Canine Genetics Progress Report

Breed: Lancashire Heelers and Miniature Bull Terriers

Condition: Primary Lens Luxation (PLL)

Date: 14.04.2008

Recent / Current Funding:

1. *Funding Body:* Kennel Club Health Foundation Fund
   Amount: £83,281 (including £8000 from Miniature Bull Terrier Breed Club) this grant was to study four inherited conditions, one of which was PLL
   Start Date: March ’03, 24 months
2. *Funding Body:* Kennel Club Health Foundation Fund
   Amount: £49,823 (including £2000 from Lancashire Heeler Breed Club)
   Start Date: March ’05, 24 months
3. *Funding Body:* Canine Health Foundation (American Kennel Club)
   Amount: $9586
   Start Date: January ’05, 12 months
4. *Funding Body:* Canine Health Foundation (American Kennel Club)
   Amount: $12927
   Start Date: February ’07, 12 months
5. *Funding Body:* Miniature Bull Terrier Club
   Amount: £12,000
   Start Date: February ’08, 12 months

6. *Funding Body:* Belgian Tibetan terrier Club; Individual donations from overseas

   Amount: €500; approximately £550

   Periode: January 01, 2008 - present

The Primary Lens Luxation research project is currently collaboration between Cathryn Mellersh (AHT), David Sargan (University of Cambridge) and David Gould (Davies Veterinary Specialists).

Progress Update

As we have reported previously, considerable progress has been made recently to identify the region of the genome that contains the mutation responsible for PLL. We have identified a region, on chromosome 3, that is shared between all dogs that are affected with PLL. Slowly but surely we have been narrowing the region and now have reduced it to around 300,000 nucleotides or letters of DNA, which is less than a tenth of 1% of the canine genome. This is called the 'PLL critical region'

We have identified a gene within the critical region that is a very good candidate for PLL that we would now like to sequence (i.e. read letter by letter). Unfortunately the sequence and structure of the DNA in this region makes it techniquely challenging to sequence and this has meant that our progress with this gene has been slower than it would be for other, more normal, regions of the genome. Because of our sequencing difficulties we have recently sent cloned Doberman DNA from the critical region to be sequenced by a company offering a new sequencing technology, in the hope this would shed light on the sequence of this gene in a normal (PLL unaffected) dog. We received the sequencing data a week ago and are currently in the process of analysing it. This is not a trivial task as we received 2,375,962 'reads' of DNA, each of which is 33 nucleotides (or letters) of DNA long. We have to align all these reads to obtain a consensus DNA sequence that will tell us the sequence of nucleotides in the normal gene. Hopefully, once we have the sequence of the normal gene we will be able to investigate the DNA from PLL affected dogs to see if mutations in this gene account for PLL.

We have also started to explore the possibility of developing a 'linkage-based' DNA test that would use the DNA within the PLL critical region to determine whether dogs are affected, carrier or clear of PLL. Because linkage-based tests do not assay for the presence or absence of the causal mutation, but rather rely on nearby 'linked' DNA, they are not 100% accurate, but if carefully designed they can

achieve levels of accuracy in excess of 95% and would represent a useful tool with which breeders can start to reduce the incidence of this condition until the mutation itself is identified.

A PhD student, Elena Hernández Merino, who is supervised by David Sargan at Cambridge University is currently examining markers from the PLL critical region in affected and known carrier dogs, to identify any that could form the basis of a linkage-based DNA test. This work is ongoing but must be given close attention; if a linkage-based DNA test is to be made available it is critically important that our selection of markers is prudent to minimise incorrect diagnoses.

Having a dedicated member of staff working on this project, means the project is moving forward as quickly as it can and the principal investigators meet on a regular basis, to monitor progress and discuss results. Identifying the mutation responsible for this condition continues to be a major focus for all concerned with this project.

Sample Collection

The research has progressed sufficiently well that we are now only targeting samples from dogs, of any breed, that are *affected with PLL*. Samples from additional affected dogs will continue to play a valuable role in the research right up until the point at which we find the mutation and can develop a DNA test.

We would also like to thank everybody who has made a financial donation to support our research studies. As a charity the AHT relies heavily on donations, whilst all research performed at the University of Cambridge is also funded solely through external donations and competitive grants, and not through support from the higher education funding system. All donations to support our research are truly appreciated by both organisations.