Summary of Flatcoated research, March 2018

Details below are taken from the final report submitted to PetPlan in March 2018, to summarise the major findings from the grant awarded to James Oliver and Cathryn Mellersh in September 2014 entitled:

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

Each Objective from the scientific proposal is listed below, in italics, and our findings stated below, together with a lay interpretation.

Objective 1.

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

FCRs were enrolled during gonioscopy screening sessions at different locations across the United Kingdom between September 2013 and December 2014. To the best of our ability, we recruited FCRs onto our study that were representative of the UK population of the breed. We achieved this by performing gonioscopy screening sessions in different locations around the UK and at different types of event, including dog shows, 'fun days' and breed information days. The gonioscopy screening was promoted by a variety of different mechanisms, including correspondence from the Kennel Club to the owners of Kennel Club registered dogs of each breed, via breed club websites and via social media. All dogs that were volunteered for screening were accepted, regardless of their age, ancestry or Kennel Club registration status.

We performed gonioscopy on 205 UK FCRs and published the data from 170 of these in *Canine Genetics and Epidemiology*. One hundred and 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 (rho = 0.34, $P < 0.01$), which is consistent with the condition being progressive. No correlation was observed between PLD and IOP (rho = -0.02, P = 0.85). These findings were also presented at the European College of Veterinary Ophthalmologist's annual congress in Helsinki, 2015.

These data have been published, paper attached.

Objective 2.

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

It was particularly challenging to collect DNA from PG cases of this breed compared to the other breeds we have been investigating despite employing multiple mechanisms of sample recruitment. Presumably, this relates to the reduced prevalence of PG in this breed compared to that reported 20 years ago. As PG cases, by their nature, would not be expected to present at gonioscopy screening sessions as described in **Objective 1**, different methods of sample recruitment were employed for these compared to PLD cases. Veterinary ophthalmologists were contacted on an international scale and invited to contribute clinical information/samples via online professional forums, advertisement in the veterinary press and via veterinary ophthalmology meetings and conferences. The study was also

promoted to FCR owners by the UK FCR clubs via social media. Finally, owners of FCRs examined under the BVA/KC/ISDS Eye Scheme were contacted via post.

We have performed GWAS investigations using DNA from 167 FCRs, comprising i) 89 FCRs with normal eyes ≥ 5 years old (controls); and ii) 78 with PLD (59) or PG (19) (cases). Data were first assessed for potential population stratification using quantile-quantile plots (Q-Q plot) to compare the distribution of observed test statistics with the distribution expected under the null. There was inflation of the observed findings across the distribution (genomic inflation factor = 1.21), indicative of population stratification or cryptic relatedness without compelling evidence for an excess of disease associations. Population stratification was corrected using the freely available GWAS software GEMMA (Genome-wide Efficient Mixed Model Association). GEMMA fits a univariate linear mixed model for marker association tests using a relationship matrix generated from the genome-wide SNP data to account for population stratification and relatedness amongst dogs. After correction, the genomic inflation factor was 1.03. Manhattan plots were created to display the GWAS findings with respect to their genomic positions to highlight any signals of potential interest. None of the SNPs reached genome wide significance (P = 0.05/number of SNPs (5.4×10^{-7})).

Lay interpretation of the above:

We have compared genome-wide markers between FCRS with glaucoma, PLD and healthy eyes, in all combinations, and unfortunately we haven't found any markers that are associated with either glaucoma or PLD when we analyse FCRs alone.

We have performed similar GWAS investigations in three further breeds: Basset Hound (BH) (37 controls, 25 PG cases, 57 PLD cases), Welsh Springer Spaniel (WSS) (33 controls, 36 PG cases, 54 PLD cases) and Dandie Dinmont Terrier (DDT) (51 controls, 32 PG cases, 21 PLD cases). We conducted a combined GWAS analysis across breeds using meta-analysis of summary statistics for each SNP (log odds ratios and standard errors; analysed using GEMMA) from each individual breed GWAS. We used a fixed effects model and inverse-variance weighted averages of regression coefficients to obtain a combined estimate of the overall odds ratio for SNPs that passed quality control filters and were informative in all breeds. No SNPs reached genome-wide statistical association when comparing controls against PLD and PG cases combined but, when comparing controls against PG cases alone, two SNPs on chromosome 28 reached a genome-wide significant level of association with PG (P-value 5.9×10^{-6}, threshold = 1.3×10^{-6}). . A FCR-specific associated locus was defined based on pair-wise linkage disequilibrium estimates ($R^2 \geq 0.5$) of the SNPs (CanFam3.1: chr28:14697561-14721865).

