Canine Genetics Progress Report

Breed: Lancashire Heelers and Miniature Bull Terriers

Condition: Primary Lens Luxation (PLL)

Date: 01.07.2008

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; donations from individuals
	Amount:	€3315; approximately £2,638
	Period:	January 01, 2008 - present

The Primary Lens Luxation research project is currently a 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 now been made 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'

Sequencing Candidate Gene In 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. It also means that this region is 'missing' from the canine *whole genome sequence*, which is most of the 2.5 thousand million nucleotides of DNA from a boxer called Tasha that have been sequenced and deposited in publicly available databases. This whole genome sequence is the 'reference sequence' that we use for many of our analyses. Because of our sequencing difficulties we 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. The task of analysing the sequencing data was not trivial, as we received 2,375,962 'reads' of DNA, each of which is 33 nucleotides (or letters) of DNA long, but the analysis is now complete. Unfortunately, because we did not have a 'reference sequence' to align the 2 million reads to (because this stretch of DNA is missing from the whole genome sequence), we were unable to align

the reads we obtained adequately to assemble them into a single consensus sequence and this means we have still not been able to determine the DNA sequence of the candidate gene we are investigating. We are currently considering alternative options.

Exclusion Of Additional Genes In PLL Critical Region

We have sequenced the entire coding regions of two additional genes inside the critical region. These genes were not particularly strong candidates for PLL but because they are located inside the PLL critical region we sequenced them to be sure neither were harbouring mutations that were responsible for PLL. Both genes have now been formally excluded from involvement with PLL.

Linkage-Based Test

We are continuing 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. Elena has recently analysed 25 affected dogs and 20 carriers with 10 new markers from within the PLL critical region. The data has been added to that we have obtained previously and is helping us establish a very detailed picture of the DNA within the critical region and also allowing us to decide which markers within the critical region will be the best ones to base a linkage test on. It is our hope that a linkage-based test will ultimately be offered to all breeds under investigation, however slight differences between breeds means the development of the test may be more straight forward in some breeds than others. As a consequence we might be able to offer a test to some breeds ahead of others but this is not a reflection of the time or effort being invested in any of the breeds.

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.