coefficient of an individual is defined as the probability that any two homologous alleles (same forms of the genetic locus) are identical by descent. That is, they were transferred from an ancestor to the current generation. Inbreeding often occurs the deeper you trace a pedigree. It is almost impossible to avoid inbreeding in a limited population, especially when the population has experienced a bottleneck. Severe inbreeding could result in shorter lives and problems of fitness, including hip dysplasia. The level of inbreeding has continuously accumulated in US pure-breed dogs over the past 40 years, with higher inbreeding occurring generally in the breeds with total populations and therefore smaller breeding populations.

Questions & Answers About the Application of Hip and Elbow Estimated Breeding Values and Inbreeding Coefficients to the Breeding and Selection of a Pup

(taken from the Cornell Hip EBV website for the Labrador retriever)

Once the new EBVs for other breeds are uploaded, then similar strategies for purchase and breeding decisions will apply to other breeds.

Why is this search function to find Labrador retrievers with better hip breeding values useful? The breeding values and inbreeding coefficients recorded in this website enable me to find dogs with low hip score breeding values that belong to the current and recent generations. The use of the dogs in the lower part of the breeding value range for breeding will likely improve the hip quality of my breeding stock and puppies they produce. Purchase of puppies produced by the sires and dams with the lower breeding values will likely produce puppies with better hips than if I based breeding decisions on hip radiographs alone. The reason is that the selection of dogs based on breeding values means that consideration has been given to both the dog's genetic (pedigree) information and hip radiographic information combined. Selection of dogs based on radiographs alone is very useful, but faster genetic gain toward better hip conformation accrues when breeding decisions are made based on genetic information as well.

Why does negative breeding value mean a better hip? The question arises due to the ambiguity of the word "value," which usually suggests the higher value the better. The breeding value is an indicator for the genetic basis of the hip score variation. Consequently, breeding values take the same unit and direction as the original phenotype - the OFA score. An OFA score of 1 is for an excellent hip, and an OFA score of 7 is for the most severe hip dysplasia.

What is the difference between expected progeny difference (EPD) and breeding value? The breeding value is the prediction of the genetic basis of an individual OFA score. Half of the genetic basis is contributed from one parent, and half from the other. If an individual is mated randomly, the expected difference of the progeny from the average (base) will be half of the breeding value. Therefore, half of the breeding value is called the EPD. For example, sires A and B have breeding values of -0.1 and 0.20, their EPDs will be -0.05 and 0.1. The progeny of sire A is expected to be 0.15 lower than the progeny of sire B.

Will an inbred dog definitely have progeny with high inbreeding? Not really. The progeny may not be inbred if the mate you select is not its relative. The inbreeding of an individual depends on if the parents are relatives or not.

Why can a breeding value be negative? The current reported breeding values were the direct output of the solutions for each dog in the mixed linear model. The base of the breeding value is

the average breeding value among all the dogs evaluated. The base is a "floating" base, which can vary by adding new dogs that have better hips.

I wish to choose a pup from a litter, and I know the parents who produced this litter. How should I use the information in the hip EBV database? Once you decide the qualities of the parents you prefer, then gather the information about any inherited traits and diseases segregating in the pedigree that you can. For a pup's genetic potential to grow up with good hip quality, go into the database and look at the hip breeding values for the dogs you like. Then you can rank those dogs based on their potential to produce pups with good hip conformation (the lowest hip breeding value indicates the dog with the genetic potential to produce the best hip conformation based on the OFA score). If only one parent is found in the database, then that's the best you can do. Secondly, you can rank the parents according to their inbreeding coefficients. You should try to choose pups produced from litters whose parents have the lowest inbreeding coefficients.

I wish to choose a pup from a litter, but I don't have information about the hip scores of either parent. You can ask the breeder for any pertinent radiographic information they have about their dog. They may have PennHIP information. They may not use the OFA method. They may not do orthopedic screening at all. We also know that elbow dysplasia is a problem in the Labrador retriever breed. If you can obtain no information about orthopedic disease in a dog's pedigree, then I suggest you try another breeder.

I wish to choose or buy a male dog as a potential breeder. How should I use the information in the database? Once you have selected the potential male dogs based on all the breed qualities you prefer, then rank the dogs based on their genetic potential to produce offspring with good hip conformation and on their inbreeding coefficients. Always breed to a female dog with the best hip conformation and lowest inbreeding coefficient you can find along with all the best qualities you can ascertain, orthopedic or otherwise.

I wish to choose or buy a female dog as a potential breeder. How should I use the information in the database? Once you have selected the potential female dogs based on all the breed qualities you prefer, then rank them based on their genetic potential to produce offspring with good hip conformation and on their inbreeding coefficients. Always breed to a male dog with the best hip conformation and lowest inbreeding coefficient you can find along with all the best qualities you can ascertain, orthopedic or otherwise.