Lay interpretation of the above:

When we analyse the FCR genome-wide marker data with that of other breeds (Basset Hounds, Welsh Springer Spaniels and Dandie Dinmont terriers, we do find two markers, on chromosome 28 that are statistically associated with primary glaucoma. We do not necessarily believe these markers themselves are causing glaucoma, but they are located in a region of the genome where a 'real' risk variant lies.

Objective 3.

To identify genetic variants that confer susceptibility to PLD and PG in the FCR.

Whole genome sequencing (WGS) data from two FCR with PG (funded by alternative sources) were assessed for the top two SNPs identified in **Objective 2**. One of these was found to carry one copy of each risk allele (SNP). WGS from this dog was compared with those from 96 dogs of other breeds

(from the AHT WGS data bank) not thought to be predisposed to PLD/PG. The locus defined in **Objective 2** was interrogated for variants present only in the FCR PG case and absent in the other 96 breeds. One intergenic (between *HSP6* and *LDB1*) SNP variant met these criteria (chr28:14624548). This SNP was genotyped in the FCR GWAS cohort to test its association with PG by Sanger sequencing. The level of association of this SNP with PCAG was the same as for the two top SNPs from the meta-analysis. We have also assessed the other three breeds in the meta-analysis for variants underlying the chromosome 28 association but none of these reached the level of association of the top SNPs. Identified regions were screened for structural variants by visual inspection in the Integrated Genome Viewer (IGV). None were found.

We have also used RNA sequencing methods as an alternative route to identifying genetic variants associated with PLD and/or PG in the FCR. This work is being done alongside our investigations with other breeds referred to above, and is funded by alternative sources. We have extracted RNA from iridocorneal angle (ICA) tissues of dogs with normal/healthy eyes that were euthanised for welfare reasons (n = 4, comprising one Golden Retriever, one Great Dane, one Beagle and one FCR) and one FCR with PG. We have performed ribosomal RNA depletion on these RNA samples followed by next-generation sequencing, and have compared RNA expression levels between the FCR eye affected by PG and the healthy control eyes. In these analyses, we found no difference in gene expression across the locus identified in Objective 2. However, when comparing gene expression in PG-affected dogs of all 4 breeds (n=7), one gene in this locus was differentially expressed. This gene has previously been associated with retinal ganglion cell protection and therefore is a promising candidate gene for further investigation into the genetics of canine PG.

Lay interpretation of the above:

We have sequenced the whole genome of two FCRs with glaucoma, in an attempt to find variants that are more highly associated (and therefore more likely to be causal) than the two variants described above. Unfortunately we didn't find any that were more highly associated with glaucoma, meaning we haven't as yet identified the precise position in the DNA that is causing glaucoma, but we know the region where at least one important risk factor is located.

We have also looked at differences gene expression (i.e. which genes are switched on and off and by how much) in the eyes of FCRs with glaucoma and compared them to dogs with healthy eyes. We have found evidence of one gene whose expression is altered between dogs with and without glaucoma; this same gene is associated with eye disease in humans and so is a promising candidate for further investigation.

Summary

We have shown that PLD almost certainly progresses in FCRs . This means that repeated gonioscopy screening is recommended throughout life and this replaces previous advice that screening should be a once-in-a-lifetime event.

We have found a position in the genome where a genetic risk variant(s) for glaucoma in FCRs is located. As yet we have not identified the precise position in the DNA that is responsible for the increased risk so at this stage we are not in a position to offer any form of DNA test. We have also identified a gene that is worth further investigation because its expression appears to be different between dogs with glaucoma and dogs with healthy eyes.

Our findings quite clearly indicate that PLD and glaucoma are complex conditions, and as such investigations to tease apart the genetic basis of these conditions will take time and continued research.

James Oliver is now writing up his thesis, so will spend much of the next six months preparing for his PhD examination and also preparing a scientific paper detailing his findings, which will be submitted for publication, also within the next six months. We hope that once our findings have been published we will be able to secure additional funding to continue our investigation of glaucoma in FCRs as well as other breeds.