

Update on CA research in Kelpies at University of New South Wales. (January 2011)

Previously we have reported that we have localized the ataxia mutation in affected kelpies to a region of 5 million bases (0.2% of the dog genome), by using the SNP arrays funded by the donation from Terry Snow. This region contains 44 genes, but none of these genes have known link with ataxia in other model organisms. In an effort to identify the causative mutation, the next generation sequencing facility at the Ramaciotti Centre at UNSW was employed to get the entire sequence for all 44 genes within the candidate region, from two affected dogs and one unaffected control dog. A total of 2017 differences were identified between affected dogs and the control dog, in which 440 differences can be eliminated as they also occur in other breeds. Any of the remaining 1577 differences could be the cause of the ataxia. All 14 differences that can result in a change in proteins made by these genes, were investigated as a possible cause of CA in kelpies by DNA sequencing 96 kelpie DNA samples from affecteds and controls to see if the difference is inherited exclusively with the disease. Unfortunately, these 14 differences have also been eliminated as causing CA in kelpies. This leaves us with more than a thousand differences to check in order to find the cause. These are not differences in the genes but between the genes in regions that may affect the amount of the gene product produced.

Recent work has been to look for more differences within regions that code for protein production, between ataxia affected dogs and unaffected control dogs. Using the automated DNA sequencing technology has allowed us to obtain a more complete sequence for seven genes that were within the candidate region, in five affected dogs and five control dogs. These seven genes have shown no differences between affecteds and unaffected controls were identified. In addition, 17 regulatory differences identified between the affected samples and control sample (by the next generation sequencing) were investigated as a possible cause for CA in kelpies. This is done by checking 49 kelpie DNA samples to see if any of these differences is associated exclusively with the disease and unfortunately, none were and these 17 differences have also been eliminated now as causing CA in kelpies.

By employing a candidate gene approach, we have now decided to focus our attention on 20 genes (out of the 44 genes) that are most likely to have a disastrous effect on the brain and resulted in CA, if a mutation is found in the gene. These 20 genes were chosen for their high expression profile in the human and mouse brain. While we have chosen to focus our attention on these 20 genes, it is possible that the causative mutation is not within one of these 20 genes and we may have to look at the rest of 24 genes to find the mutation causing CA. This will begin very shortly and should be completed by the end of May. The genetic cause of CA looks to be very difficult to identify but we will continue until the causative mutation is found and a DNA test is developed. The search has taken longer than we had hoped as the cause is not in any of the obvious places. When there is no obvious mutation, the search takes longer as other genes in the region and DNA between the genes will need to be looked at and other techniques will need to be adopted.

The continuing research has been supported by a grant from the Working Kelpie Council and a donation from the Swedish Kelpie Club. Annie Pan has taken over the research from Jeremy Shearman who has completed his PhD and moved to Thailand. Jeremy's research has been published in the scientific journal, *Animal Genetics*. The data can be accessed online.

A further development has been to establish a collaboration with Professor Rosanne Taylor and her student, Jessica Fletcher, at Sydney University. One problem with disease research is

distinguishing animals affected with CA and those with one of the many other conditions producing similar symptoms. If animals are included in the study that is suffering from other conditions it can confuse the research and make results difficult to interpret. The only way to be sure is to examine brain tissue under the microscope and look at the changes and ensure that they are consistent with CA. We have obtained 6 samples from animals euthanized because of severe ataxia that is suspected to be caused by CA. Samples come from Tasmania, NSW, Victoria and Canada. We are adding samples like this to the testing panel as they become available. The more samples we have the easier our task.

We are continuing the research into CA in the hope of producing a DNA test. It requires more hard work and a little luck.

Online access to ataxia paper and data

<http://web.science.unsw.edu.au/~awilton/AtaxiaPaper.pdf>

<http://web.science.unsw.edu.au/~awilton/AtaxiaSuppData.zip>

This report has been produced by researchers Annie Pan and her supervisor Dr. Alan Wilton.