

Glaucoma Project Report from James Oliver, AHT February 2017

“Identification of genetic risk factors for glaucoma and pectinate ligament dysplasia in the Flatcoated Retriever and development of DNA tests to reduce disease prevalence”

Aims and objectives of the project

Objectives of the study

1. To provide robust and current prevalence data for pectinate ligament dysplasia (PLD) in the Flatcoated Retriever (FCR) in the UK
2. To collect DNA from i) PLD cases ii) PG cases and ii) controls and perform genome-wide association analyses for PLD and PG
3. To identify genetic variants that confer susceptibility to PLD and PG in the FCR
4. To enhance the understanding of the relationship between PLD and PG in the FCR
5. To develop genetic tests based on the mutations or genomic regions we identify
6. To investigate which other breeds share the FCR genetic risk factors we identify and are thus able to benefit from the genetic tests we develop

Summary of the scientific achievements to date.

Objective 1.

To provide robust and current prevalence data for pectinate ligament dysplasia (PLD) in the Flatcoated Retriever (FCR) in the UK

We have now performed gonioscopy on 205 UK FCRs and published the data from 170 of these in *Canine Genetics and Epidemiology*; a copy of the manuscript is submitted along with this report. One hundred six of the 170 (62.4%) FCR we reported on were affected by PLD (ordinal grades 1-3); 70 (41.2%) being mildly affected (grade 1), 36 (21.2%) moderately affected (grade 2) and 0 severely affected (grade 3). A significant positive correlation was observed between PLD and age which is consistent with the condition being progressive. These findings were also presented at the European College of Veterinary Ophthalmologist's annual congress in Helsinki, 2015.

Objective 2.

To collect DNA from i) PLD cases ii) PG cases and ii) controls and perform genome-wide association analyses (GWAS) for PLD and PG

We were awarded funds to undertake a GWAS with 96 PLD cases and 96 controls, with the aim of identifying regions of the genome significantly associated with PLD. We have now performed GWAS investigations with DNA from 166 FCRs, comprising i) 89 FCRs with normal eyes (controls) ii) 77 with PLD or PG (cases).

In our previous report (November 2015) we summarised the results of a combined GWAS we had done with GWAS data we had at that time from 148 FCRs and also for 92 Welsh Springer Spaniels comprising i) 24 with normal eyes (controls) ii) 68 with PLD or PG. That analysis revealed a single locus on chromosome 11 that reached a genome-wide significant level of association with disease.

Since our previous report we have repeated a combined GWAS with i) the data from the 166 FCRs we now have (described in this report) and ii) 116 WSSs we currently have genome-wide genotyping data for and unfortunately the locus on chromosome 11, described above, no longer demonstrates a significant association with either PLD or PG. This non-significant result, obtained with a larger data set than before, indicates that our previous association was likely a false positive, obtained as a result of population structure within one or both of the breeds under investigation. We are currently exploring alternative methods to correct for population structure that are more appropriate for binary traits than the fixed effect model we used previously.

Concurrent investigations in our laboratory with other breeds at risk from PLD and PCAG have similarly failed to identify loci associated with PLD, but have successfully revealed two loci significantly associated with PCAG that we are currently following up. For the other breeds we

have studied we have larger numbers of samples from dogs affected with PCAG than we have for the FCR. Of our 77 FCR cases 65 are dogs with PLD but without PCAG whereas only 12 have PLD and PCAG; these numbers reflect the fact that the prevalence of PLD (and consequently PCAG) has decreased in the FCR over recent years, probably as a result of extensive eye screening by breeders (reference) and the very modest number of samples we have been able to include in our analyses from FCRs affected with PCAG might explain our difficulty in identifying regions associated with PCAG in this breed.

RNA and whole genome sequencing

Although outside the work described in our original proposal we are currently using RNA and whole genome sequencing methods as an alternative route to identifying genetic variants associated with PLD and/or PCAG in the FCR. This work is being done alongside our investigations with other breeds referred to above, and are funded by alternative sources. Briefly, we have extracted RNA from iridocorneal angle (ICA) tissues of dogs with normal/healthy eyes that were euthanized for welfare reasons (n = 4, comprising one Golden Retriever, one Great Dane, one Beagle and one FCR) and one FCR with PCAG. We have performed ribosomal RNA depletion on these RNA samples followed by next-generation sequencing, and are currently comparing RNA expression levels between the FCR eye affected by PCAG and the healthy control eyes. To our knowledge this study is the first to investigate RNA expression in the ICA of both normal canine eyes and those affected by PCAG. Genes which are preferentially expressed/suppressed in the PCAG vs control eyes will be further interrogated for candidate causal variants by assessing whole genome sequencing data from FCRs affected with PCAG. We already have whole genome sequencing data from 2 FCR cases in place to enable this.

Publications and presentations arising from the project.

- A manuscript has been published:

Oliver JAC, Ekiri A, Mellersh CM Prevalence of pectinate ligament dysplasia and associations with, age, sex and IOP in the Basset Hound, Flatcoated Retriever and Dandie Dinmont Terrier. **Canine Genetics and Epidemiology.** (2016) Mar 12;3:1.

- Our findings have also been presented orally:

Oliver JAC, Ekiri A, Mellersh CM (2016) Prevalence of pectinate ligament dysplasia and associations with, age, sex and IOP in the Basset Hound, Flatcoated Retriever and Dandie Dinmont Terrier. Annual Meeting of the European College of Veterinary Ophthalmologists, Helsinki, Finland. DOI 10.1186/s40575-016-0033-1