Canine Genetics Progress Report

Breed: Lancashire Heelers and Miniature Bull Terriers

Condition: Primary Lens Luxation (PLL)

Date: 25.10 **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 a collaboration between Cathryn Mellersh (AHT), David Sargan (University of Cambridge) and David Gould (Davies Veterinary Specialists).

Progress Update

As we have reported previously, we have identified a region, on chromosome 3, that is common to 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'

Linkage-Based Test

Since the last report we have continued 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.

We now know the genetic 'appearance' of the chromosome that is carrying the PLL mutation, and it is more or less the same in Miniature Bull Terriers, Lancashire Heelers and both Jack and Parson Russell terriers. Any linkage test we develop will look for the presence of this 'PLL' chromosome in any dog that we test and predict the dog's PLL genotype accordingly. However, for a linkage test to be robust, we need to make sure that there aren't any 'normal' chromosomes present in the population that look like the PLL chromosome. We also need to be sure that affected dogs always have two PLL chromosomes, carriers always have a single PLL chromosome and clear dogs don't ever carry a PLL chromosome.

We have continued to make progress with the development of the linkage test but the two breeds on which we have focused (Lancashire Heelers and Miniature Bull Terriers) have both presented us with their own particular difficulties, which are explained below. We encourage all readers, be they Miniature Bull Terrier or Lancashire Heeler 'supporters' to read both sections of this document, to gain a full appreciation for the full range of issues that we are currently considering.

Miniature Bull Terriers

The Problem

We know from our research that MBTs are relatively inbred compared to other breeds of dog, which means that there aren't as many different versions of each chromosome present in the MBT population as there are in other breeds. Because of these high levels of inbreeding we are concerned that there might be normal chromosomes segregating in the MBT population that are very similar to the PLL chromosome. This situation could have arisen if, when the PLL mutation arose in the MBT, there were lots of other copies of the same chromosomes present in the population. These other chromosomes did not gain the mutation, but because we have not yet been able to identify the PLL mutation we can't tell the difference between the genuine PLL chromosomes and other 'normal' chromosomes that do not carry the mutation, but are the same in other respects. To put this simply, imagine the founding dog (the dog in which the PLL mutation initially arose) had a **red** and a **blue** chromosome and the PLL mutation arose on the **red** chromosome (now denoted as **red***). The **red** chromosome would have been inherited from either the founding dog's mother or father, and thus approximately half of the founder's brothers and sisters would also have inherited the **red** chromosome, as well as around half of the parent's siblings (and other related dogs as well). Any dogs that carried the **red** chromosome and that subsequently reproduced would have passed the **red** chromosome onto future generations. But only the **red*** chromosome, generated within the founder and passed on to his/her ancestors, would carry the mutation. In other words, there could be **red**, and **red*** chromosomes present in the population, but until we identify the actual PLL mutation we can't tell them apart.

The Solution

We need to obtain an accurate estimate of how many dogs that are NOT affected with PLL carry two copies of the **red** chromosome. This will enable us to estimate the frequency of the **red** chromosome (as opposed to the **red***) in the current MBT population**.** If the percentage of genuinely unaffected dogs carrying two copies of the **red** chromosome is very low, then we will know that the linkage test

will have a high degree of accuracy – however, if the number of unaffected dogs that are homozygous for the **red** chromosome is high, the linkage test will have a lower level of accuracy (and we will be able to estimate the error rate fairly precisely).

Before we can undertake this estimation we need to confirm which MBT samples we hold are from dogs that NEVER developed PLL. Some of the samples we hold were submitted from dogs when they were quite young and although they were PLL free at the time of submission we can't be sure they remained so. We can't use young dogs for this calculation because we can't be certain young dogs are genuinely unaffected.

We request that anybody and everybody who has ever sent the AHT a DNA sample from a MBT that is now over the age of 10 (or that lived to be greater than 10) that has never developed PLL PLEASE to let us know. If the dog was formally examined by an ophthalmologist and declared free of PLL in later life then even better! Please email Bryan McLaughlin (bryan.mclaughlin@aht.org.uk) with any relevant update information, stating the dog's KC registered name, the age he/she is now, (or the age to which they lived), and evidence they were unaffected by PLL (this might be your knowledge they never developed PLL, or an ophthalmologist's report etc.). The sooner we obtain this update information the sooner we can begin to estimate the frequency of 'normal' **red** chromosomes in the MBT population and the consequent accuracy of any potential linkage test.

