

Canine Osteosarcoma and Hemangiosarcoma: The Challenge of the Dog Disease Mapping Project

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Several years ago, the Dog Genome Project, spearheaded by the Broad Institute of MIT and Harvard together with the canine genetics community, completed the sequencing of the dog genome. This has given us an in depth understanding of the canine genome (genome = entire length of DNA in a dog), and led to the development of a powerful tool called SNP array (su-nlp array), which allows researchers to look for mutations that give dogs their specific traits, including inheritable diseases.

Our collective hope in writing this article is to raise awareness of osteosarcoma and hemangiosarcoma among Irish Wolfhound owners, and to ask you to participate in our Dog Disease Gene Mapping Project.

What are Hemangiosarcoma (HSA) and Osteosarcoma (OSA)?

Hemangiosarcoma (HSA) is a malignant tumor of vascular endothelial cells (i.e., cancer of blood vessels) that affects dogs more than any other species. Although HSA affects all breeds, the incidence is considerably higher in certain breeds, including Golden Retrievers, Labrador Retrievers, German Shepherd Dogs, and Boxers, Leonbergers and Great Pyrenees to a lesser extent. HSA has the potential to arise at any type of tissue/organ in the body, but the four most common sites are the spleen, heart (right atrium or auricle), skin/subcutaneous tissues and liver. Other less frequent primary sites include kidney, muscle, bone, oral cavity, bladder and lung. It is a very aggressive type of cancer that can spread to multiple organs/tissues at a very early stage, either through blood vessels or via local seeding following tumor rupture.

Being a cancer of blood vessels, the tumor itself is usually filled with blood. The most problematic aspect of the disease is that tumors originating in internal organs can develop into large masses asymptotically, thus going undetected. The tumor can rupture suddenly and cause severe internal bleeding at times resulting in death. For this very reason, HSA is often referred as a “silent killer.” It is emotionally devastating for family members to face such a crisis, often requiring an immediate surgery and aggressive supportive care. It is equally frustrating for clinicians who can offer only limited treatments in cases with an advanced stage.

Osteosarcoma (OSA) is the most common malignant bone cancer in dogs and the second one in humans. In humans it is most frequent among young adults and it primarily occurs during adolescence in conjunction with rapid bone growth. In dogs it is an important health concern, accounting for 5-6% of all canine neoplasms. In the United States, 8,000–10,000 cases are reported annually. Canine OSA shows strong clinical, histological and cytological similarities to the human cancer. It is a very aggressive cancer and in the majority of cases, metastasis and death follows within a few months or years. The median survival time for dogs treated with amputation plus chemotherapy is 12 months, with only 20% surviving 2 years. While osteosarcoma can occur in dogs of any size, some large and giant dog breeds have a much higher risk of developing OSA within their

lifetime than other breeds (three to twenty fold increased risk compared to the average in dogs) including the long-limbed hounds: Irish wolfhounds (with the highest frequency, 12% of all 10 year old dogs had been diagnosed in a recent Swedish study), Great Dane, Scottish Deerhound, Rhodesian Ridgeback, Great Pyrenees and Borzoi and mastiff-type breeds (Rottweiler, Labrador Retriever, Flat-coated Retriever, Golden Retriever, Mastiff, Bullmastiff, Saint Bernard, Irish Setter and Newfoundland). The average age at diagnosis is seven and a half years old.

