Genome-wide Association Analyses for Primary Glaucoma and Pectinate Ligament Dysplasia in the Flat Coated Retriever in the United Kingdom

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Background

In the UK, the Flat Coated Retriever is known to be affected by primary glaucoma (PG). In this breed, PG is strongly related to the degree of pectinate ligament dysplasia (PLD) which, in turn, has been shown to be highly heritable.[1] Although PLD has not been proven to be the sole cause for PG it is, at the very least, a very useful clinical marker for the risk of developing it. The phenotypic observations on PLD are thus used as a guide for breeding and are assessed by gonioscopy which is performed most commonly, in the UK, by a panellist of the BVA/KC/ISDS Eye Scheme. Over the last decade, there has been a marked acceleration in the discovery of genes and chromosomal loci that are associated with or, less commonly, causal for PG in man. The only published studies into the molecular genetics of primary PG in dogs, to the authors' knowledge, have been into primary open angle glaucoma (POAG) in the Beagle. In this breed a Gly661Arg mutation of *ADAMTS10* has been shown to be the likely cause of primary glaucoma.[2]

In this study we aim to perform a genome-wide association analysis for PG and PLD in the Flat Coated Retriever, to identify region(s) of the genome that contain mutations that increase an individual dog's risk of developing PLD and PG. These regions will be investigated in depth, with the aim of pinpointing the precise mutations that are involved. An important aim of the study will be to develop a DNA tool with which breeders can estimate an individual dog's risk of developing PG and of passing it on to its offspring.

Aims and Objectives

- To perform a genome-wide association analysis for PG and PLD in the Flat Coated Retriever in the UK
- To identify sequence variants that confer susceptibility to PG and PLD
- To enhance the understanding of the relationship between PLD and PG in this breed i.e. is PLD likely to be causal or not for PG?
- To confirm or exclude this breed of dog as a spontaneous model for PG in man

Materials and Methods

- A total of 240 Flat Coated Retrievers

- Cheek swabs will be taken, following written owner consent, from Flat Coated Retrievers examined by a panellist of the ECVO or BVA/KC/ISDS Eye Scheme and submitted for genome-wide analysis along with copies of Eye Scheme Certificate. If feasible, the same

examiner will perform all eye tests to reduce the influence of subjectivity on assessment of the iridocorneal angle.

- A full ophthalmic examination will be performed and the protocol for gonioscopy as published by Read et al (1998) will be followed with use of a slit-lamp biomicroscope.[3]

- There will be three data sets comprising the following:
    1. Ninety-six animals free from glaucoma and with less than 25% of the ICA circumference affected with PLD (judged as 'normal'). **CONTROLS**
    2. Ninety-six animals free from glaucoma and with greater than 25% of ICA circumference affected with PLD (judged as PLD-affected and at risk of developing primary glaucoma). **PLD CASES**
    3. Forty-eight animals with primary glaucoma (uni or bilateral). An assessment of degree of PLD in the fellow eye will be performed in unilateral cases. **GLAUCOMA CASES**

- It is also hoped that tissue from the iridocorneal angle of Flat Coated Retrievers with primary glaucoma will be obtained. This tissue will be derived from eyes that have to be removed owing to irretractable primary glaucoma (i.e. blind and painful eyes that show no response to medical and/or surgical therapy). The tissue will need to be immediately preserved in RNAlater, which will be provided by the AHT, before transport to our laboratory for analysis. Any owners or veterinarians who are in a position to contribute iridocorneal angle tissue should contact the Animal Health Trust (contact details are at the end of this document) prior to surgery, to request some RNAlater for this purpose. Gene transcripts and levels of expression of relevant genes in this tissue will be compared with that from tissue of the iridocorneal angle of a normal canine eye.

- Statistical analysis
    1. Genome-wide association (GWA) analysis: We will use the analysis package PLINK to conduct basic unadjusted GWA analyses. We will also assess for the presence of population substructure in our samples using multidimensional scaling in PLINK and correct our data for any stratification using a mixed model implemented in the statistical package R. This tool is designed to take into account the familial and pedigree structure of the sample set.
    2. Analysis of identified variants (associated GWAS markers (SNPs) or putative mutations) for PLD and PG. In our primary tests for association with PLD and PG we will use logistic regression to calculate odds ratios and 95% confidence intervals for both general and linear per allele models. To test the linearity of the association between identified variants and risk of PLD or PG we will use log likelihood ratio tests by comparing a general model with the linear per allele model. This will give us an indication of how the mutation is expressed (i.e. in a recessive, dominant or co-dominant manner). If multiple mutations are identified we will use log likelihood ratio tests to assess the independence of each mutation

on conferring disease risk and to assess for the presence of genetic interaction between mutations.

Funding

This project is currently unfunded. Funding for the collection of DNA from 240 FCRs will be provided by the Kennel Club Genetics Centre. When the DNA sample is complete (or nearing completion) funding to support the GWAS will be applied for, but in the experience of the investigators such an application is much more likely to be successful if the DNA samples are in hand prior to applying for funding.

Timeframe

The study will commence when funding has been secured and when the DNA sample collection is complete. If collection of DNA from 48 GLAUCOMA CASES proves difficult we may consider initiating the study when we have collected DNA from 96 CONTROLS and 96 PLD CASES.

Publications

1. Read RA, Wood JLN, Lakhani KH. Pectinate ligament dysplasia (PLD) and glaucoma in Flat Coated Retrievers. I. Objectives, technique and results of a PLD survey. *Veterinary Ophthalmology* 1998; 1: 85-90
2. Kutchey J, Kunkel J, Esson D et al. Screening ADAMTS10 in dog populations supports Gly661Arg as the glaucoma-causing variant in beagles. *Investigative Ophthalmology and Visual Science* 2013; 54: 3
3. Wood JLN, Lakhani KH, Read RA. Pectinate ligament dysplasia (PLD) and glaucoma in Flat Coated Retrievers. II. Assessment of prevalence and heritability. *Veterinary Ophthalmology* 1998; 1: 91-99

Contact Details:

To request a DNA swab collection kit or RNAlater please contact:

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