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Application for Funding

Project Title: Identification of germ-line osteosarcoma susceptibility mutations in Irish Wolfhounds

Date of Application: 04.12.15

Principal Investigator: Dr M. Starkey (Head Molecular Oncology, AHT)

Co-Investigators: Dr C. Mellersh (Head Canine Genetics, AHT)

Description of the Project:

Identifying the inherited genetic alterations that cause Irish Wolfhounds to have an increased risk of developing osteosarcoma, and trying to understand their effect, are essential steps towards developing interventions to try and prevent osteosarcoma development, or treat the cancer in a 'targeted manner' should it arise. We propose to 'decode' the string of 2.4 billion letters that comprise the DNA (a procedure referred to as 'whole genome DNA sequencing') from 4 Wolfhounds with osteosarcoma and 1 unaffected Wolfhound (which does not carry a chromosome 11 genetic marker strongly associated with osteosarcoma in US Greyhounds). We will attempt to identify 'functionally significant' variant DNA sequences present in the genomes of affected Wolfhounds that are absent from the genomes of a large number of breeds unaffected by osteosarcoma. This approach is viable because we have access to the genome sequences of dogs representing at least 25 breeds that are being compiled by the AHT's Canine Genetics Group via KC funding and the AHT's 'Give a Dog a Genome' project.

Budget:

Preparation of DNA for sequencing	£291.40
Construction of 'Sequencing libraries'	£1,052.52
Illumina HiSeq4000 DNA sequencing	£6,080.40
Total Direct Costs	£7,424.32

Budget justification: .....

Time Line: See the following page

Further information about the proposed study

Identifying inherited genetic risk factors for complex diseases such as cancer is not straightforward. It is difficult to discriminate between genetic alterations that are associated with disease and those that represent inconsequential random variations between individuals. Genetic alterations that are more likely to be associated with disease are more readily identifiable when they occur within 'genes' (the functional units within DNA), because a functionally significant consequence can be envisaged. However, there is increasing evidence that inherited genetic risk factors for complex diseases such as cancer are more likely to occur between genes, in sections of the DNA that are presumed to regulate how specific genes behave. Such genetic risk factors are much more difficult to identify because the sections of the DNA in which they occur may not currently be recognised as being 'regulatory regions', and so the functional consequences of genetic alterations in such regions are not readily apparent. The upshot is that any DNA sequencing study attempting to identify inherited genetic risk factors for a cancer is unlikely to deliver results quickly. However, where a conventional 'genetic mapping approach' ('genome-wide association study') has failed to pinpoint regions of the DNA that contain inherited genetic risk factors for a cancer (as a possible consequence of one or more risk factors being 'fixed' in the DNA), a whole genome sequencing approach is the only option to search for inherited genetic risk factors for the cancer. The computational analysis of whole genome sequence data to search for inherited genetic risk factors associated with a cancer is the first step in the process. The second and third steps (respectively) are to investigate whether other affected dogs carry the variant DNA sequences, and to try to experimentally demonstrate that variant DNA sequences predicted to be 'functionally significant' do actually have an effect that may be functionally significant. It is impossible to predict the cost of the experimental work required to validate potentially functionally significant variant DNA sequences at the onset of a whole genome sequencing study because the work involved is dependent upon the nature and number of the potentially functionally significant variant DNA sequences identified by the computational analysis of the genome sequence data.

Irish Wolfhound whole genome DNA sequences

The Irish Wolfhound whole genome DNA sequences generated during this study could be of use to any research study of inherited disease in the Wolfhound. We would anticipate analysing the whole genome DNA sequence data over a period of about 6 months before making the sequence data available in a publicly accessible database. Consistent with common practice in the world of academic research, unless we were entirely confident that we had derived all of the 'information' (vis-à-vis osteosarcoma) that we conceivably could from the sequence data, at the point of submission we would not 'label' the DNA sequences submitted to the publicly accessible database with the 'status' ('osteosarcoma', or 'unaffected by osteosarcoma') of the dogs whose DNA samples were sequenced. However, we would divulge the status of the dogs whose DNA samples were sequenced to an investigator from another institution who wished to utilise the DNA sequences for a non-osteosarcoma-focussed research study on a collaborative basis (in essence, they would acknowledge the role of the Animal Health Trust in any scientific publication describing the work that utilised DNA sequences generated by the Animal Health Trust). Once we had submitted for publication in a scientific journal a description of our analysis of the whole genome sequence data for the identification of genetic risk factors associated with osteosarcoma in Irish Wolfhounds we would add the status of the dogs concerned to the DNA samples previously submitted to the publicly accessible database.