If anybody has a 10 year old dog that has never developed PLL, but that never been sampled, please consider submitting a sample now. Swab kits can be requested from Bryan at the above address.

Lancashire Heelers

The Problem

We know now that affected MBTs and LHs all carry copies of the PLL chromosome (described as the **red*** chromosome above). However, it has come to our attention that not all affected dogs carry two copies of the PLL chromosome – a small fraction carry only one PLL chromosome and a copy of a different chromosome (i.e. they are heterozygous, as opposed to homozygous, for the PLL chromosome). There are two possibilities to explain this anomaly; either the non-PLL chromosomes that these affected dogs carry are in fact identical to the PLL chromosome over a tiny region that lies in between the markers we have analysed (so we haven't found it yet) or that some dogs can in fact develop PLL when they carry only a single copy of the mutation. This is in contrast to the assumption that PLL is a recessive condition. Most of the affected LHs we have analysed do carry two copies of

the PLL (**red***) chromosome (consistent with a true recessive condition), so it appears that the heterozygous dogs may be unusual in some way that we don't understand at this time. It does appear as if some, but not all, the heterozygous dogs developed PLL quite late in their lives. Obviously the possibility that heterozygous (carrier) dogs can develop the disease has serious implications for the development of a linkage test.

The Solution

We need to find out if the non-PLL chromosomes that some affected dogs carry are in fact identical to the PLL chromosome over a small region. Imagine the chromosomes are like strings of beads, where each bead is a genetic marker we have analysed, and the beads on the PLL chromosome are red and those on non-PLL chromosomes are many different colours. Most affected dogs carry two chromosomes, both of which carry red beads. But the small number of heterozygous affected dogs carry one chromosome with red beads and one chromosome with different coloured beads. We have not currently analysed every bead along the length of the chromosome, so it is possible that the chromosomes carrying different coloured beads do in fact carry a very short stretch of red beads that lie in between the beads (markers) we have analysed. If they do, and we can find this tiny region where the beads are in fact all red (i.e. where the seemingly heterozygous dogs are in fact homozygous) then we will have identified the tiny region where the PLL mutation lies. If they don't, then we will know for certain that PLL is not a true recessive condition and that carriers can, under certain circumstances, develop PLL. Either conclusion would obvious have important implications for understanding of PLL and the development of a DNA test.

The solution to this question is for us to analyse virtually every bead (marker) on the PLL chromosome and experiments to achieve this are already being planned. To enable us to analyse markers at this density we will make use of highly sophisticated and expensive technology but, due to the generosity of a great many individual owners and breeders, we have the funds in place to undertake this investigation. We will carry out the experiments with DNA from both LHs and MBTs that are affected and unaffected with PLL, to gain an unprecedented, in-depth view of the PLL chromosome. Whether we identify a tiny region of the PLL chromosome that is in fact homozygous in all affected dogs (of either breed) or whether we conclude that some dogs with PLL do in fact have two different chromosomes our understanding PLL will be enormously enhanced.

In Conclusion

The experiments described above will take around three months to plan, execute and analyse, which will take us into the start of 2009. It was always the hope to make a linkage test available to breeders by the end of 2008 but realistically this is now unlikely to happen. Our estimated timeframe was based on the length of time similar projects have taken to deliver a result, but this time I am afraid many different factors have meant that the anticipated timeframe was not realistic for this project. Confounding factors include the fact that the region of the PLL chromosome we are working with is missing from the Whole Genome Sequence, that Miniature Bull Terriers are particularly inbred, and that PLL isn't behaving as a well-behaved recessive condition should, in Lancashire Heelers at least. But unfortunately this is what real research is like sometimes.

But the collaboration is committed to making a PLL test available to the dog breeding community. I personally am now being kept awake at night with this one, because I know how much this means to all you committed and dedicated breeders, as well as all the ophthalmologists who see affected dogs suffering with this horrible condition. This is what keeps me going and I am still supremely confident we will develop a DNA test within the near future.

The collaboration would 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.