THE KENNEL CLUB GENETICS CENTRE

25 January 2023

Flatcoated Retriever research update report – March 2022

Histiocytic Sarcoma project

In January 2021 researchers in the United States published the details of two regions of the genome (called loci) that are associated with histiocytic sarcoma (HS) in Flatcoated Retrievers (FCRs) (Evans et al. 2021). This means that some dogs carry genetic variants in these regions that increase their risk of developing HS. Although the precise variants that confer risk have not been confirmed yet, identifying regions of the genome where risk variants are located is an important first step in understanding the genetic basis of HS better.

One of the loci is on chromosome 5, and overlaps with susceptibility loci for two hematopoietic cancers, hemangiosarcoma and B-cell lymphoma, in the closely related golden retriever breed. The other locus is on chromosome 19 and is unique to the FCR. Together the authors report that these two loci account for about 35% of all disease risk which, if confirmed, is an extremely high value.

Researchers from the Kennel Club Genetics Centre (KCGC) have agreed to collaborate with the paper's authors, Dr Jacquelyn Evan and Dr Elaine Ostrander to achieve two objectives:

1. Confirm that the two loci described in the paper are indeed associated with HS risk in an independent cohort of FCRs. This is standard procedure for research studies investigating the genetics of genetically complex diseases such as cancer. DNA from a new cohort of FCRs with and without HS (details below) will be genotyped for markers within the two risk loci and statistical analysis will be used to determine whether markers on the risk *versions* of chromosome 5 and chromosome 19 are found in cases more often that controls. These risk *versions* are known as risk *alleles*.
2. Calculate the frequency of the risk alleles in FCRs from the UK. Assuming the association is confirmed (1 above) then knowing the frequency of the risk alleles will be essential before we can begin to consider developing a breeding tool based on either or both of these risk loci. If the risk alleles are very common within the UK FCR population it will be much more difficult to apply selective pressure against them, and reduce their frequency, than if they are less common.

Study cohort

Researchers from the KCGC are working with Jane Dobson and Anna Hollis from the Queen's Veterinary Hospital at the University of Cambridge to undertake this project. Between us we have identified a cohort of around 30 new HS cases and over 70 potential controls that have not yet been genotyped for the two risk loci described by Evans et al. These samples are from dogs with a confirmed diagnosis of HS that were patients of Dr Dobson or whose DNA was submitted directly to the KCGC, or who were reported by their owners to be free from HS over the age of 10. As part of this study, we will shortly be contacting the owners of the potential controls to confirm that these dogs did not in fact develop HS during their lifetimes. The KCGC will extract DNA from all of the above samples and send it to Dr Jacquelyn Evans, who worked within Dr Elaine Ostrander at the time the original findings were published but who has now established her own laboratory at Cornell University. Jacquelyn will genotype all the new cases and controls for markers within the risk alleles

to (i) confirm association with HS and (ii) determine the frequency of the risk alleles within the new cohort of FCRs.

Timeline

The DNA extraction and follow up of controls will start imminently and it is expected that the genotyping will take place over the summer, with preliminary results anticipated by early Autumn.

Acknowledgements

We would like to thank Luna's Legacy for providing vital funds to support this research. We would like to thank all the Flatcoated retriever owners who have contributed information, samples from their dogs and funds over the years – we wouldn't be in a position to do any of our research without you.

For all enquiries, please contact Cathryn Mellersh (cm935@cam.ac.uk) or Bryan McLaughlin (bryan.mclaughlin@thekennelclub.org.uk

Update January 2023

The work described above has been completed by the Evans lab and the results are summarised below.

Risk allele on chromosome 5

Eighty-two FCRs (26 HS cases and 56 unaffected controls) were successfully genotyped for the risk allele on chromosome 5. The frequency of the risk allele in the whole cohort was 0.42, meaning about 42 out of 100 FCR chromosomes carry the risk allele. The frequency among the cases was slightly higher than among the controls (0.46 compared to 0.40). This trend is what we would expect for an allele that is associated with HS. However the difference was not statistically significant, probably due to the relatively small numbers of dogs that were genotyped, and means we were not able to confirm association with HS in the UK population. The results also indicate that the risk allele is quite common among the controls as well as the affected dogs. However the frequency of the Chromosome 5 risk allele is lower in FCRs from the UK than in US FCRs (0.42 vs 0.66) confirming that different populations of the same breed can differ genetically, and that findings should be validated in specific populations.

Risk allele on chromosome 19

Seventy-four FCRs (18 cases and 56 unaffected controls) were successfully genotyped for the risk allele on chromosome 19. The frequency of the risk allele in the whole cohort was 0.9, meaning that nine out of ten FCR chromosomes carry the risk allele. The frequency among the cases was ever so slightly higher than among the controls (0.916 compared to 0.89) but the difference was not statistically significant. The frequency of the chromosome 19 risk allele is higher in UK FCRs compared

to FCRs from the US (0.9 vs 0.73). The high frequency of the chromosome 19 risk allele in the UK means it has no potential to be used as a selective breeding tool in the UK population.

Summary of results

The results demonstrate that the chromosome 19 risk allele described by Dr Jacquelyn Evan and Dr Elaine Ostrander is very common in the UK FCR population and should not therefore be used as a selective breeding tool in the UK FCR population.

Use of the chromosome 5 risk allele as part of a selective breeding tool has not been ruled out; however its association with HS in the UK population remains to be confirmed and will require a larger cohort of samples to do so. The frequency of the chromosome 5 risk allele is quite high among controls as well as cases, whereas some cases did not carry this risk allele, demonstrating that HS is a complex disease and additional risk factors for this disease remain to be identified.