I bought a pup already but just found this website. How should I use the information in the database to decide if this puppy is at risk of hip dysplasia? If you can identify the parents in the database, look at the OFA breeding values of the parents. If they are above 0, then the pup has a higher chance of developing hip dysplasia than if the breeding values are below 0. The closer the breeding value is to 1, the greater the susceptibility to develop hip dysplasia. If you decide the pup is susceptible, it should be examined regularly for hip instability by your veterinarian. Depending on the dog's age, medical or surgical intervention may be an option. This is especially important if your dog has clinical signs of hip dysplasia, like reluctance to jump, bunny-hopping gait behind at speed (both hind legs moving forward together), soreness or stiffness after exercise, a "wobbly" hind-limb gait, poor muscle mass development behind compared to its forequarter, difficulty getting up, placing extra bodyweight on its forelimbs with a hunched back, a clicking sound when it walks, or reluctance to allow you to pet near its hips. Any pup susceptible to hip dysplasia or any developmental orthopedic disease should be watched for

rapid bodyweight gain, and if it is too fat, its food intake should be restricted under advice of your veterinarian.

If a puppy is at risk for hip dysplasia based on the breeding value of its parents, what should I do about it? Ask your veterinarian to examine your puppy's hips regularly. This is especially important if your dog has clinical signs of hip dysplasia, like reluctance to jump, bunny-hopping gait behind at speed (both hind legs moving forward together), soreness or stiffness after exercise, a "wobbly" hind-limb gait, poor muscle mass development behind compared to its forequarter, difficulty getting up, placing extra bodyweight on its forelimbs with a hunched back, a clicking sound when it walks, or reluctance to allow you to pet near its hips. Any pup susceptible to hip dysplasia or any developmental orthopedic disease should be watched for rapid bodyweight gain, and if it is too fat, its food intake should be restricted under advice of your veterinarian.

I wish to choose a male dog for my female dog to produce a litter of pups with the best hips I can. How do I select a dog from this database? Rank the male dogs based on their OFA hip breeding value scores and their inbreeding coefficients. Choose the dog with the qualities you like as well as the best genetic potential to produce offspring with good hip conformation and lower inbreeding coefficient.

Once I have identified a litter for puppy selection or a dog to which I'd like to breed, how do I locate the owner or breeder? We can suggest trying Google, other Labrador retriever owners, breed/trade magazines like "Just Labs," contacting the Labrador retriever breed clubs or the AKC, etc. You can also purchase a pedigree from the AKC, and this will have an owner's name on it. Eventually estimated breeding values and inbreeding coefficients for OFA hip scores will be available for many breeds.

Genomic Reference Panel and Genomic Prediction

State-of-the-art for predicting the dogs that carry the best combination of alleles at the genes that contribute to hip dysplasia is called genomic prediction. By genomic, I mean a method that interrogates the whole genome of the individual dog. No gene has yet been identified that contributes substantially, say 20%, to the overall genetic variation of the full range of hip dysplasia. However, if the density of genetic markers or variants for which a dog is genotyped is sufficient to capture every gene that "lives" near a marker, then we can use the marker genotypes as a surrogate for the genes. The marker(s) is so close to the gene that the form of its alleles is always inherited with the gene, i.e., recombination does not interfere with this relationship. There are a couple of strategies that can be used to undertake the genomic prediction. A subset of genetic markers called single nucleotide polymorphisms (SNPs) that span the genome are jointly selected for their contribution to CHD (or any other complex trait). Or a set of SNPs that are each significantly associated with the trait are used to build a multivariate linear model in a forward or backward method, keeping the markers in the model that accounts for the most variation but eliminating redundant markers.

We employed the joint marker or Bayesian approach for our first effort. We used the Norberg angle, which is highly phenotypically and genetically correlated with the OFA hip score. A reference population was established of dogs belonging to breeds susceptible or resistant to hip dysplasia that have undergone genome-wide SNP genotyping and that have accompanying estimated hip breeding values calculated. A new dog of a breed that is in the reference population is genotyped either across the genome or at the best subset of SNPs, and its estimated breeding value for optimal hip quality is estimated from the dogs in the reference panel based on its own SNP genotypes. Modest correlations can also be made with the raw Norberg angle. The best estimates of the genetic potential of two dogs to produce offspring with optimal

hip quality will be based on gene mutation tests, but it will take resources and time to discover the genes that contribute to CHD. In the meantime, SNP-based selection will have to suffice, to which we will later add the mutations to improve the prediction model.

Currently, the largest reference population for genomic prediction we have available is for the Labrador retriever (Guo *et al.* 2011). For 180 Labrador retrievers genotyped on the Illumina version 1, 22K mapping array, genomic hip breeding values for the Norberg angle were calculated in a Bayesian framework (Guo *et al.* 2011). This statistical method uses all the available genotypes to explain the variability in the Norberg angle. The estimated hip breeding values of these Labrador retrievers were correlated with their genomic breeding values using the most predictive (effective) 280 SNPs of the 22,000 markers in the version 1 array. Thirty percent of the variation in the Norberg angle of 108 Labrador retrievers not used to develop the reference genomic panel was explained by the genomic prediction. The accuracy for a true phenotype is about as expected, because the heritability of HD as measured by the Norberg angle is only about 0.2–0.3. The accuracy of the genomic prediction for estimated hip breeding values on a subset of the 108 naïve dogs was moderate at 57% of the variation. Ongoing research would combine genomic prediction with the true hip radiographs of a genotyped dog to improve the accuracy of the prediction by including newly genotyped and phenotyped dogs into the reference panel. Other breeds might be added on which to predict genetic quality of hips. Recalculation of the genomic prediction algorithm based on more individuals and denser genotyping using the Illumina HD array should improve accuracy of the prediction. This iteration would be repeated over and over.

Speaker Information
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