Skeletal Dysplasia Project Update- November 2017

We began working on what we called skeletal dysplasia in Tollers in 2003. This is a summary of our recently published work on this research project and how it affects the NSDTR (1). We recently identified the change in DNA sequence responsible for "chondrodystrophy" in dogs. Based on this research we now know that Tollers have chondrodystrophy specifically rather them some new or novel leg shortening gene. Please notice the term used is chondrodystrophy and no longer "chondrodysplasia" or "skeletal dysplasia". Chondrodystrophy in veterinary medicine is a very specific term defined in the 1950s (2) to describe dogs with short legs and abnormal intervertebral discs, while chondrodysplasia and skeletal dysplasia are much more general terms used to describe animals with short legs. A number of mutations responsible for forms of chondrodysplasia have been identified in dogs (3-7). None of those are responsible for shorter legs in the Toller (Bannasch, unpublished data). A similar situation has occurred in human medicine where the original classification of forms of dwarfism was clarified once genes and mutations were identified that distinguished them (8).

There is a great deal of variation of leg length within the NSDTR breed. We started on this project in Tollers in 2003, however, many dogs described as normal or unaffected by breeders have radiographic evidence of abnormal leg length making it very challenging to classify dogs as affected or not. In this study, we utilized the more severely affected NSDTRs, based on radiographs (N=13) and compared them to the least severely affected (N=15) in order to map this trait. Dogs were categorized as affected if they had short legs with radiographic signs of radial bowing and physeal (growth plate) widening. We identified a statistically significant association on chromosome 12 using these criteria. It is a region where the affected dogs were homozygous (the two copies of their chromosome are identical). When we looked for other breeds with the same DNA sequence in the region we identified the other known chondrodystrophic breeds (Beagles, Cocker Spaniels, French Bulldogs and Dachshunds). They were also homozygous across this region and shared the same DNA sequence as the affected Tollers.

Hansen (2) first described type I intervertebral disc disease (IVDD) in the dog using five breeds, which he classified as chondrodystrophic: French bulldog, Dachshund, Pekingese, Dachsbrache and the Spaniel. Dogs from these breeds have abnormal long bone growth plates (i.e., radius, ulna, humerus, femur, tibia and fibula), abnormal vertebral growth, and abnormal intervertebral discs based on histopathology of these tissues. He showed that by 1 year of age the discs had all degenerated in a manner similar to much older dogs of other breeds. Later, Braund performed systematic histopathological analysis of the Beagle breed and classified it as chondrodystrophoid-like with the same abnormalities in the intervertebral discs and long bones (9). While the discs are abnormal at a cellular level by 1 year of age (2, 9) it is not until the dogs are older that some of them show clinical signs of disc degeneration. Those clinical signs can be acute herniation into the spinal canal, or more chronic with changes to the disc and ultimately collapse of the disc.

We next compared DNA of dogs (N=36) with type I intervertebral disc disease (IVDD) to DNA of dogs (N=31) with no history of neurologic (spinal cord) abnormalities across breeds and including mixed breeds and the same region on chromosome 12 was the most highly associated region across all the chromosomes. The causative change in the DNA of dogs with IVDD, which is the same as in the short legged tollers was identified within this region on chromosome 12 (called CDDY for genetic testing purposes). It has an odds ratio of 51.23 (95% CI = (46.69, 56.20)) for Hansen's Type I disc disease across all dog breeds. All dogs (n=74) with mineralized thoracolumbar discs or IVDD based on surgery were either heterozygous or homozygous for this mutation (28 had 1 copy and 46 had two copies of the mutation- however 20 of those were dachshunds which appear to be virtually homozygous for the mutation) except one Rottweiler. We next tested 7 NSDTR with type I IVDD and they were either heterozygous= 1 copy of CDDY (N=5) or homozygous= two copies CDDY (N=2).

The actual mutation is an extra copy of the *FGF4* gene. This extra copy on chromosome 12 is highly expressed (turned on) in the developing intervertebral disc and vertebral body in puppies. The normal version of the *FGF4* gene is specifically expressed during embryonic development (12). In mice, where this type of information is available, *FGF4* is highly expressed in the limb buds, developing spine (somites) and the ear buds (13-14). One receptor for *FGF4* is the *FGF3* Receptor, which when activated is the main cause of human achondroplasia (15).

Mode of inheritance

Leg length:

Within the NSDTR the mutation decreases leg length in an additive manner. One copy causes leg shortening and 2 copies causes more severe leg shortening. The leg is shorter based on shortening (and bowing) of the radius, ulna and humerus. Below the pastern, the foot turning out (valgus) does not appear to be due to CDDY. The extent of the bowing and shortening is variable and overlapping between the three genotype classes (N/N, CDDY/N and CDDY/CDDY). It is also not the only thing that changes the length of legs in dogs. Many other genetic factors can govern overall size and height, just like in people.

Type I IVDD:

Across dog breeds, animals were identified with IVDD that had one copy of CDDY indicating that it only takes one copy to predispose to intervertebral disc disease. This was also true in the NSDTR. 7 tollers with type I IVDD (confirmed by surgery) were identified and 5 were heterozygous for the mutation (1 copy) and 2 had two copies. It appears that across dog breeds only 1 copy of this mutation is necessary to cause abnormal discs and therefore susceptibility to IVDD. This is based on the Tollers, as well as 74 dogs of other breeds used in the published study (1).

