Identification of genetic risk factors for osteosarcoma in Irish Wolfhounds

Mike Starkey, Head Molecular Oncology

October 2019

At the Irish Wolfhound Health Group Health Seminar in November 2017, I presented an update on our progress in identifying inherited genetic risk factors for osteosarcoma in Irish Wolfhounds. At that time generous support from the Irish Wolfhound Health Group had recently enabled us to decode ('*sequence*') the DNA ('*genome*') of 4 Wolfhounds with osteosarcoma and 1 unaffected Wolfhound (a dog that died aged 12, and did not have cancer). The genome of a 5th Wolfhound with osteosarcoma was sequenced as part of the Animal Health Trust's '*Give a Dog a Genome*' project (GADAG; https://www.aht.org.uk/research/give-a-dog-a-genome). We identified an average of 7.3 million 'letters' ('*genetic variants*') at particular positions in a Wolfhound genome that were different from those found in the 'reference Boxer dog genome'. In order to try to predict which of the millions of genetic variants present in the Wolfhound genomes may be associated with osteosarcoma development, we looked to see which of the variants were absent from the genomes of 401 dogs belonging to 93 breeds that do not develop osteosarcoma. (The genomes of these 401 dogs were sequenced as part of the GADAG project, or by researchers who, along with the Animal Health Trust, are members of a collaborative international consortium)
We identified 19 genetic variants that were (1) present in the genome of at least one of the 6 Wolfhounds and absent from the genomes of the 93 non-susceptible breeds, and (2) were predicted by computer programs to be potentially 'harmful' changes in the DNA. In addition, we identified 173 genetic variants that were unique to the Wolfhound genomes, but which were not predicted to be harmful changes in the DNA. A proportion of these 173 genetic variants are likely to be 'Irish Wolfhound-specific genetic variants' which are not associated with osteosarcoma. However, it is very difficult to predict which genetic variants are harmless letter changes in the DNA and which may be associated with osteosarcoma.

Research on 'inherited cancers' in humans has shown that inherited genetic risk factors for cancer may be 'subtle changes' in the DNA which are not predicted to have a harmful consequence. Furthermore, we have recently identified a genetic variant associated with the development of mast cell tumours by Labradors and Golden Retrievers (https://journals.plos.org/plosgenetics/article?id=10.1371 /journal.pgen.1007967) that was not predicted to have any consequences. For this reason, we could not exclude any of the 192 Wolfhound-specific genetic variants as potential osteosarcoma risk factors solely on the basis of computer prediction as to their likely effect. In order to try to establish which of a large number of genetic variants are potential inherited genetic risk factors for a cancer in a susceptible breed we would typically like to do two things:

1) Test DNA from large numbers of dogs with the cancer and unaffected dogs to identify which of the variants are present significantly more often in the DNA of the affected dogs than the DNA of unaffected dogs
2) Test 'tissue' from dogs that have the variants in their DNA to investigate which of the variants have a 'biological effect'

Unfortunately, neither of these options was possible at the time. Our previous research has suggested that inherited genetic risk factors for osteosarcoma are present in the genomes of a large proportion of Wolfhounds that both have, and do not have, osteosarcoma. Consequently, comparing the frequencies of each of the 192 Wolfhound-specific genetic variants in Wolfhounds with osteosarcoma and unaffected Wolfhounds is unlikely to be effective at identifying those variants that are associated with osteosarcoma development. In addition, we do not currently have the 'tissue samples' (for example, osteosarcoma biopsies collected in a special 'preservative', or blood samples collected in a special 'blood sampling tube') required to investigate the possible 'biological effects' of the 192 Wolfhound-specific genetic variants.

Consequently, we have recently adopted a different strategy to try to identify genetic variants in the DNA of Irish Wolfhounds that are associated with osteosarcoma development. We are working on the basis that inherited genetic risk factors for osteosarcoma that are carried by Irish Wolfhounds may also be carried by other large and giant dog breeds that are susceptible to developing osteosarcoma, and are using to good effect the genome sequences of other breeds of dog with osteosarcoma that were generated as part of the GADAG project. We have recently identified 8.5 million genetic variants (differences identified by comparison with the reference Boxer dog genome sequence) amongst the 5 Wolfhounds with osteosarcoma, and 1 Leonberger, 1 Newfoundland, and 1 Pyrenean Mountain Dog with osteosarcoma, respectively, and 149 small, non-susceptible breeds. We have compared the frequency of each of these variants in the 5 Wolfhounds plus the 3 other giant breed dogs with osteosarcoma with their frequency in the 149 small, non-susceptible breeds. (We decided to exclude the genome sequence of the unaffected Wolfhound from the analysis because even though the dog lived to the age of 12 and did not have cancer, it is highly likely that the dog had 1 or more genetic risk factors for osteosarcoma in its DNA) Irrespective of the computer prediction of the biological effect of a genetic variant, variants which are present much more often in the DNA of the dogs with osteosarcoma (both Wolfhounds and the 3 other osteosarcoma-susceptible breeds) than in the DNA of the 149 non-susceptible breeds are likely to be associated with osteosarcoma, or some other trait (e.g. size) that is shared by the Wolfhound, Leonberger, Newfoundland, and Pyrenean Mountain Dog, and not by the 149 small, non-susceptible breeds. Due to their presence in Wolfhounds and at least one other osteosarcoma-susceptible breed, we can exclude the possibility that such variants are merely Wolfhound-specific genetic variants that are not associated with osteosarcoma. An initial screen of the variants which are present at a significantly higher frequency in the DNA of the Wolfhounds and the 3 other osteosarcoma-susceptible breeds suggests that few of the variants are located at positions in the DNA close to '*genes*' (the 'units' within the DNA that have a biological function) that are associated with 'size'. Consequently, we are optimistic that there are plausible genetic variants that confer an increased risk of osteosarcoma development amongst the variants that are present at a significantly higher frequency in the DNA of the Wolfhounds and the 3 other osteosarcoma-susceptible breeds. We are currently analysing the variants associated with osteosarcoma to identify variants that are predicted to have the greatest 'biological effect' and/or are located in the DNA close to genes that have functions relevant to bone biology, or have been shown to contain genetic variants that have been implicated as genetic risk factors for inherited human cancers.

In addition to the ongoing computational analyses, we need to further assess the likelihood that each osteosarcoma-associated genetic variant is a genetic risk factor for osteosarcoma by investigating *how* each variant could be a risk factor for osteosarcoma development. To do this we

will need to look at the 'biological effect' of each of the variants in 'tissue samples' from Wolfhounds that have variants in their DNA.

Irish Wolfhound samples required for further research

Osteosarcoma biopsies (5mm^3 or smaller pieces of tissue) collected immediately after surgery in a special preservative (called 'RNAlater'), and surplus blood samples (2.5ml) from both Wolfhounds with osteosarcoma and unaffected Wolfhounds collected in special tubes (called 'PAXgene tubes'). We can provide 'RNAlater' tubes in advance of surgery, and 'PAXgene tubes' ahead of an appointment in which it is expected that there may be some blood left over from that to be collected for a clinical purpose. Please contact us to request an RNAlater tube or a PAXgene tube by telephoning 01638 751000 ext. 1214, or by E-mail to oncologyres@aht.org.uk.