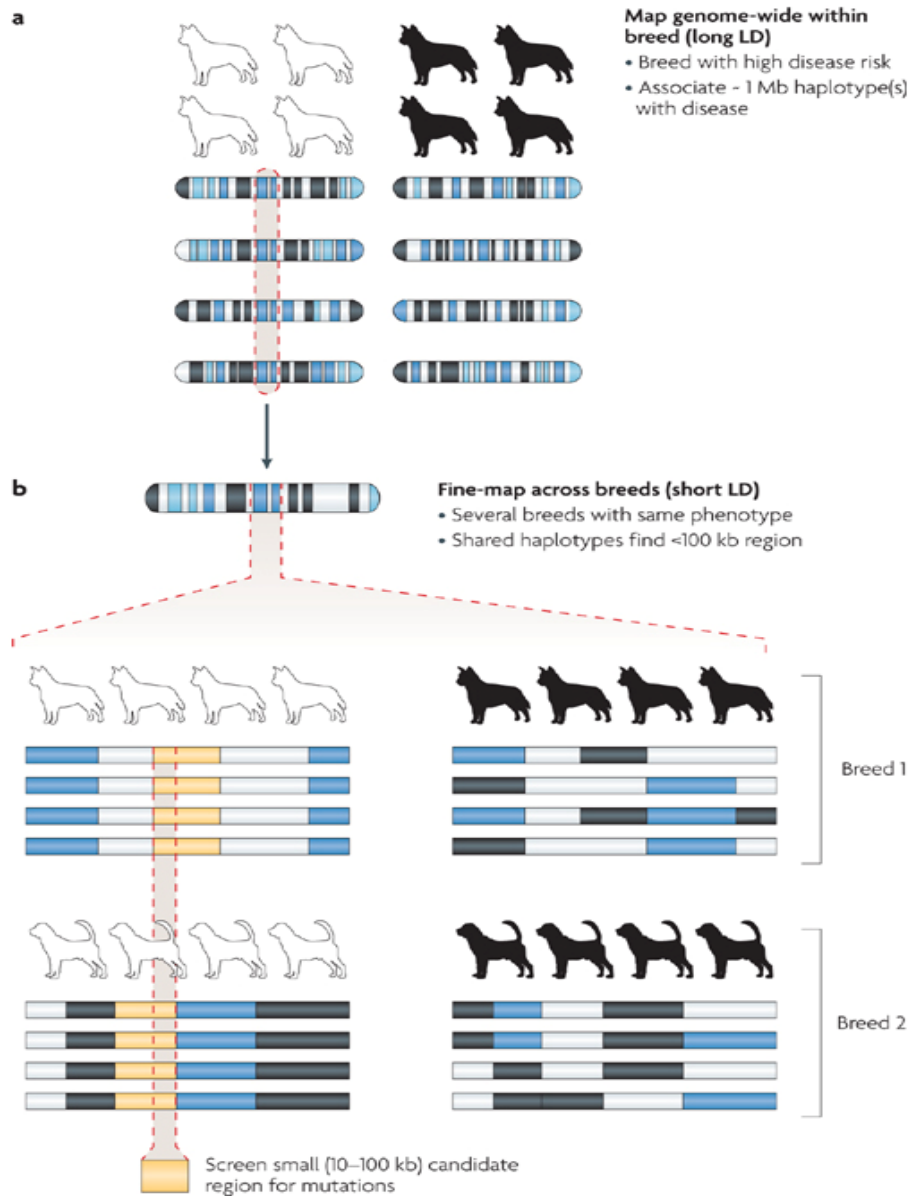
Finding the cause of OSA and HSA requires identifying mutations in genes that may make certain breeds, and certain individuals within a breed, susceptible to these devastating diseases. This is a challenging task that we, members of the dog disease mapping team at the Broad Institute of MIT and Harvard (www.DogDNA.org), together with numerous collaborators throughout the US and worldwide have been working on in the past few years. Identifying causative mutations/genes will subsequently enable the development of DNA tests capable of detecting susceptible individuals, and individuals that may pass these genes on to offspring. This will allow owners/veterinarians to more closely follow the susceptible dogs for tumor occurrence before possible life-threatening symptoms. Identification of the genetic basis of OSA and HSA also will allow scientists to better understand the biology of the disease in the long run, which may lead to the development of preventative measures and effective new treatments.

About OSA/HSA disease mapping projects

The identification of genetic abnormalities in a cancer is the first step in the development of new, and often more effective/targeted therapy. Genome-wide association study to find mutations causing cancer has been carried out in human medicine for quite some time, but because there are most likely several mutations that can increase the risk of a cancer, finding those mutations has been a great challenge. Because of the unique genomic structure of dogs due to their domestication and breed creation history, mapping those cancer-causing mutations in dogs should be easier than in humans, but it is still a challenging task.

Briefly, the process of finding cancer-causing mutations is as follows (Fig. 1): The first step is called “genome-wide association mapping”, in which the entire length of DNA (a.k.a. whole genome) is scanned using a SNP array, which now contains ~170,000 SNP markers. Those SNP markers come in different flavors in different individuals. The “flavor” of the marker is called “haplotype” in genetic terms. The haplotypes of the SNP markers of each individual at each location are compared between groups of affected (with cancer) and unaffected (healthy) dogs. By doing so, we can locate regions that come in a common haplotype in the affected dogs, and are different from the unaffected dogs. For the OSA and HSA project, we are performing the initial genome-wide association mapping within a single breed, Greyhounds and Golden Retrievers, respectively.

