Animal ***Health*** Trust
Oncology Research Group

Animal Health Trust research study (2005 -) to identify inherited genetic alterations that confer an increased risk of Irish Wolfhounds developing osteosarcoma

In collaboration with scientists in Sweden and the United States, we have conducted a large, conventional 'genetic case-control association' investigation to look for significant differences between the frequency of genetic markers (called 'SNPs') present in the DNA from 151 Wolfhounds with osteosarcoma and DNA from 136 unaffected dogs (aged at least 6 years old). The DNA samples were from dogs in the UK and Ireland, Europe (mainly Sweden), and the United States (and there is no difference between the overall 'genetic profiles' of Wolfhounds from Europe and the United States). Unfortunately, we found no genetic markers that were present more often (than would be expected by chance) in the DNA from Wolfhounds with osteosarcoma. Working on the premise that dogs that develop osteosarcoma at a 'young age' may carry one (or more) additional genetic risk factors for osteosarcoma to dogs that develop osteosarcoma in middle-old age, our Swedish collaborators undertook a second comparison of 28 dogs (less than 6 years old) with osteosarcoma and 62 unaffected dogs (aged at least 7 years old). This comparison identified regions of 4 different chromosomes containing genetic markers which are believed to be close (in the DNA) to genetic alterations that confer an increased risk of developing osteosarcoma. However, subsequent efforts have yet to identify genetic alterations in these 4 regions which could conceivably cause an increased risk of developing osteosarcoma.

The most likely explanation for why the 151 'affected dog' v 136 'control dog' comparison failed to identify genetic markers associated with osteosarcoma is that genetic markers which are located close (in the DNA) to genetic alterations that confer an increased risk of developing osteosarcoma are carried by all, or nearly all, Irish Wolfhounds. Some evidence that supports this hypothesis comes from a study on genetic risk factors for osteosarcoma in US Greyhounds that was conducted in parallel with the Irish Wolfhound research project. A genetic marker strongly associated with osteosarcoma in US Greyhounds was identified on chromosome 11, and 95% of <u>all</u> the Irish Wolfhounds (both with and without osteosarcoma) analysed carry two copies of the genetic marker concerned. Furthermore, a substantial portion of the DNA in Wolfhounds is 'fixed' - in other words, in these regions the sequence of letters that comprises the DNA is the same in all, or nearly all, Wolfhounds. If genetic alterations that confer an increased risk of developing osteosarcoma are located in these 'fixed regions' (for example, in the region on chromosome 11 that contains the genetic marker associated with osteosarcoma in US Greyhounds) they are impossible to find by 'conventional' genetic case-control association studies. In principle, a 'DNA test' could be used to identify which genetic alteration(s) associated with osteosarcoma are carried by a Wolfhound, and therefore predict the extent of the dog's increased risk of developing osteosarcoma. However, if genetic alterations that confer an increased risk of developing osteosarcoma are carried by all, or nearly all, Irish Wolfhounds, the frequency of the genetic alterations in the Irish Wolfhound breed can only be reduced by outbreeding to a breed/s that does not carry the same genetic alterations.

We still believe that it is important to identify the inherited genetic alterations that cause an increased risk of Irish Wolfhounds developing osteosarcoma in order to try to understand how the alterations contribute to the development and progression of osteosarcoma. Understanding the effects of the inherited genetic alterations is the first step in developing interventions to try and prevent the development of osteosarcoma, or treat the cancer in a 'targeted manner' should it arise. We believe that the most cost-effective way to try to identify the inherited genetic alterations that confer an increased risk of developing osteosarcoma is now to 'decode' the string of 2.4 billion letters that comprise the DNA (a procedure referred to as 'whole genome DNA sequencing') from several Wolfhounds with osteosarcoma and several unaffected Wolfhounds. The aim would be to identify differences between the 'DNA sequences' of affected and unaffected dogs that could disrupt processes involved in 'bone biology'. The DNA sequences of affected Wolfhounds would be compared with (1) the 'reference Boxer DNA sequence'; (2) DNA sequences of unaffected Wolfhounds that did not carry the chromosome 11 genetic marker associated with osteosarcoma in US Greyhounds, and (3) DNA sequences from other breeds (being generated at the AHT) unaffected by osteosarcoma. Whole genome DNA sequencing currently costs between £2,000 - £4,000 per dog, and so there is a considerable challenge to find sufficient funds to make this approach achievable. The 'whole genome DNA sequences' created by this exercise would be placed in the public domain, and would represent a hugely valuable resource for further studies to identify causes of inherited diseases in Irish Wolfhounds.

In parallel with the search for inherited genetic alterations that cause an increased risk of developing osteosarcoma, we would also like to try to identify the genetic alterations that arise in the osteosarcomas themselves and are responsible for the transition from '*an inherited predisposition to osteosarcoma*' to '*actual development*' of the cancer. The strategy would again involve 'whole genome DNA sequencing', but on this occasion we would be comparing the 'DNA sequence' of an osteosarcoma with the 'normal DNA sequence' (e.g. from a cheek swab) from the same dog, in order to identify the genetic alterations in the tumour associated with osteosarcoma development and progression. However, before we will be able to apply for research funding to undertake this investigation, it is necessary for us to collect 5 - 10 osteosarcoma biopsies (5mm cubes), within a special preservative, as soon as possible following surgery, or within 12 hours of a dog being put to sleep if a biopsy is collected post-mortem.

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