IVDD in chondrodystrophic dogs occurs due to the intervertebral discs degenerating between birth and 1 year of age (2, 9). The Intervertebral disc is composed of an outer ring called the annulus fibrosis and an inner gelatinous region called the nucleus pulposus. In chondrodystrophoid dogs this inner area becomes abnormal at a cellular level by 1 year of age. It then loses its ability to act as a cushion in the spine. A disc can move and cause back pain.

Sometimes the disc herniates into the spinal canal impinging on the spinal column causing acute pain, bleeding and paralysis. Surgery to correct this problem is very expensive and 24% of Dachshund owners opt to euthanize rather than do the surgery (4). Diagnosing the root cause of back pain or herniated disc is expensive and not always performed, making it a challenge to know the actual numbers of dogs with clinical signs as a consequence of chondrodystrophy.

Test Interpretation:

CDDY follows basic rules of inheritance:

CDDY/CDDY X CDDY/CDDY will produce 100% CDDY/CDDY

CDDY/CDDY X CDDY/N will produce 50% CDDY/CDDY and 50% CDDY/N

CDDY/N X CDDY/N will produce 25% CDDY/CDDY, 50% CDDY/N and 25% N/N

CDDY/N X N/N will produce 50% CDDY/N and 50% N/N

N/N X N/N will produce 100% N/N

Allele Frequency:

The allele frequency is a measure of how common a mutant allele is in a population. It is used to allow the comparison of genotype percentages over time or between groups. The allele frequency will change over time with implementation of genetic testing and clearing dogs by parentage. Ideally we will determine how common CDDY is based on a random sampling of breeding NSDTR (grant pending with AKC-CHF). However we do have ~ 300 dogs that were used for other genetic studies in the laboratory and that previous work allowed us to estimate how common CDDY is in those dogs (11). Often these samples included related animals and small families, not necessarily breeding dogs. In that sample 20% appear to have two copies of CDDY, 54% appear to have one copy of CDDY, and 26% are normal which is an estimated allele frequency of 47% for the CDDY allele (11). The Veterinary Genetics Laboratory provided the allele frequency of the CDDY mutation of 39% based on 106 dogs tested (14% CDDY/CDDY, 50% N/CDDY and 36% N/N). Some of those animal were related to each other so based on owner reported sire and dam information, unrelated animals were evaluated and there were 62 samples with an allele frequency of the CDDY mutation of 36% (13% CDDY/CDDY, 45% N/CDDY and 42% N/N) (Kindly provided by Dr. Bellone, Director of the UCDavis VGL).

It is important to be aware that CDDY is very common in the breed when considering its use in breeding decisions. Since the frequency of the mutation appears to be very high in the breed, breeders should attempt to reduce the frequency slowly over time in order to not adversely affect diversity within the breed.

The identification of chondrodystrophy and IVDD in the Toller, along with the CDDY test provides important information for the owner and/or breeder. There are still many questions to be answered, such as:

1. How often do Tollers with the mutation have disc disease?

This question is difficult to answer properly. Ideally a set of 500-1000 puppies with 1 or 2 copies of CDDY would be followed for their lifetimes and have appropriate diagnostics to evaluate their discs if warranted clinically. This has not been done for any breed even though chondrodystrophy is a serious medical concern in many breeds. Information that is easier to obtain includes the breed breakdown of IVDD cases or the % of IVDD cases among dogs of that breed presenting to a particular clinic. The majority of numbers found in the literature are of these two types. In an attempt to get some information useful for breeding decisions, breed clubs conduct health surveys. These are snapshots in time of what diseases are in the breed. They do not provide a relative risk. For example some dogs whose owners fill out the survey are 2 years old and some are 14 years old. Risk is still present in the 2 year old (it could go on to have disease X) but it will be counted as normal. The second challenge of breeder health surveys is that the diagnostic criteria applied are not always the same since they are owner reported. That being said the following are the numbers available from the available health surveys in Tollers:

The original 2002 Canadian Toller club health survey reported 13/1180 (1.1%) with herniated disc disease, slipped disc, calcified disc, degenerative disc disease. For comparison this survey also found 1.02% of dogs had Addison's disease.
http://www.toller.ca/tollerhealth/SurveySummary.html

The Kennel Club (UK) health survey reported 0/130 affected with disc disease and none affected with Addison's disease.
http://www.toller-club.co.uk/toller_health.php

2014 Toller health survey (UK) reported 13/214 or 6.1 % of the dogs whose owners filled out the survey had back problems. For comparison this survey also found 1 dog (0.5%) had Addison's disease.
http://www.toller-club.co.uk/toller_health.php

At the UC Davis Veterinary Medical Teaching Hospital 20 Tollers were seen in the last 15 years and 4 had type I IVDD, none had Addison's disease.

2. Do two copies of CDDY cause greater abnormalities or earlier age of disease in discs then 1 copy of CDDY? We know that dogs can have a herniated disc with just one copy of CDDY but we do not know if having two copies is worse. We are currently working on this question in the laboratory by comparing age of disc herniation in dogs of many breeds with 1 copy versus 2 copies. A previous study in the Dachshund showed that this region on chromosome 12 was associated with higher numbers of radiographically mineralized discs in 2 copies versus 1 copy

(16). Since this disease has been extensively studied in other breeds, the NSDTR can benefit from that work.

References

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