When we find regions that are associated with OSA or HSA in the initial screening, we then compare the regions by more densely picked SNP markers across several related breeds that suffer from the same disease, including the Irish Wolfhounds. The step is called “fine-mapping” across breeds. We suspect that at least some of these breeds will share the same mutation as the breed used in the genome-wide association step. Since each breed has its own characteristics at any given location of the genome, searching for a smaller region that is shared among affected dogs across breeds will allow us to rapidly narrow down the region and identify disease-associated mutations.



Nature Reviews | Genetics

Figure 1 (Karlsson and Lindblad-Toh, Nature Rev Genetics 2008, 7:713)

A two-stage approach takes full advantage of the long linkage disequilibrium (LD) within breeds and the short ancestral haplotypes shared across breeds, allowing traits to be mapped with relatively few samples. In this example, dogs with a mutation for loss of pigmentation (white) are compared with normally pigmented dogs (black). a | In stage 1, genome-wide association analysis within a breed uses dozens to hundreds of samples and greater than or equal to 15,000 SNPs to identify one or more associated regions that are approximately 1 Mb long. b | In stage two, fine-mapping with a much denser set of SNPs in multiple breeds that share the same phenotype refines the association to a discrete region of 10–100 kb. This candidate region, which corresponds to the shared ancestral haplotype that carries the causative mutation, is screened for functional variants.

Where we are in the OSA/HSA disease mapping projects

We have been working very hard to collect a sufficient number of cases and control dogs in the past few years. We initially analyzed ~100 HSA cases and ~100 controls (Golden Retrievers), and ~120 OSA cases and ~120 controls (Greyhounds and Rottweilers) on one type of SNP array. Based on the data we obtained from the initial SNP array analysis, we have also moved onto the fine-mapping stage. Unfortunately, our in depth analysis revealed that the initial SNP array we were using did not have the sufficient marker density to accurately pinpoint the regions where we should look for the mutations.

We still learned a lot from this experience, and we have developed a new type of SNP array that can scan the dog genome more densely and evenly. We are happy to report that we have just analyzed 145 HSA cases and 113 controls (Golden Retrievers) and 155 OSA cases and 120 controls (Greyhounds) on the new type of SNP array. We are also happy to report that the results are looking very promising. We are in collaboration with Mike Starkey at the Animal Health Trust in UK to perform a genome wide scan in Irish wolfhounds as well. We have submitted 17 OSA cases with 30 controls from US, plus 24 cases and 24 controls from our collaborators in Sweden, that in addition to the UK collection will add up to a good number of samples. At the same time we continue to analyze other breeds for these variants, as well as in detail investigate the genomic regions for potential mutations.

From our first mapping experience, though, we also learned that we need more cases and controls from each breed to participate and aid us to find the mutations accurately.

Therefore, please read on and learn how you can help us succeed in identifying mutations that give dogs the susceptibility to HSA and OSA.

How to participate in the OSA/HSA disease mapping projects – Please note: we need older dogs (8y+) with no history of cancer to participate, too!!!

As we mentioned above, in order for us to successfully identify a gene (or genes) that is associated with any given disease, it is very important to recruit a high enough number of participants. We are enrolling Irish Wolfhounds (and other pure breed dogs) that fall into any of the following categories:

- 1) Has HSA or OSA (presumptive diagnostics is OK)
- 2) Over 8 years old and without cancer
- 3) Has other types of cancer/hereditary diseases (a list available at www.DogDNA.org)

We only need 5ml (= 1 teaspoon) of blood in a purple top tube (EDTA tube). If your dog received blood transfusions due to excessive hemorrhage, then we would accept cheek swab samples. Blood sample gives us much higher quality of DNA, so if you suspect your dog has HSA and would like to participate in our study, please get the blood sample taken before any blood transfusion. The sample can be mailed in at room temperature, as long as it arrives within a week from the time it was taken. Please make sure it is well protected against potential breakage, especially because the tube for blood sample is made of glass. We need a consent form signed by the owner to be sent in with the

sample. We are asking for pedigree and health information of the dog on the consent form. The consent form can be found at www.DogDNA.org by clicking on the link "Printable brochure" (PDF). We are asking the pedigree information only because we are looking at genes that are all inherited from the parents, and need to know the "relatedness" of each dog enrolled in the study. A copy of your dog's pedigree is helpful, but if your dog (or any of his/her parents/siblings) is registered with any organization (e.g. AKC), you can just provide us the registration number and organization. All the information regarding your dog is kept strictly confidential, and the genetic disposition of any dog is never to be made public. The details of "how to" can be found at www.DogDNA.org. You can also contact us by e-mail at dog-info@broadinstitute.org

We are looking forward to your participation!!!